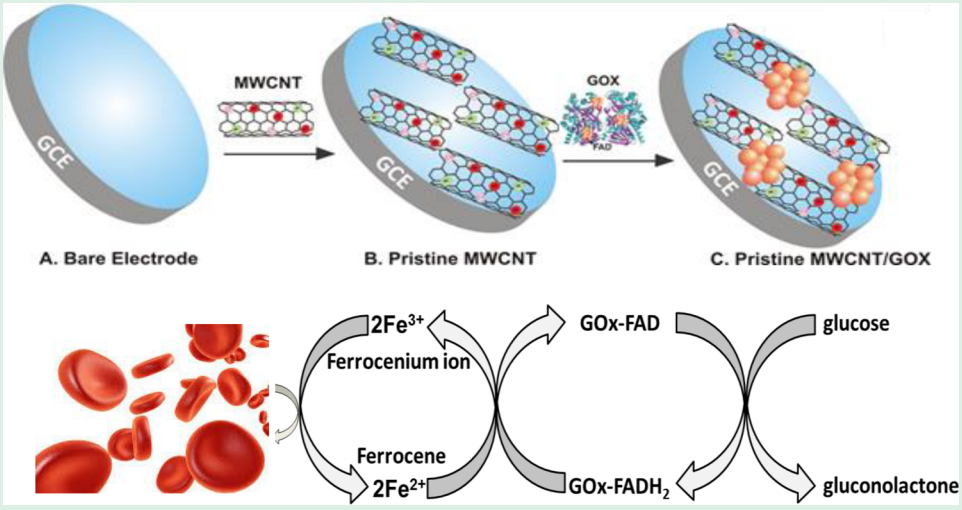
ACS APPLIED BIO MATERIALS
Cite This: ACS Appl. Bio Mater. 2019, 2, 1740−1750
Article
www.acsabm.org

Achieving a Stable High Surface Excess of Glucose Oxidase on Pristine Multiwalled Carbon Nanotubes for Glucose Quantification

Bose Dinesh,* K. S. Shalini Devi, and Uma Maheswari Krishnan*

Centre for Nanotechnology & Advanced Biomaterials (CeNTAB), School of Chemical and Biotechnology, SASTRA Deemed University, Thanjavur 613 401, India



ABSTRACT: In this study, glucose oxidase (GOx) immobilization onto ten different types of carbon modified GCEs and its direct electron transfer (DET) were investigated. A maximum amount of GOx immobilization (Γ_{GOx}) of 2.9 nM/cm^2 was achieved on the pristine multiwalled carbon nanotubes (PMWCNT) with high stability. Furthermore, the coefficient value for electron transfer (0.5) and the rate constant of 3.16 s^{-1} were measured from scan rate studies on PMWCNT/GOx. The derived electro-analytical parameters were superior in PMWCNT system than those of several CNT based nanocomposite materials published in the literature. The PMWCNT/GOx displayed a standard potential ($E^{0\prime}$) of −444 mV with perfect redox peaks, and appreciable peak separation (ΔE_p) value of 22 mV in neutral electrolyte medium was noted. Glucose quantification was made using the mediator, ferrocene monocarboxylic acid (FMCA), and quantification was done with dissolved oxygen (O_2) reduction caused by the glucose oxidase-mediated enzymatic catalysis of glucose. Sensor calibration results revealed a broad range from 0.2 to 5.8 mM with a lower limit of determination found to be 45 μM for glucose. A strong affinity between PMWCNT/GOx and glucose was assessed with Michaelis−Menten constant (2.24 mM). The proposed biosensor had excellent sensitivity and remained unaffected by the presence of other electroactive groups. This work demonstrates that pristine MWCNT can be used directly as an immobilization matrix for biosensing applications without cumbersome electrode preparation steps and the introduction of dopants.

KEYWORDS: *pristine MWCNT, glucose oxidase, immobilization, biosensors, glucose*

1. INTRODUCTION

A flavin enzyme of glucose oxidase (GOx) with a mass value of 150−180 kDa remains among the extensively employed biomolecule to monitor the progression of diabetes in patients with dysregulated glucose levels. The immobilization of GOx is essential for fabricating biosensors, clinical devices, and bioreactors. Several strategies like cross-linking, physical adsorption, and covalent entrapment have been employed for the immobilization of GOx. Flavin adenine dinucleotide (FAD) is interiorly embedded in the protein folds of GOx.[1,2] As a result, DET (direct electron transfer) of GOx is not possible on solid electrodes due to the weak electrical wiring between the FAD moiety and the bare substrate surfaces.[3,4] There have been numerous efforts made to advance DET between the FAD of GOx and the substrate using a plethora of materials including metal oxides, polymers, quantum dots, mesoporous silica, metal nanoparticles, and carbon-based materials such as CNTs and graphene-based nanocomposites for the GOx immobilization.[5,6] Among these, CNT-based materials are capable of creating molecular wiring with GOx that can reduce the electron tunnelling distance, thereby promoting DET. This might be due to the ability of the

Received: February 18, 2019
Accepted: March 25, 2019
Published: March 25, 2019

DOI: 10.1021/acsabm.9b00145
ACS Appl. Bio Mater. 2019, 2, 1740−1750

nanotubes with a diameter around 1 nm to enter the protein folds and get closer to the redox moiety of the enzyme.[7] A mixture consisting of CNT, GOx, and ethanol−water was coated on glassy carbon electrode for glucose sensing, which exhibited reasonable performance over GOx modified electrode indicating the advantage of CNT incorporation on the working electrode.[8]

Several reports are available on CNT-based electrode materials where the nanotubes have been dispersed in various matrix materials. Different types of matrixes such as the polycation polyethylenimine,[9] surfactants 3-aminopropyltriethoxysilane,[10] and cetyltrimethylammonium bromide (CTAB),[11,12] the ionic liquid alkylated imidazolium acetate,[13] and biopolymers cellulose, gelatin, and chitosan have been investigated for GOx entrapment.[14,15] Both single and multiwalled CNTs have been employed as the dispersed phase in these matrices. Majority of the reports have employed multiwalled CNTs owing to their ease of synthesis and low cost. The SWCNT-coated electrode was used for mixtures of GOx and horseradish peroxidase enzyme on a pyrrole film for glucose detection.[16] Similarly, poly-L-lysine on SWCNT-coated pyrolytic graphite electrode was explored for glucose biosensor applications.[17] Other studies have employed nitrogen and boron doped MWCNT modified electrode[18,19] and Ag nanoparticle incorporated polydopamine decorated CNT electrode[20] for GOx immobilization.

Surface functionalized CNTs offer a better reactive surface for retention of the enzymes, and several studies have focused on CNT functionalization strategies for enzyme immobilization. Acid-treated functionalized MWCNT[21] further modified with polypyrrole[22] and the cross-linking agent, namely alkylated carbodiimide,[23] poly(vinyl alcohol), and pyrrole based compounds have also been exploited for GOx immobilization.[12,24] In an interesting variation, electrospun Au fibers were deposited on an electrode modified with carboxylated MWCNT thorough electrophoretic deposition, which was employed for GOx DET studies.[25] Metal nanoparticle-decorated CNT based electrodes have also been investigated for enzyme immobilization. Polymeric materials such as PDDA (polydiallyldimethylammonium chloride) served as a capping agent for Au and Pt nanoparticle-decorated CNTs[26] that enabled the nanocomposite to retain its nanodimensions. Other composite matrices such as MWCNTs-Au nanorods incorporated composite membrane,[27] MWCNT-alumina-coated silica (ACS), and Pt nanoparticle-linked MWCNT-ACS[28,29] carbon-paste electrode modified using PDDA capped Pt nanoparticles incorporated in a CTAB matrix containing MWCNT,[29] etc., have been developed for GOx immobilization. Recently, graphene-based materials have garnered attention for sensing applications due to their biocompatibility and conducting nature. Chen et al., reported few CNT-graphene based composite materials, such as GO-MWCNT hybrid, electrochemically reduced rGO-MWCNT, and Au nanoparticle-decorated graphene-CNT hybrid.[30−32] Other graphene-based composites that have been reported in the literature for GOx immobilization include a graphene-CNT hybrid film grown by 3D seamless chemical vapor deposition,[33] PDDA-capped Au nanoparticles incorporated on functionalized graphene-MWCNT nanocomposite,[34] a 3D graphene layer as a platform for GOx-MWCNT composite immobilization,[35] and rGO-CNTs-carbon fiber felt-based electrode.[36] Although these CNTs based electrode materials have shown reasonable sensitivity, they suffer from some several drawbacks such as cumbersome methodology to fabricate the electrode film and requirement of expensive chemicals, surfactants, ionic liquids, and polymers as well as Pd, Pt, Au, and Ag metal nanoparticles that require extensive procedures made to activate modified electrode. The above-mentioned matrices have exhibited a surface coverage value in the range of 0.001 to 3.8 nM cm^{-2} and direct electron transfer behavior. However, effective GOx immobilization in a time-efficient approach still remains a challenge. The present work attempts to address this problem and explores the possibility of employing pristine MWCNT as a dynamic host matrix for GOx immobilization.

Pristine MWCNT contains about 2−5% of carbonaceous and iron impurities that may have a crucial play in altering the belongings of pristine MWCNT. In a seminal work, Compton et al. have investigated the electrocatalytic effect of pristine MWCNT with hydrazine,[37] glucose,[38] and hydrogen peroxide,[39] which were attributed to the residual metallic impurities within CNTs. Later, Pumera et al.[40−46] extensively investigated and proved that the nanographitic and metallic impurities in CNTs have an intense effect on the electrochemistry of CNTs, which proves that the contaminants within the CNTs overlook their electrochemistry rather than the bare CNTs. This aspect was further substantiated by the work of Senthil Kumar et al.[47] where a bipyridyl immobilized MWCNT was developed for electrochemical biosensing of the purine bases in DNA. The iron impurities present in the MWCNT formed a Fe-bipyridyl complex that was responsible for the detection. Another related study employed chitosan conjugated MWCNTs containing iron contaminants that contributed to the peroxidase-like catalytic activity toward H_2O_2 sensing.[48] In the context of protein-based sensing, there has been only one report on the use of MWCNT containing iron and graphitic impurities[49] for the electric wiring of the metalloprotein, that is, hemoglobin and its application toward H_2O_2 biosensor. Hence, the present study aims to employ pristine MWCNT (PMWCNT) as an active host matrix for glucose oxidase entrapment for glucose detection. The time-efficient electrode preparation was made without the use of any surfactants, linkers, functionalization, or composite/hybrid materials. For comparison of the immobilization efficiency as well as sensing performance, GOx was immobilized onto another nine different carbons, and their surface coverage and redox behaviors were compared with PMWCNT, a facet that remains hitherto unexplored. The results of the study could open up new vistas to DET with redox active enzymes/metalloproteins employing a carbon-enzyme modified surface.

2. EXPERIMENTAL SECTION

2.1. Materials. MWCNT and Nafion (5%) were obtained from Sigma-Aldrich, USA. Further dilution of the as-received 5% Nafion was made with ethanol for the electrodes fabrication. Analytical grade reagents were used as-received for the entire experiments. *Aspergillus niger* based GOx (100 U/mg solid) was procured from HiMedia, India. Using Na_2HPO_4 and NaH_2PO_4 salts, 0.1 M phosphate buffer electrolyte solution (PBS) was prepared with pH 7. Prior to performing the entire electrochemical measurements, the PBS electrolyte was purged with N_2 gas for about 15 min for deoxygenation. All electrochemical analysis was carried out with CHI 440B workstation (CH Instruments, USA). An electrochemical cell comprising GCE (0.07 cm^2) modified with the sensing element was employed to quantify the analyte and hence became the working electrode. Besides, Ag/AgCl (saturated) reference and Pt ring counter electrodes were employed for the present study. Morphology and elemental analysis of the PMWCNT and PMWCNT/GOx were

DOI: 10.1021/acsabm.9b00145
ACS Appl. Bio Mater. 2019, 2, 1740−1750

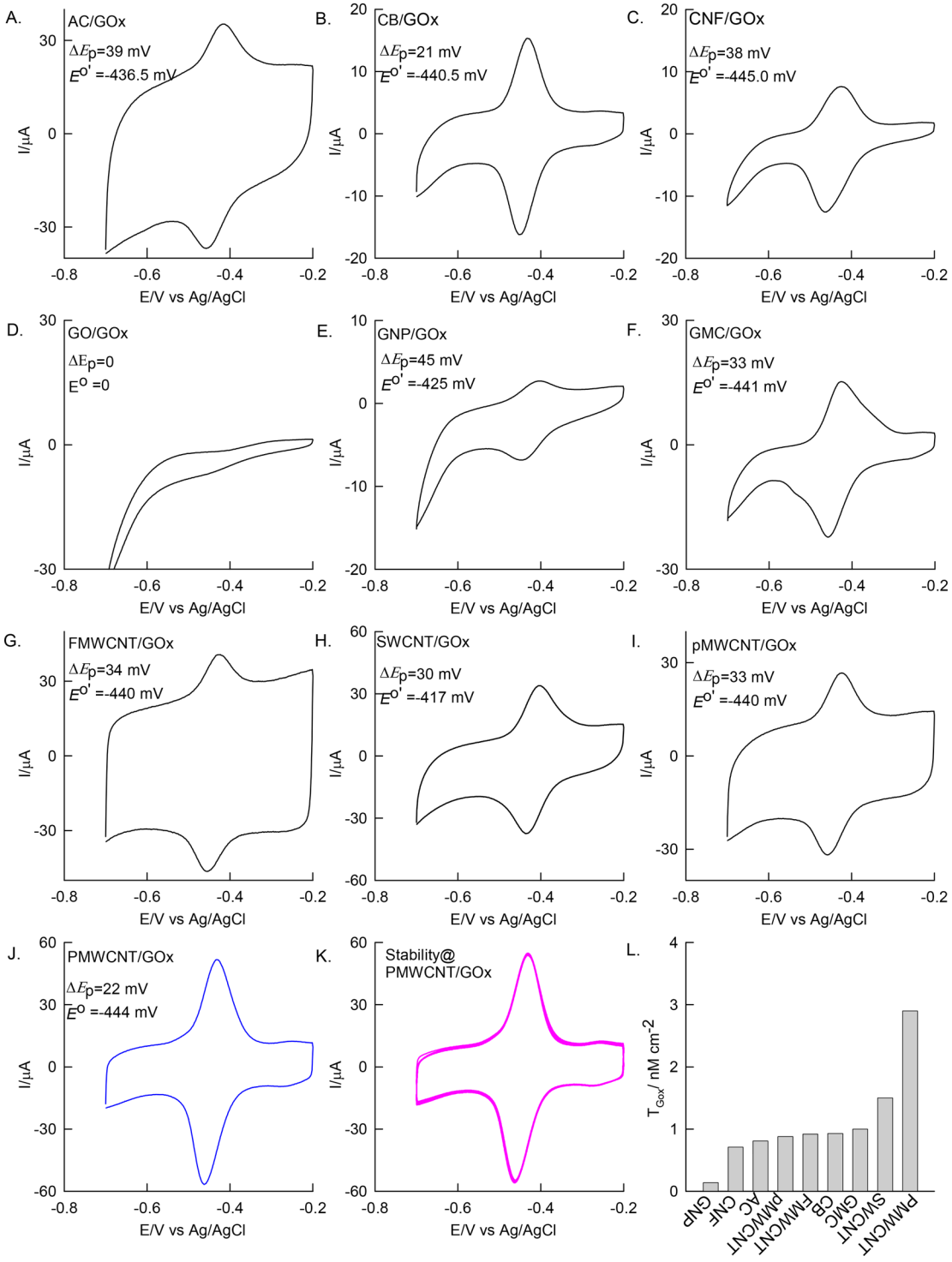


Figure 1. Cyclic voltammetry response of GOx immobilized (A) AC, (B) CB, (C) CNF, (D) GO, (E) GNP, (F) GMC, (G) FMWCNT, (H) SWCNT, (I) purified MWCNT, (J) PMWCNT. (K) Stability CVs at GOx*PMWCNT in PBS PH7 at 50 mV s^{-1}. (L) Bar diagram between different carbons modified GCEs and surface coverage value (Γ).

measured using scanning electron microscopy (FESEM) (JSM 6701F, JEOL, Japan) and energy dispersive analysis of X-ray (EDAX, INCA CENTA-X3, Oxford Instruments, UK) respectively.

2.2. Preparation of GOx Immobilized Electrodes. Purified and functionalized MWCNTs were prepared by following methodologies, that is, 0.2 g of PMWCNTs mixed with 6 M (purification) and 15 M HNO_3 (functionalization) were refluxed for a half day at 140 °C in an oil bath. The residue was filtered and washed with H_2O repeatedly until neutralization of supernatant solution and then dehydrated at 80 °C using hot air oven. All other carbons like single-walled (SWCNT), activated carbon (AC), graphene oxide (GO), graphite nanopowder (GNP) carbon nanofiber (CNF), and graphitized mesoporous carbon (GMC) were procured from Sigma-Aldrich, USA and used as-procured without further modification. Carbon black (N330 grade) was received as a gift sample (Phillips tire industry. Kochi, India). For the electrode preparation, carbon samples were prepared by blending 1 mg of different carbon material with 0.5 mL of ethanol by sonication for 15 min. The carbon dispersion (5 μL) was coated onto the GCE

DOI: 10.1021/acsabm.9b00145
ACS Appl. Bio Mater. 2019, 2, 1740−1750

Table 1. Literature Comparison of Glucose Sensor Data with PMWCNT/GOx[a]

S. no.	modified electrodes	$E^{0\prime}$ (V)	ΔE_p (mV)	Γ (nM/cm^2)	sensitivity μA/mM/cm^2	linear range (mM)	LOD (μM)	ref
1	PyBA/MWCNT/GOx	−0.438	27	1.15		0.5−3.5		2
2	GOD/MWCNT/TP	−0.4	42					9
3	APTES/MWCNT/GOx	−0.45	35				5	10
4	CNT/GOx/GCE	−0.490	38	0.102	3.40			11
5	Cellulose-MWCNT/Gox/GCE	−0.371	29		6.57	0.5−1.0		12
6	GOx/MWCNT/CTAB	−0.46	32		53.5			13
7	GOD/MWCNT-ACS	−0.466	44		0.127	0.1−0.8	17.5	14
8	GCNT/GOx/GAD	−0.4	47	3.88	2.47	6.3−20		15
9	MWCNT/GOD/AuNRs/Au	−0.384	38	0.07	38.2	0.28−5.88	0.2	16
10	Au/SWCNT/GOD/HRP/PPy				7.01	0.005−1		17
11	PLL-SWCNT/GOx/GCE	−0.5	19					18
12	CNX-MWCNT/Gox/GCE	−0.459	27	0.752	13	0.02−1	100	19
13	BCNT/GOx/GCE	−0.477	26	1.94	111.51	0.05−3	100	20
14	Nf/GOD/Ag-Pdop@MWCNT/GCE	−0.48	28		3.1	0.05−1.1	17	21
15	PPY-Nf/MWCNT/CH-GOx	−0.25	136		2860.3	0.01−4.7	5	23
16	GOx/CNT/DHP/GCE	−0.418				0.020−15	9	24
17	PVA-MWCNT/GOx/GCE	−0.402	48	3.98	8670	0.1−20	150	25
18	CMWCNT-GOx/Gold fibres			0.001	0.47	1−30	4	26
19	CNT-PDDA-PtNPs/CPE	−0.37			24.7	0.1−3	15	27
20	HPt-CNTs/GOD/GCE	−0.44				0.0012−8.4	0.4	29
21	MWCNT-GO hybrid/GOx/GCE	−0.420	36		0.266	0.05−23.2	28	30
22	ERGO/MWCNT/GOx-Nf	−0.421	26	0.358	1.908	0.1−6.5	4.7	31
23	GR-MWCNT/AuNPs/GOx	−0.40	36		0.238	0.01−5.2	950	32
24	G-CNT/GOx	−0.459			19.31	0.02−8		33
25	PDDA/AuNPs/GCNT/GOD	−0.454	37	0.222	29.72	0.05−2.1	4.8	34
26	3DG-MWCNT-GOD/GCE	−0.410	40		49.58	0.1−16	1000	36
27	PMWCNT/GOx	−0.444	22	2.90	6600	0.2−5.8	45	this work

[a]MWCNT = Multiwalled carbon nanotubes, GOD/GOx = glucose oxidase, GCE = glassy carbon electrode, SWNT = single walled nanotubes, APTES = 3-aminopropyl triethoxysilane, Nf = nafion, PLL = poly lysine, TP = Toray carbon paper, GCNT = gelatin multiwalled carbon nanotubes, GAD = gluteraldehyde, HPt = hollow platnium, DHP = dihexadecyl phosphate film, Pdop = poly dopamine, ACS = alumina coated silica, PDDA = poly(diallyl-ammonium chloride, CH-GOx = chitosan glucose oxidase, PVA = poly vinyl alcohol, PyBA = 4(pyrrole 1-yl) benzoic acid, HRP = horse radish peroxidase, ERGO = electrochemically reduced graphene oxide, GR = graphene, AuNPs = gold nanoparticles, Au = gold, AgNPs = silver nanoparticles, BCNT = boron doped nanotubes.

working area and subjected for aeration at ambient conditions. The carbon sample-modified GCEs were subjected to recordings in the potential range from −0.8 to −0.2 V in pH 7 PBS purged with N_2 using cyclic voltammetry. GOx dispersion was prepared by mixing 10 mg of GOx in 0.1% Nafion-ethanol (1 mL) and stored in the icebox for further experimental usage. Nafion was used as a dispersant for GOx to form a uniform film on the carbon modified surfaces, which are also expected to inhibit the leakage of GOx from the PMWCNT and thus to improve the stability of PMWCNT/GOx.

3. RESULTS AND DISCUSSION

3.1. DET Behavior of GOx Immobilized Different Carbon Samples. Different types of carbon (AC, CB, CNF, GMC, GO, GNP, SWCNT, fMWCNT, and PMWCNT)-modified GCEs were used for the GOx immobilization followed by cyclic voltammetry analysis at 50 mV s^{-1} for the DET measurements. Figure 1A shows that the GOx/AC exhibits a redox peak with a ΔE_p value of 39 mV as a result of the FAD/FADH$_2$ redox center present in GOx and the $E^{0\prime}$ was found to be −0.436 V for the system. Interestingly, GOx/CB/GCE shows a reversible DET behavior at an $E^{0\prime}$ = −0.440 V with a ΔE_p of 21 mV indicating an efficient redox response with a favorable electron transfer (Figure 1B). The −OH and −COOH functional groups along with the negligible metal impurities present on the spherical carbon nanoparticles contribute to the effective redox response of GOx/CB modified electrode.[50] Recently, Senthil Kumar et al. employed the same grade of CB for efficient entrapment of organic redox mediators like quinoid of curcumin, sesamol, and Coomassie brilliant blue dye for electrochemical sensing applications and reported similar response toward the analyte.[51−53] The GOx immobilized CNF show a feeble reversible redox peak at −0.445 V with a ΔE_p value of 38 mV (Figure 1C). The GOx immobilized GO modified electrodes show an irreversible nonfaradaic response (Figure 1D). This response is on expected lines as the nonconducting GO might not efficiently mediate the electron transfer between the substrate and enzymes. In contrast, both the GOx immobilized GNP and GMC modified electrodes (Figure 1E,F) exhibit a reversible redox response due to the graphitic disorders and their porosity. However, they displayed poor peak current stability and hence may not be suitable for enzyme immobilization.

As claimed earlier, to increase the DET of GOx, functionalized MWCNT electrodes and its further modifications by carboxylation, polymers, cross-linking agents, surfactants, and metal nanoparticles have been explored.[19−23] In the present study, GOx immobilized fMWCNT showed a redox peak current with a large background signal and low peak current magnitudes (Figure 1G). Meanwhile, the pristine SWCNT-GOx (Figure 1H) electrode was also checked for the suitability of enzyme immobilization. Interestingly, unlike the other types of carbons, SWCNT-GOx system shows an appreciable redox peak at an $E^{0\prime}$ of −0.417 V with a ΔE_p of 30 mV. Extended

DOI: 10.1021/acsabm.9b00145
ACS Appl. Bio Mater. 2019, 2, 1740−1750

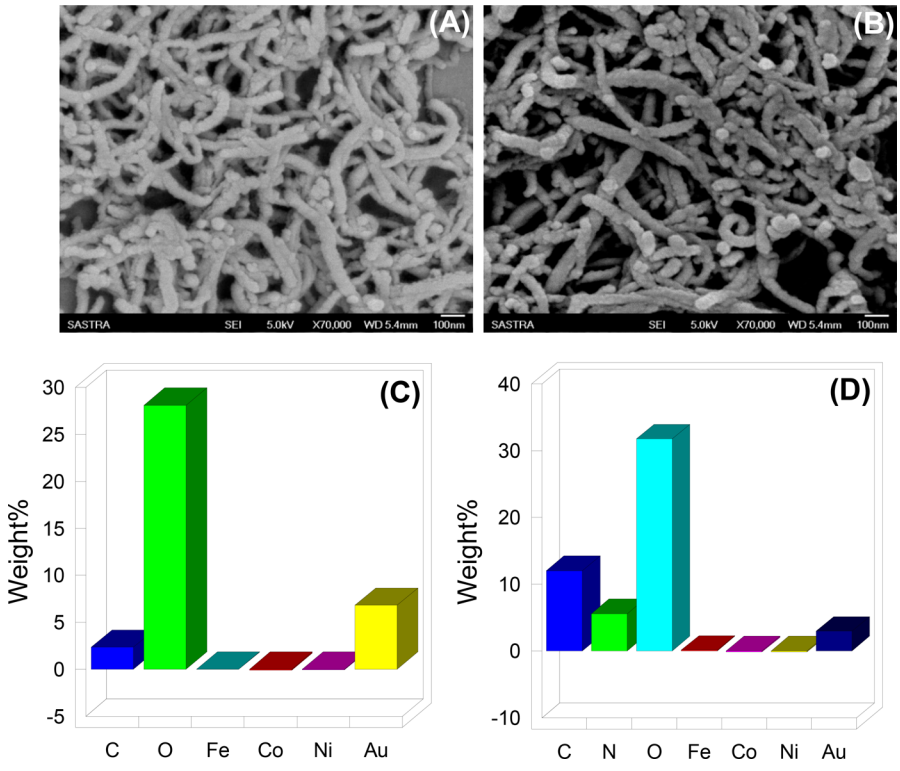


Figure 2. FESEM images of the (A) pristine MWNCT and (B) GOx immobilized pristine MWCNT. EDX results of (C) PMWCNT and (D) PMWCNT/GOx.

experiments with purified MWCNT-GOx modifier (Figure 1I) also yielded good redox behavior with $E^{0\prime} = -0.440$ and ΔE_p = 33 mV, but the stability of the electrode film was poor. Finally, the GOx entrapped PMWCNT modified electrode was subjected to CV analysis (Figure 1J). The CV response shows well-defined direct electrochemistry with a redox behavior at $E^{0\prime} = -0.440$ V and a relatively low ΔE_p value of 22 mV. The stability of the GOx/PMWCNT/GCE is investigated by performing 50 consecutive CVs at 50 mV s^{-1} (PBS pH 7) (Figure 1K). It was estimated that 99.2% of redox peak current magnitude (cathodic current and anodic current) of the initial cycle was observed in the 50th cycle, which inferred that the GOx/PMWCNT was extremely stable and GOx was firmly attached to the PMWCNT. The surface entrapment of GOx on various carbons was calculated from the following eq 1:[27]

$$\Gamma = Q/nFA \quad (1)$$

Where Q represents the charge of anodic peak integration, n refers to electrons that participated in the electrochemical reaction, F is the Faraday's constant (96480 C/mol), and A (0.071 cm^2) is the geometric area of the working surface. The surface excess values (mol cm^{-2}) of GOx immobilized on different carbons are sequenced as PMWCNT (2.9×10^{-9}) > SWCNT (1.5×10^{-9}) > GMC (1.0×10^{-9}) > CB (0.93×10^{-9}) > f-MWCNT (0.92×10^{-9}) > pMWCNT > (0.88×10^{-9}) > AC (0.81×10^{-9}) > CNF (0.71×10^{-9}) > GNP (0.14×10^{-9}) > GO (nil response). The bar chart for different carbons and their surface excess (Γ) values is shown in Figure 1L. Among the GOx immobilized carbon electrodes studied, the maximum GOx surface excess value (Γ = 2.9 nM cm^{-2}) was achieved for the PMWCNT modified electrode, which provided a large surface area and suitable biocompatible environment for the GOx retention. Table 1 lists the parameters reported in the literature for CNT based GOx immobilized electrodes. It is seen that only the report on GOx immobilization on gelatin-MWCNT through glutaraldehyde cross-linking by Chen et al.[13] exhibited a surface coverage value of 3.88 nM/cm^2 that is slightly larger than the present GOx immobilized PMWCNT. However, the ΔE_p value of 47 mV is higher than that of the present work indicating that the former system has slower electron transfer. The obtained ΔE_p and surface coverage values in the present study for GOx-PMWCNT combine are superior to that of other CNT based electrode materials reported in the literature.

The enhanced reversible redox response of the GOx entrapped PMWCNT might be due to the following phenomena mentioned in the literature. The plausible reason behind improved DET is the trace metal impurities (Fe, Co, and Ni), negligible metal oxide, and carbonaceous (amorphous and graphitic carbons) species encapsulated into PMWCNT. These impurities may play a role in mediating good interactions with GOx, which, in turn, facilitate effective DET, improved O_2 reduction behavior and glucose oxidation in the presence of a mediator. FESEM images of the PMWCNT (Figure 2A) reveal that the average nanotube diameter was found to be 50 nm, while after immobilization of GOx the nanotube morphology remained intact albeit with an increased diameter of 80 nm (Figure 2B). The EDX results of PMWCNT show the presence of elements C and O and metals such as Fe, Co, and Ni, indicating the occurrence of a trace level of metal impurities in the PMWCNT (Figure 2C). Likewise, the EDX spectrum of PMWCNT/GOx also exhibits the aforementioned elements along with N indicating the GOx immobilization on PMWCNT (Figure 2D).

DOI: 10.1021/acsabm.9b00145
ACS Appl. Bio Mater. 2019, 2, 1740−1750

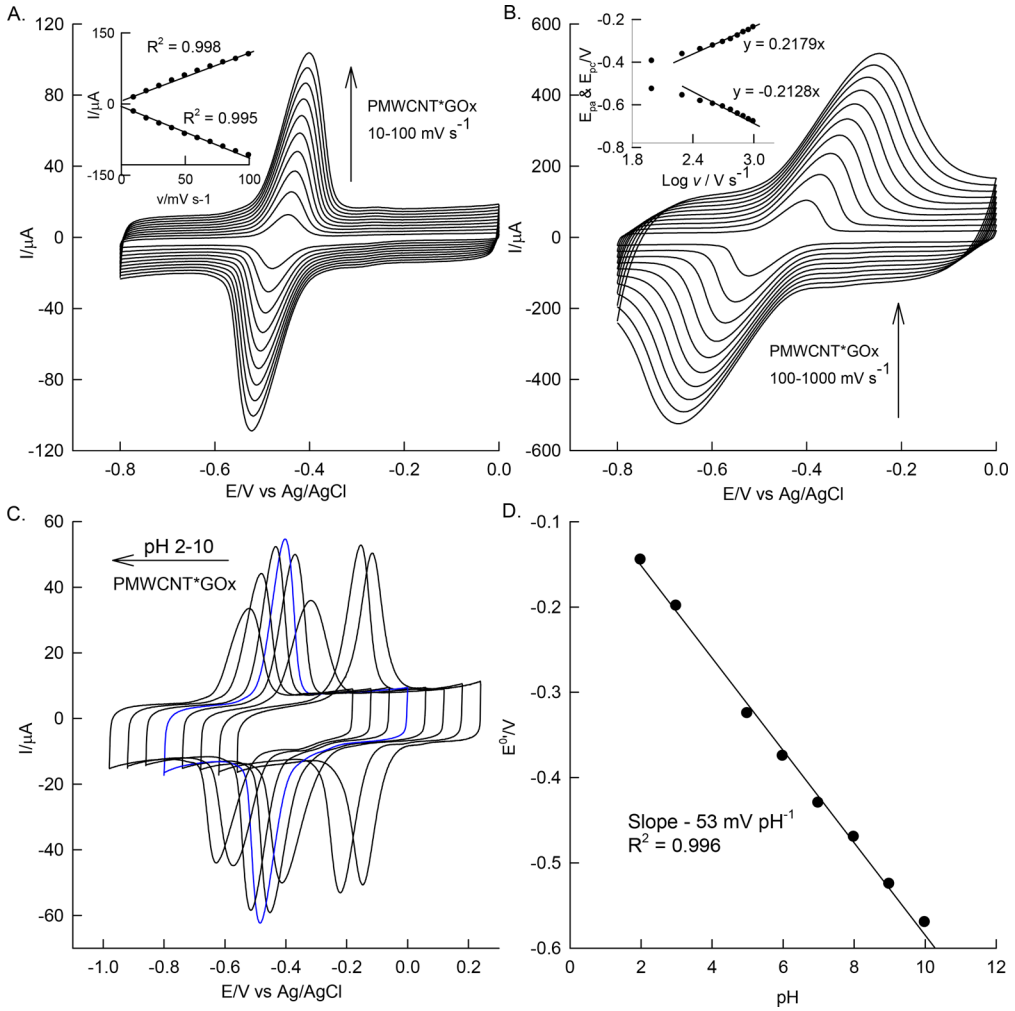


Figure 3. CVs of various scan rate (A) 10−100 mV s^{-1} and (B) 100−1000 mV s^{-1} at PMWCNT/GOx in pH 7 PBS. Inset of A: Plot of anodic (i_{pa}) and cathodic peak current (i_{pc}) versus scan rate. Inset B: Plot of E_{pa} and E_{pc} versus log of scan rate. (C) Effect of solution pH (2−10) on the CV response of PMWCNT/GOx at a fixed scan rate of 50 mV s^{-1}. (D) Plot of $E^{0\prime}$ versus pH.

3.2. Scan Rate and pH Effect on PMWCNT/GOx Modified the Electrode. Figure 3A and B show CVs of the PMWCNT/GOx in the deoxygenated electrolyte (pH 7) at different sweep cycling. Both i_{pa} and i_{pc} increments were observed with progressive increase in the scan rates for GOx redox peaks. Peak separation (ΔE_p) values were also increased in the sweep ranges of 10 to 1000 mV s^{-1}, indicating a pseudoreversibility, arising due to internal resistance between the interface and electrodes.[33,34] The i_{pa} and i_{pc} are observed as the same magnitude at all the scan rates. The ratio of i_{pa}/i_{pc} close to unity suggests that the PMWCNT/GOx involves the surface-controlled process. A linear relationship is observed between E_{pa} and E_{pc} and log v (Figure 3B, inset). The slopes of anodic ($2.303RT/(1-\alpha)nF$) and cathodic ($-2.303RT/(1-\alpha)nF$) peak potentials were calculated from the linear plot. Charge transfer coefficient estimated for an electrochemical surface adsorption controlled phenomena using Laviron equation (eq 2):[31]

$$\frac{k_a}{k_c} = \frac{\alpha}{1-\alpha} \tag{2}$$

where k_a is the slope value of E_{pa} versus log v (0.217), and k_c is the slope value of E_{pc} versus log v (0.212), and α is the coefficient of electron transfer, which is 0.5. The obvious rate constant (k_s) for electron transfer can be acquired using eq 3:[31] where n is 2, R, T, and F are their standard values, while

$$\log k_s = \propto \log (1-\propto) + (1-\propto) \log \propto - \log \frac{RT}{nFv} - \frac{\propto (1-\propto)\, nF\Delta E_p}{2.3\ RT} \tag{3}$$

ΔE_p is the separation between anodic and cathodic potential (0.131 V) at 0.1 V s^{-1}. The calculated k_s value of 3.16 s^{-1} for the PMWCNT/GOx is larger than that reported for PVA-MWCNT (1.58 s^{-1}),[24] GOx-CTAB-CNT (1.14 s^{-1}),[12] GOx-MWCNT-alumina-coated silica (2.10 s^{-1}),[29] GOx-Nf-MWCNT (1.53 s^{-1}),[11] GOx-B-MWCNT (1.56 s^{-1}),[18] and several other GOx immobilized electrodes, which are tabulated in Table 1.

The influence of electrolyte pH on DET of PMWCNT/GOx was investigated. Figure 3C displays the CVs at 50 mV s^{-1} obtained from the PMWCNT/GOx at various pH values between pH 2 and pH 10. Formal potential of the GO_X redox response shifts toward the negative direction with increasing pH. The slope of $E^{0\prime}$ versus pH (Figure 3D) was found to be −53.0 mV/pH (R^2 = 0.99), which matched with the theoretical value of −59.0 mV/pH (Nernstian slope), suggesting that two protons and electrons were contributed during the electrochemical response of FAD/$FADH_2$ as described in eq 4:[36]

DOI: 10.1021/acsabm.9b00145
ACS Appl. Bio Mater. 2019, 2, 1740−1750

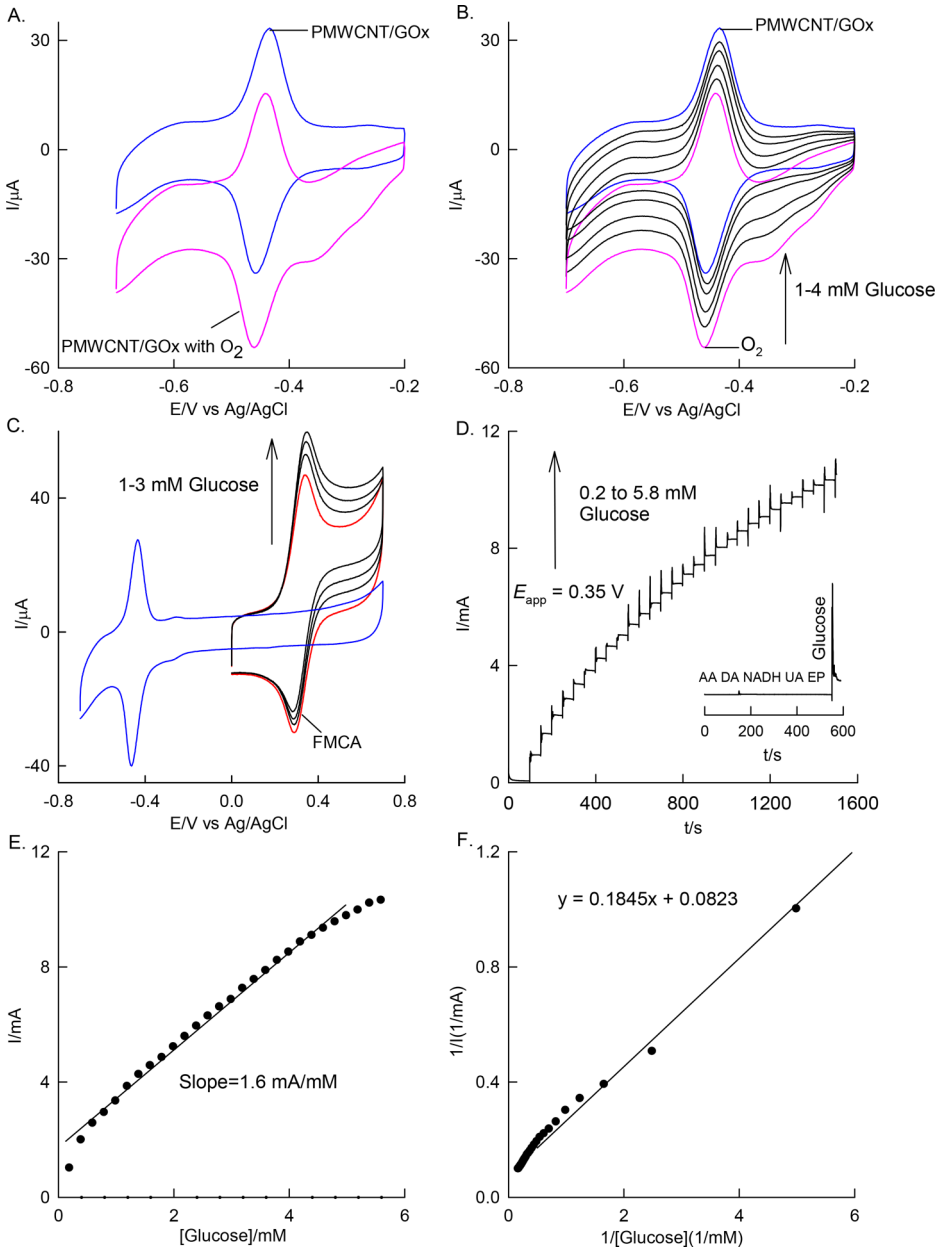


Figure 4. (A) CVs of PMWCNT/GOx @N_2 saturated and PMWCNT/GOx @O_2 saturated in PBS. (B) CVs of PMWCNT/GOx with different concentrations of glucose (1−3 mM). (C) CVs of GOx/PMWCNT in PBS containing 2 mM of FMCA mediator and difference concentrations of glucose. (D) Amperometric *i*−*t* response of PMWCNT/GOx modified GCE upon sequential additions of 0.2 mM glucose in PBS (pH 7) containing 2 mM FMCA at 0.35 V. Inset, interference effect. (E) Plot of calibration between glucose concentration and steady state current. (F) Michaelis menton plot.

$$GOx - FAD + 2e - +2H^+ \leftrightarrow GOx - FADH_2 \quad (4)$$

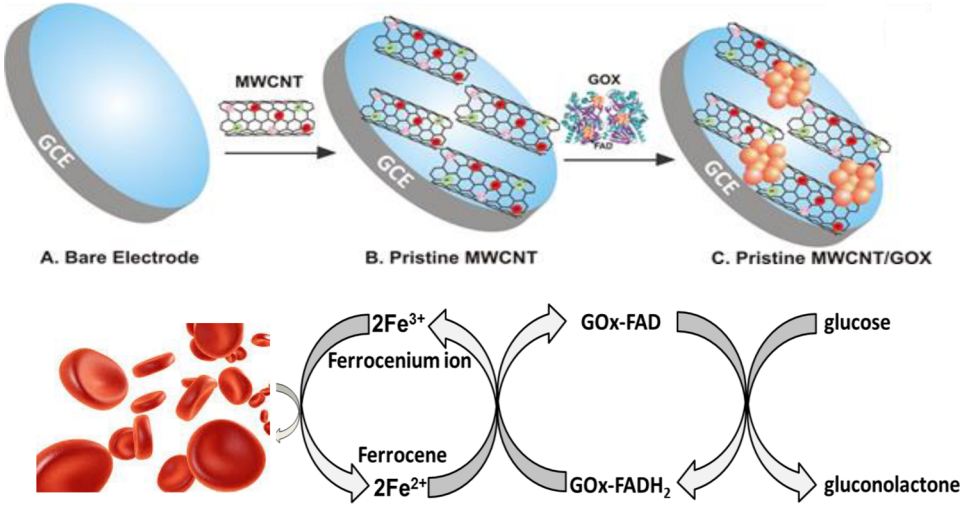
3.3. Electrochemical Quantification of Glucose via O_2 Reduction and with Mediator Assistance. DET-based glucose sensing was checked by monitoring the dissolved O_2 reduction with the enzyme-based reaction as shown in the eqs 4 and 5.[18] From Figure 4A, characteristic reversible peaks were obtained in both aerated (O_2) and deaerated (N_2) saturated PBS at 20 mV s^{-1}. However, the i_{pc} of the PMWCNT/GOx in dissolved oxygen-containing PBS was larger than that found in a N_2 environment, and the corresponding oxidation peak current was lower. This result proves that catalytic dissolved O_2 reduction happens at the PMWCNT/GOx modified electrode as described in eq 5:[31]

$$GOx - FADH_2 + O_2 \rightarrow GOx - FAD + H_2O_2 \quad (5)$$

CVs of PMWCNT/GOx in dioxygen saturated PBS with four glucose concentrations, that is, 1 to 4 mM, are presented in Figure 4B. The i_{pc} attributed to the dissolved O_2 reduction was observed to correlate well with the increasing glucose concentrations. The serial decrement in the i_{pc} proves the efficient O_2 reduction at PMWCNT/GOx. Thus, PMWCNT/GOx showed enhanced catalytic response toward the indirect glucose determination, that is, O_2 consumption. DET on glucose oxidase-immobilized CNTs and various forms of graphene is still not convincing. This is because GOx redox peaks are usually observed on CNTs and graphene because of adsorbed flavin and not due to FAD/$FADH_2$ within the protein folds. Also, literature reports provide supporting

DOI: 10.1021/acsabm.9b00145
ACS Appl. Bio Mater. 2019, 2, 1740−1750

Scheme 1. (A) Bare GCE, (B) PMWCNT Modified GCE, (C) GOx Entrapped PMWCNT/GCE Preparation and Its Bioelectrocatalysis Pathway for Glucose Analysis



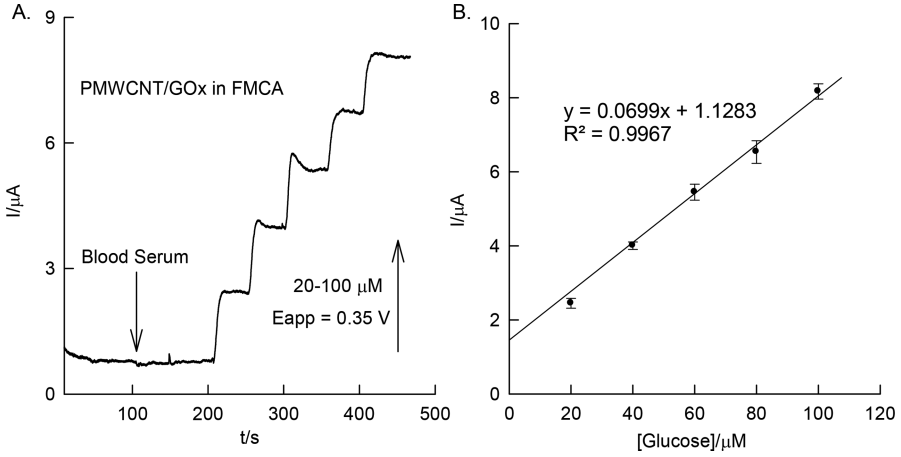


Figure 5. (A) Amperometric $i-t$ response of PMWCNT/GOx modified GCE upon human serum samples spiking with sequential additions of 20 μM glucose in PBS (pH 7) containing 2 mM FMCA at 0.35 V. (B) Calibration plot between steady state current versus glucose concentration.

evidence that adsorbed FAD or contaminations present in commercially available GOx exhibit a vital role in DET-based glucose sensing applications.[54]

Figure 4C displays the CVs of PMWCNT/GOx in pH 7 electrolyte solution having various glucose concentrations (1–3 mM) in the presence of FMCA. A redox response recorded at +0.37 V is a resultant of the FMCA mediator. Linear increments of i_{pa} and a sequential decrease in i_{pc} due to FMCA were observed with increasing concentrations of glucose. This result proves that the PMWCNT/GOx is a promising candidate to catalyze the glucose reduction with the aid of a mediator. This is depicted by Scheme 1.[33]

3.4. Amperometric Determination of Glucose on PMWCNT/GOx. Chronoamperometry, being one of the most widely used techniques for evaluating glucose sensors, has been employed to study the bioelectrocatalytic behavior of PMWCNT/GOx with the aid of FMCA. Figure 4D shows the well-behaved amperometric response obtained at PMWCNT/GOx-modified GCE at a potential (E_{app}) of 0.35 V and a stirred condition in deaerated PBS (pH 7). The amperometric steady state current increased linearly with increasing of glucose levels. The linear calibration was obtained between 0.2 mM and 5.8 mM of glucose with the regression coefficient close to unity (R^2 = 0.98) (Figure 4E). The detection limits (LOD) for the linear segments were estimated using eq 6:[50]

$$\mathrm{LOD} = 3S_b/\mathrm{m} \tag{6}$$

where S_b is derived from the five repeated blank signals current values resulting standard deviation, m is the slope of the sensor, and the LOD was found to be 45 μM. The sensitivity of the PMWCNT/GOx is 6.6 mA/mM/cm^2. The apparent Michaelis Menton constant (K^{app}_M) was derived from the Lineweaver–Burk equation (eq 7):[24]

$$\frac{1}{I_{SS}} = \frac{I}{I_{max}} + \frac{K^{app}_M}{I_{max}C} \tag{7}$$

where I_{ss} stands for the current response of glucose, I_{max} is the catalytic current of glucose oxidation, and C represents the glucose concentration. The derived K^{app}_M of 2.24 mM proves the high affinity enzyme–substrate kinetics and identical sharing of the GOx on the surface for glucose oxidation via DET (Figure 4F). The lower K_M value indicates that the GOx entrapped pristine CNT networks possess a higher enzyme activity for glucose oxidation with FMCA as a mediator. Table 1 shows a compilation of glucose biosensor data based on GOx immobilized MWCNT composites. It may be noted that many

DOI: 10.1021/acsabm.9b00145
ACS Appl. Bio Mater. 2019, 2, 1740–1750

of these sensors have the limit of detection in the higher range of glucose concentration, whereas in the present work, a very low LOD has been achieved. Further PMWCNT/GOx has shown a very impressive current sensitivity as well. The plausible explanation for this interesting bioelectrocatalytic behavior of the PMWCNT/GOx can be due to the metal impurities that provide a large number of active sites to entrap GOx without compromising its enzymatic activity.

3.5. Selectivity Study and Recovery Test. Selectivity of the PMWCNT/GOx electrode for the glucose sensing was assessed with possible interference from molecules like dopamine, NADH, epinephrine, ascorbic acid, and uric acid, which are commonly encountered in serum samples. These samples were diluted 100 times prior to measurements, and the corresponding data are shown in the inset of Figure 4D. The presence of these interferents did not affect the electrode performance, which indicated that the PMWCNT/GOx can serve as a good interface for the selective sensing of glucose.

To prove the real applicability of the assembled biosensor in clinical analysis, human serum samples (with 10-fold dilution) were collected and employed for detection of glucose levels using the PMWCNT/GOx modified electrode. The precision of sensor was determined by standard protocol, and the respective amp $i-t$ results are shown in Figure 5A and B. Five different glucose concentrations were spiked in the serum sample, and the recovery was calculated between 88 and 98% (n = 3), showing an excellent precision in the given samples. Table 2 shows the recovery data from which it can be inferred that the PMWCNT@GOx is a reliable sensor for serum sample analysis.

Table 2. Recovery Data for Human Serum Samples on PMWCNT/GOx

S. no.	added (μM)	found (μM)	recovery (%)	RSD (%)
1	20	17.60	87.9	5.44
2	40	39.51	98.7	2.50
3	60	59.84	99.7	3.96
4	80	75.14	93.9	4.68
5	100	98.15	98.1	2.55

3.6. Repeatability, Reproducibility, and Stability. The current sensitivity was measured for N_2 purged PBS containing 0.1 mM glucose at PMWCNT/GOx modified electrode for five recordings under optimized amperometric $i-t$ conditions for reproducibility. Additionally, the current response in dioxygen purged PBS containing 0.1 mM of glucose was analyzed using five different modified electrodes for repeatability. The RSD values of 3.12% and 1.8% show appreciable reproducibility and repeatability respectively at PMWCNT/GOx. Furthermore, PMWCNT/GOx was performed with one-month storage in the refrigerator for stability assessment. The fabricated system retained 94.3% of its original response suggesting excellent storage stability of the fabricated biosensor.

4. CONCLUDING REMARKS

Pristine MWCNT is found to be a suitable biocompatible electrode material for the effective GOx immobilization. PMWCNT avoids extensive preparation procedures and time-consuming chemical treatments with surfactant, linkers, and polymers. Nanographite, metal, and metal oxide impurities in the PMWCNT assisted the strong interaction between the GOx and PMWCNT. Here we investigated several nanocarbons for enzyme immobilization. Surface coverage and DET behavior of the PMWCNT/GOx is several-fold higher than the other carbon structures as well as other CNT based hybrids published in the literature. The simple biosensor constructed in a time-efficient approach was used for glucose detection by O_2 consumption and with FMCA as a redox mediator. Chronoamperometric data provide an excellent linear range, low detection limit, and good substrate and enzymatic activity with good selectivity and rapid response of 3 s. This has the potential to be explored further for enzyme immobilization applications including biosensing of clinically and industrially relevant materials.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: dinesh@scbt.sastra.edu.

*E-mail: umakrishnan@sastra.edu. Phone: +(91) 4362 264101 Ext 3677. Fax: (+91) 4362 264120.

ORCID

Uma Maheswari Krishnan: 0000-0001-6508-4485

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors acknowledge SASTRA Deemed University for infrastructural support. K.S.S.D. and U.M.K. acknowledge RCUK for financial support (MRC ref: MR/P027881/1).

REFERENCES

(1) Wang, J. Electrochemical Glucose Biosensors. *Chem. Rev.* **2008**, *108*, 814−825.

(2) Kowalewska, B.; Jakubow, K. The Impact of Immobilization Process on the Electrochemical Performance, Bioactivity and Conformation of Glucose Oxidase enzyme. *Sens. Actuators, B* **2017**, *238*, 852−861.

(3) Willner, I.; Katz, E. Integration of Layered Redox Proteins and Conductive Supports for Bioelectronic Applications. *Angew. Chem., Int. Ed.* **2000**, *39*, 1180.

(4) Barsan, M. M.; Ghica, M. E.; Brett, C. M. A. Electrochemical Sensors and Biosensors Based on Redox Polymer/ Carbon Nanotube Modified Electrodes: a Review. *Anal. Chim. Acta* **2015**, *881*, 1−2.

(5) Rakhi, R. B.; Sethupathi, K.; Ramaprabhu, S. A Glucose Biosensor Based on Deposition of Glucose Oxidase onto Crystalline Gold Nanoparticle Modified Carbon Nanotube Electrode. *J. Phys. Chem. B* **2009**, *113*, 3190−3194.

(6) Tian, K.; Prestgard, M.; Tiwari, A. A Review of Recent Advances in Nonenzymatic Glucose Sensors. *Mater. Sci. Eng., C* **2014**, *41*, 100−118.

(7) Guiseppi-Elie, A.; Lei, C.; Baughman, R. H. Direct Electron Transfer of Glucose Oxidase on Carbon Nanotubes. *Nanotechnology* **2002**, *13*, 559−564.

(8) Gutierrez, F.; Rubianes, M. D.; Rivas, G. A. Dispersion of Multiwall Carbon Nanotubes in Glucose Oxidase: Characterization and Analytical Applications for Glucose Biosensing. *Sens. Actuators, B* **2012**, *161*, 191−197.

(9) Ivnitski, D.; Branch, B.; Atanassov, P.; Apblett, C. Glucose Oxidase Anode for Biofuel cell based on Direct Electron Transfer. *Electrochem. Commun.* **2006**, *8*, 1204−1210.

(10) Luong, J. H. T.; Hrapovic, S.; Wang, D.; Bensebaa, F.; Simard, B. Achieving Direct Electrical Connection to Glucose Oxidase Using Aligned Single Walled Carbon Nanotube Arrays. *Electroanalysis* **2004**, *16*, 38−46.

(11) Cai, C.; Chen. Direct Electron Transfer of Glucose Oxidase Promoted by Carbon Nanotubes. *Anal. Biochem.* **2004**, *332*, 75−83.

DOI: 10.1021/acsabm.9b00145
ACS Appl. Bio Mater. 2019, 2, 1740−1750

(12) Hyun, K.; Han, S. W.; Koh, W.-G.; Kwon, Y. Direct Electrochemistry of Glucose Oxidase Immobilized on Carbon nanotube for Improving Glucose sensing. *Int. J. Hydrogen Energy* **2015**, *40*, 2199−2206.
(13) Wu, X.; Zhao, F.; Varcoe, J. R.; Thumser, A. E.; Rossa, C. A.; Slade, R. C. T. Direct Electron Transfer of Glucose Oxidase Immobilized in an Ionic Liquid Reconstituted Cellulose−Carbon Nanotube Matrix. *Bioelectrochemistry* **2009**, *77*, 64−68.
(14) Periasamy, A. P.; Chang, Y.-J.; Chen, S.-M. Amperometric Glucose Sensor Based on Glucose Oxidase Immobilized on Gelatin-Multiwalled Carbon Nanotube Modified Glassy Carbon Electrode. *Bioelectrochemistry* **2011**, *80*, 114−120.
(15) Li, Y.; Wang, F.; Huang, F.; Li, Y.; Feng, S. Direct electrochemistry of Glucose Oxidase and its Biosensing to Glucose Based on the Chit-MWCNTs−AuNRs Modified Gold Electrode. *J. Electroanal. Chem.* **2012**, *685*, 86−90.
(16) Zhu, L.; Yang, R.; Zhai, J.; Tian, C. Bienzymatic Glucose Biosensor Based on Co-Immobilization of Peroxidase and Glucose oxidase on a Carbon nanotubes Electrode. *Biosens. Bioelectron.* **2007**, *23*, 528−535.
(17) Vaze, N.; Hussain, C.; Tang, D.; Rusling, J. L.; et al. Biocatalytic Anode for Glucose Oxidation Utilizing Carbon Nanotubes for Direct Electron Transfer with Glucose Oxidase. *Electrochem. Commun.* **2009**, *11*, 2004−2007.
(18) Deng, C.; Chen, J.; Chen, X.; Xiao, C.; Nie, L.; Yao, S. Direct electrochemistry of Glucose Oxidase and Biosensing for Glucose Based on Boron-Doped Carbon Nanotubes Modified Electrode. *Biosens. Bioelectron.* **2008**, *23*, 1272−1277.
(19) Deng, S.; Jian, G.; Lei, J.; Hu, Z.; Ju, H. Glucose Oxidase-Graphene-Chitosan Modified Electrode for Direct Electrochemistry and Glucose Sensing. *Biosens. Bioelectron.* **2009**, *25*, 373−377.
(20) Wang, Y.; Liu, L.; Li, M.; Xu, S.; Gao, F. Multifunctional Carbon Nanotubes for Direct Electrochemistry of Glucose Oxidase and Glucose Bioassay, Biosensors. *Biosens. Bioelectron.* **2011**, *30*, 107−111.
(21) Xue, C.-H.; Zhou, R.-J.; Shi, M.-M.; Wu, G.; Zhang, X.-B.; Chen, H.-Z.; Wang, M. Electrochemistry of Glucose Oxidase Immobilized on Carbon Nanotubes Noncovalently Functionalized by Multihydroxyl and Multicarboxyl Groups. *J. Electroanal. Chem.* **2010**, *642*, 92−97.
(22) Shrestha, B. K.; Ahmad, R.; Mousa, H. M.; Neupane, M. P.; Park, C. H.; Kim, C. S.; Kim, J. I.; et al. High-performance Glucose Biosensor Based on Chitosan-Glucose Oxidase Immobilized Polypyrrole/Nafion/Functionalized Multi-Walled Carbon Nanotubes Bio-Nanohybrid film. *J. Colloid Interface Sci.* **2016**, *482*, 39−47.
(23) Janegitz, B. C.; Pauliukaite, R.; Ghica, M. E.; Brett, C. M. A.; Filho, O. F. Direct Electron Transfer of Glucose Oxidase at Glassy Carbon Electrode Modified with Functionalized Carbon Nanotubes within a Dihexadecylphosphate Film. *Sens. Actuators, B* **2011**, *158*, 411−417.
(24) Gupta, S.; Prabha, C. R.; Murthy, C. N. Functionalized Multi-walled Carbon Nanotubes/Polyvinyl Alcohol Membrane Coated Glassy Carbon Electrode for Efficient Enzyme Immobilization and Glucose Sensing. *J. Environ. Chem. Eng.* **2016**, *4*, 3734−3740.
(25) Jose, M. V.; Marx, S.; Murata, H.; Koepsel, R. R.; Russell, A. J. Direct Electron Transfer in a Mediator-free Glucose Oxidase-Based Carbon Nanotube-Coated Biosensor. *Carbon* **2012**, *50*, 4010−4020.
(26) Amatatongchai, M.; Sroysee, W.; Chairam, S.; Nacaprich, D. Amperometric Flow Injection Analysis of Glucose using Immobilized Glucose Oxidase on Nano-Composite Carbon Nanotubes-Platinum Nanoparticles Carbon Paste Electrode. *Talanta* **2017**, *166*, 420−427.
(27) Tsai, M.-C.; Tsai, Y.-C. Adsorption of Glucose Oxidase at Platinum-Multiwalled Carbon Nanotube-Alumina-Coated Silica Nanocomposite for Amperometric Glucose Biosensor. *Sens. Actuators, B* **2009**, *141*, 592−598.
(28) Wang, Y.; Yuan, R.; Chai, Y.; Li, W.; Zhuo, Y.; Yuan, Y.; Li, J. Direct Electron Transfer: Electrochemical Glucose Biosensor Based on Hollow Pt Nanosphere Functionalized Multiwall Carbon Nanotubes. *J. Mol. Catal. B: Enzym.* **2011**, *71*, 146−151.
(29) Wu, W.-C.; Huang, J.-L.; Tsai, Y.-C. Direct Electron Transfer and Biosensing of Glucose Oxidase Immobilized at Multiwalled Carbon Nanotube-Alumina-Coated Silica Modified Electrode. *Mater. Sci. Eng., C* **2012**, *32*, 983−987.
(30) Palanisamy, S.; Cheemalapati, S.; Chen, S.-M. Amperometric Glucose Biosensor Based on Glucose Oxidase Dispersed in Multi-walled Carbon Nanotubes/Graphene Oxide Hybrid Biocomposite. *Mater. Sci. Eng., C* **2014**, *34*, 207−213.
(31) Mani, V.; Devadas, B.; Chen, S.-M. Direct Electrochemistry of Glucose Oxidase at Electrochemically Reduced Graphene Oxide-Multiwalled Carbon Nanotubes Hybrid Material Modified Electrode for Glucose Biosensor. *Biosens. Bioelectron.* **2013**, *41*, 309−315.
(32) Devasenathipathy, R.; Mani, V.; Chen, S.-M.; Huang, S.-T.; Huang, T.-T.; Lin, C.-M.; Hwa, K.-Y.; Chen, T.-Y.; Chen, B.-J. Glucose Biosensor based on Glucose Oxidase Immobilized at Gold Nanoparticles Decorated Graphene-Carbon Nanotubes. *Enzyme Microb. Technol.* **2015**, *78*, 40−45.
(33) Terse-Thakoor, T.; Komori, K.; Ramnani, P.; Lee, I.; Mulchandani, A. Electrochemically Functionalized Seamless Three-Dimensional Graphene-Carbon Nanotube Hybrid for Direct Electron Transfer of Glucose Oxidase and Bioelectrocatalysis. *Langmuir* **2015**, *31*, 13054−13061.
(34) Yu, Y.; Chen, Z.; He, S.; Zhang, B.; Li, X.; Yao, M. Direct Electron Transfer of Glucose Oxidase and Biosensing for Glucose based on PDDA-Capped Gold Nanoparticle Modified Graphene/Multi-walled Carbon Nanotubes. *Biosens. Bioelectron.* **2014**, *52*, 147−152.
(35) Bagheri, S.; Termehyousefi, A.; Mansouri, N.; Babadi, A. A.; Abd karim, M. S.; Adib Kadri, N. Carbon-Based Nanobiohybrid Thin Film for Amperometric Glucose Sensing. *ACS Biomater. Sci. Eng.* **2017**, *3*, 2059−2063.
(36) Goncales, V. R.; Colombo, R. N. P.; Minadeo, M. A. O. S.; Matsubara, E. Y.; Rosolen, J. M.; Córdoba de Torresi, S. I. Three-Dimensional Graphene/Carbon Nanotubes Hybrid Composites for Exploring Interaction between Glucose Oxidase and Carbon based Electrodes. *J. Electroanal. Chem.* **2016**, *775*, 235−242.
(37) Banks, C. E.; Crossley, A.; Salter, C.; Wilkins, S. J.; Compton, R. G. Carbon Nanotubes Contain Metal Impurities which are Responsible for the Electrocatalysis seen at Some Nanotube-modified Electrodes. *Angew. Chem., Int. Ed.* **2006**, *45*, 2533−2537.
(38) Batchelor-McAuley, C.; Wildgoose, G. G.; Compton, R. G.; Shao, L. D.; Green, M. L. H. Copper Oxide Nanoparticle Impurities are Responsible for the Electroanalytical Detection of Glucose seen using Multiwalled Carbon Nanotubes. *Sens. Actuators, B* **2008**, *132*, 356−360.
(39) Sljukic, B.; Banks, C. E.; Compton, R. G. Iron Oxide Particles are the Active Sites for Hydrogen Peroxide Sensing at Multiwalled Carbon Nanotube Modified Electrodes. *Nano Lett.* **2006**, *6*, 1556−1558.
(40) Pumera, M. Carbon Nanotubes Contain Residual Metal Catalyst Nanoparticles even after Washing with Nitric Acid at Elevated Temperature Because These Metal Nanoparticles are Sheathed by Several Graphene sheets. *Langmuir* **2007**, *23*, 6453−6458.
(41) Pumera, M.; Iwai, H. Multicomponent Metallic Impurities and their Influence upon the Electrochemistry of Carbon Nanotubes. *J. Phys. Chem. C* **2009**, *113*, 4401−4405.
(42) Ambrosi, A.; Pumera, M. Nanographite Impurities Dominate Electrochemistry of Carbon Nanotubes. *Chem. - Eur. J.* **2010**, *16*, 10946−10949.
(43) Stuart, E. J. E.; Pumera, M. Nanographite Impurities of Single-walled and Double-Walled Carbon Nanotubes are Responsible for the Observed "electrocatalytic" effect Towards the Reduction of Azo Groups. *Chem. - Asian J.* **2011**, *6*, 804−807.
(44) Stuart, E. J. E.; Pumera, M. Nanographite Impurities within Carbon Nanotubes are Responsible for Their Stable and Sensitive Response toward Electrochemical Oxidation of Phenols. *J. Phys. Chem. C* **2011**, *115*, 5530−5534.

DOI: 10.1021/acsabm.9b00145
ACS Appl. Bio Mater. 2019, 2, 1740−1750

(45) Pumera, M.; Sasaki, T.; Iwai, H. Relationship between Carbon Nanotube Structure and Electrochemical Behavior: Heterogeneous Electron Transfer at Electrochemically Activated Carbon nanotubes. *Chem. - Asian J.* **2008**, *3*, 2046−2055.
(46) Stuart, E. J. E.; Pumera, M. Signal Transducers and Enzyme Cofactors are Susceptible to Oxidation by Nanographite Impurities in Carbon Nanotube Materials. *Chem. - Eur. J.* **2011**, *17*, 5544−5548.
(47) Mayuri, P.; Kumar, A. S. In Situ Derivatization of an Intrinsic Iron Impurity as a Surface-Confined Iron(ii)tris(2, 2′-bipyridine) Complex on MWCNT and its Application to Selective Electrochemical Sensing of DNA's purine bases. *Langmuir* **2015**, *31*, 5945−5951.
(48) Gayathri, P.; Kumar, A. S. An Iron Impurity in Multiwalled Carbon Nanotube Complexes with Chitosan that Biomimics the Heme-Peroxidase Function. *Chem. - Eur. J.* **2013**, *19*, 17103−17112.
(49) Senthil Kumar, A.; Gayathri, P.; Barathi, P.; Vijayaraghavan, R. Improved Electric Wiring of Hemoglobin with Impure-Multiwalled Carbon Nanotube/Nafion Modified Glassy Carbon Electrode and its Highly Selective Hydrogen Peroxide Biosensing. *J. Phys. Chem. C* **2012**, *116*, 23692−23703.
(50) Dinesh, B.; Saraswathi, R.; Kumar, A. S. Water Based Homogenous Carbon Ink Modified Electrode as an Efficient Sensor System for Simultaneous Detection of Ascorbic acid, Dopamine and Uric acid. *Electrochim. Acta* **2017**, *233*, 92−104.
(51) Dinesh, B.; Devi, K. S. S.; Kumar, A. S. Curcumin-Quinone Immobilised Carbon black Modified Electrode Prepared by in-situ Electrochemical Oxidation of Curcumin-Phytonutrient for Mediated Oxidation and Flow injection Analysis of Sulfide. *J. Electroanal. Chem.* **2017**, *804*, 116−127.
(52) Gandhi, M.; Rajagopal, D.; Parthasarathy, S.; Raja, S.; Huang, S.-T.; Kumar, A. S. In Situ Immobilized Sesamol-Quinone/Carbon Nanoblack-Based Electrochemical Redox Platform for Efficient Bioelectrocatalytic and Immunosensor Applications. *ACS Omega* **2018**, *3*, 10823−10835.
(53) Shalini Devi, K. S. S.; Anusha, N.; Raja, S.; Kumar, A. S. A New Strategy for Direct Electrochemical Sensing of a Organophosphorus Pesticide, Triazophos, Using a Coomassie Brilliant-Blue Dye Surface-Confined Carbon-Black-Nanoparticle-Modified Electrode. *ACS Appl. Nano Mater.* **2018**, *1*, 4110−4119.
(54) Bartlett, P. N.; Al-Lolage, F. A. There is no Evidence to Support Literature Claims of Direct Electron transfer (DET) for Native Glucose oxidase (GOx) at Carbon Nanotubes or Graphene. *J. Electroanal. Chem.* **2018**, *819*, 26−37.

DOI: 10.1021/acsabm.9b00145
ACS Appl. Bio Mater. 2019, 2, 1740−1750