One-pot scalable synthesis of rGO/AuNPs nanocomposite and its application in enzymatic glucose biosensor

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ABSTRACT
A nanocomposite, rGO/AuNPs, was synthesized simultaneously using one-pot approach with gold nanoparticles (AuNPs) and reduced graphene oxide (rGO) within gelatin which used as a reducing and stabilizing agent. Then, the fabricated nanocomposites were characterized by UV-Vis, transmission electron microscope (TEM) and field emission scanning electron microscope (FE-SEM). The optimal nanocomposite was determined using electrochemical approaches. After facile and eco-friendly synthesis of rGO/AuNPs nanocomposites, it was used to fabricate the bioanode of enzymatic glucose biosensors. After drop-casting the nanocomposites on a screen-printed electrode (SPE), the glucose oxidase (GOx) was immobilized on the pre-treated SPE through a protein cross-linking approach using glutaraldehyde (GA) as a crosslinking reagent and 2,5-dihydroxybenzaldehyde (DHB) as a mediator to improve the electrochemical performance. Then, electrochemical performance of enzyme immobilized nanocomposites was studied using the potentiostat. The results demonstrate that the enzymatic biosensor made of rGO/AuNPs nanocomposites showed enhanced the sensitivity of selectivity for detection of glucose.

GRAPHICAL ABSTRACT

1. Introduction
Graphene, a single layer of sp²-hybridized carbon atoms, has received tremendous attention because of its unique properties such as high surface area, high capacitance, and good mechanical strength, excellent thermal and electrical conductivities [1,2]. Graphene-based materials exhibit great potential for electrode materials of electrochemical biosensors, because it increases sensing area and improves the electron transfer rate [3,4]. Specially, reduced graphene oxide (rGO), synthesized via the chemical reduction of graphene oxide (GO), is of great interest in biosensing. rGO offers an outstanding electron transfer mediator to regulate electron transfer between nanomaterials and substrate and greater supporting matrix to engage the depositing nanomaterials [5,6]. There are various techniques that have been reported for the reduction of GO, such as chemical reduction, thermal annealing, microwave irradiation or electrochemical reduction [7]. Among these approaches, chemical reduction, which can be utilized at room temperature or moderately elevated temperature, is well-adapted for its easy handling and cost-effective properties [8]. Sodium borohydride (NaBH₄) and hydrazine (N₂H₄) are the well-known reduction agents [9]. Recently, Piao et al.
used gelatin as a reducing agent to reduce GO into rGO and this approach also improved biocompatibility of graphene due to the nature of gelatin [10].

To enhance catalytic and electrochemical activities of graphene-based nanomaterials, metal nanoparticles have been widely employed to fabricate a promising platform for direct electron transfer [3]. Among the metal nanoparticles, gold nanoparticles (AuNPs) have been extensively used due to their a good biocompatibility and low level of toxicity to develop various sensors [11]. Another advantage of using AuNPs is having a large surface area to volume ratio and moreover, the size and shape of AuNPs determines their electronic and optical properties. Thus, it is important to control the structural and morphological properties of AuNPs, for understanding the optical and electronic properties [12–14]. Sodium citrate is the most common reducing agent for gold precursor tetrachloroaurate (HAuCl4) to synthesize AuNPs which is also known as Turkevich method [15]. This method is a straightforward and reproducible technique for the synthesis of spherical AuNPs ranging from 15 to 50 nm in size [16]. There are several synthetic (polyethylene glycol-g-polyvinyl alcohol (PEG-g-PVA), poly(acrylic acid) (PAA) etc.) and natural (starch, chitosan, gelatin and so on) polymers that have been also reported as reducing/capping agents for the synthesis of AuNPs [17,18]. These natural polymers enhance the biocompatibility of AuNPs, when used as both reducing and stabilizing agents [14].

There are two accepted strategies on how to construct rGO/AuNPs nanocomposites. The first strategy starts with producing GO and AuNPs separately and subsequently the well-dispersed GO and AuNPs solutions are mixed. Finally, GO sheets are reduced using one of the above-mentioned techniques. In this strategy, GO sheets are reduced at the end because this would prevent them from restacking [19,20]. In the second strategy, a suspension containing GO and gold precursor, HAuCl4, is mixed with a reducing agent to form a rGO/AuNPs nanocomposite [21]. These reducing agents are typically organic molecules, aqueous alkaline solutions or polymers. Thirumalraj et al. reported studied a simple approach for gallic acid supported reduced graphene oxide/gold nanoparticles (GA-RGO/AuNPs) using one-pot hydrothermal method for the detection of dopamine [22]. Zhang et al. studied one-pot green synthesis of RGO–AuNPs nanohybrid at 80 °C using glucose as a reducing agent [23]. Marinou et al. reported a facile approach for AuNP/rGO synthesis for application in real proton-exchange membrane (PEM) fuel cell. In this procedure, instead of using hydrazine as the reducing agent, aqueous solution of NaBH₄ were utilized to avoid doping of graphene with nitrogen atoms [24]. Zhang et al. reported one-step approach for synthesis of rGO/AuNPs nanocomposites in the presence of the polyaldehyde (PAA) for their electrocatalytical activity towards reduction of H₂O₂ and O₂. PAA was used as reducing and stabilizing agent for the preparation of the nanocomposites [25]. Beside these two approaches, there are other in situ techniques have been reported including microwave irradiation assisted, and direct electrodeposition etc. [25,26]. However, these techniques are expensive, time consuming or not suitable for a large scale of production.

Herein, I report in situ reduction of GO and HAuCl₄ into rGO/AuNPs nanocomposites using a biopolymer, gelatin, as both a reducing and a stabilizing agent. The procedure was optimized for a better electrochemical response using cyclic voltammetry (CV), after depositing the nanocomposite on screen printed electrode (SPE). As-synthesized nanocomposites were well characterized by UV-Vis, field emission scanning electron microscope (FE-SEM) and transmission electron microscope (TEM). The nanocomposite was then used as an electrode material to provide a large number of active centers for enzyme bindings. An enzymatic matrix was prepared with the glucose oxidase (GOx), 2,5-dihydroxybenzaldehyde (DHB), glutaraldehyde (GA) for a selective and sensitive binding of glucose. In order to improve the electrochemical performance of GOx, DHB and GA was utilized as mediator and a crosslinking reagent, respectively. The enzymatic matrix then immobilized on the SPE, prefabricated with rGO/AuNPs nanocomposites. After the fabrication of the biosensor, the results demonstrate that the enzyme-based biosensor is highly sensitive and selective towards glucose. This new approach for the synthesis of rGO/AuNPs nanocomposites is facile, green and not a laborious task, so it can be employed as the platform substrate for surface-enhanced Raman scattering (SERS) and electrochemical-based biosensors.

2. Experimental

2.1. Chemicals and reagents

Glutaraldehyde (GA) solution (25% in water) was purchased from Fluka Chemica (Buchs Switzerland). Glucose, 2, 5-dihydroxybenzaldehyde (DHB), gelatin, and galactose were attained from Sigma Aldrich (St. Louis, MO, USA). Hydrogen tetrachloroaurate (III) trihydrate (HAuCl₄·3H₂O) were purchased from Acros Organics, (NJ, USA). 10 mM of HAuCl₄ solution was prepared in DI water and stored in a refrigerator before use. Glucose oxidase (GOx) were obtained from MP Biomedicals LLC.
(OH, USA). 0.1 M of PBS solution (pH 7.0) prepared using NaH$_2$PO$_4$ and Na$_2$HPO$_4$ which were purchased from Fisher (NJ, USA). GO was synthesized using modified Hummer’s method by the oxidation and exfoliation of graphite. The ultrapure water with resistivity $\geq$18.2 M$\Omega$cm from Milli-Q systems (Millipore, USA) were used to prepare all aqueous solutions.

### 2.2. Instruments and apparatus

UV-Vis (Lambda 25, Perkin Elmer, USA) was used to characterize the all samples. A field emission scanning electron microscope (FE-SEM) (LEO1530, Gemini FE-SEM, Carl Zeiss, Germany) was performed at 5 kV to observe surface morphology and transmission electron microscopy (TEM) images were obtained using FEI Tecnai TF30 (Netherlands) operated at 80 kV. For each sample, one drop of solution was placed on a carbon-coated 400 mesh copper grid and excess solution was removed using filter paper. The grid was dried at room temperature before imaging.

An electrochemical workstation (660 D, CH Instruments) was used for electrochemical measurements. The SPE was used as bare or after modification with various enzyme immobilized nanocomposites. Cyclic voltammetry (CV) measurements were performed for the electrochemical responses of the electrodes toward to glucose in 0.1 M of PBS solution (pH 7.0) as an electrolyte at room temperature.

### 2.3. Synthesis of rGO/AuNPs nanocomposites

To fabricate one-pot synthesis of rGO/AuNPs nanocomposites, gelatin (0.5%, 0.75%, 1.0%, and 1.25%) in 40 ml of GO solution (0.2 mg/ml) and 10 ml of HAuCl$_4$ (10 mM) were mixed in 100 mL flask. Then, the mixture was placed in a water bath pre-heated to 90 °C under constant stirring with various heating times (4 h, 8 h, 12 h, 24 h). Thereafter the heating process, the mixture was cooled to room temperature in an ice water bath to form rGO/AuNPs nanocomposites.

### 2.4. Fabrication of rGO/AuNPs based enzymatic glucose biosensors

The 0.5 units/µl of GOx solution was first mixed with 20 mM of 2,5-dihydroxybenzaldehyde (DHB). Then, 0.3 vol% of GA was added to GOx/DHB mixture and vortexed for 10 min. to prepare GOx/DHB/GA mixture. To fabricate rGO/AuNPs based enzymatic glucose biosensors, 5 µl of the prepare nanocomposites were drop casted on the SPE surface and dried. 20 µl of the GOx/DHB/GA mixture was added over the previous modified SPE and left to dry for 1 h at 35 °C. The air-dried electrodes were thoroughly washed with 10 mL PBS, pH 7 to remove the non-cross-linked species.

### 3. Results and discussion

#### 3.1. Characterization of materials

To the best of my knowledge, this is the first study on optimization of an eco-friendly and one-pot synthesis of rGO/AuNPs nanocomposites using gelatin as biocompatible reducing and stabilization agent for graphene oxide and Hydrogen tetrachloroaurate (III) trihydrate. The synthesis route for the nanocomposite is illustrated in Figure 1. In this process, GO acted as multi-functional crosslinkers due to the presence of oxygen containing functional groups especially, carboxylic acids, epoxy groups and alcohols while gelatin was used as reducing and stabilization agent for both GO and the gold precursor by thermal treatment [10,14]. Moreover, it is reported that at low concentrations, graphene is non-toxic and the use of a biocompatible polymer, gelatin, as a reducing agent could further reduce the toxicity of graphene at higher concentration [10]. Therefore, the as-synthesized nanocomposite can be potentially used in biomedical applications.

In order to investigate the formation of rGO and AuNPs using gelatin as reducing and stabilization agent, UV-vis spectra were utilized. The mixture of
10 ml of HAuCl4 (10 mM) and 40 ml of GO solution (0.2 mg/ml), containing 1.25% of gelatin, were stirred for 12 h at 90°C to form rGO/AuNPs nano-composite. The AuNPs and rGO were also individually synthesized under the same condition (0.5% of gelatin, under constant stirring for 12 h at 90°C), detailed in the experimental section. As shown Figure 2a, the UV-vis spectra of AuNPs and rGO revealed a sharp absorption peak 525 nm and a broad shoulder located around 270 nm due to the surface plasmon resonance of AuNPs and transition of sp2 hybridized carbon atoms, respectively [27]. The UV-vis spectra of rGO/AuNPs consists of AuNPs and rGO’s bands, which can be attributed to the attachment of AuNPs on rGO sheets.

The Figure 2b reveals a SEM image of rGO synthesized through reduction of GO using gelatin as reducing agent. It shows characteristics wrinkled and curved layers for rGO. The TEM image of AuNPs is presented in Figure 2c which shows that the size of individual AuNP varies from 18 nm up to 42 nm. Figure 2d and 2e shows SEM and TEM images of as-synthesized rGO/AuNPs nanocomposite, respectively. These results suggest the uniform dispersion and formation of AuNPs on rGO sheets which is the clear evidence for the interaction. Figure 2f is the enlarged view of Figure 2e and the average particle size of AuNPs on rGO sheets is 36 nm.

3.2. Optimization of nanocomposite

Various gelatin and heating times were applied to optimize experimental parameters for the rGO/
AuNPs nanocomposites and enhance the electrochemical behavior. First, 10 ml of HAuCl₄ (10 mM) and 40 ml of GO solution containing 1.25% of gelatin were mixed at various heating times (4, 8, 12, 24 h) under constant stirring at 90 °C. Then, these 4 nanocomposites were deposited on screen printed electrode (SPE) individually and as shown in Figure 3a the electrochemical behavior of modified SPEs were examined by CV. The results show a pair of quasi-reversible redox peaks for all electrodes and among the modified electrodes, 12 and 24 h of heating times show comparable electrochemical features, but 24 h has slightly better response, indicating fast electron-transfer kinetics with good electrocatalytic capability. Moreover, Figure 3c and 3d show the effect of heating time for 24 and 12 h, respectively. As can be seen from the figures, different heating time treatment may result in different size of the AuNPs. It may be the reason why 24 h of heating time shows better electrochemical response. The darker sheet like structure may result from RGO sheets.

In order to further improve the composition, 40 ml of GO solution (0.2 mg/ml) and 10 ml of HAuCl₄ (10 mM) in a series of gelatin solutions (0.5%, 0.75%, 1.0%, and 1.25%) were prepared in 100 mL flask. After modifying the SPE by drop casting of as-prepared nanocomposites, CVs were recorded. As shown in Figure 3b, the nanocomposite made of 1.0% gelatin deposited SPE shows a well-defined pair of quasi-reversible redox peaks, indicating a better faradaic pseudocapacitive behavior among electrodes.

Glucose biosensors are essential not only diabetes diagnosis but also food quality monitoring, bio-processing and in the development of renewable, sustainable fuel cells [28–30]. Thus, enzymatic and non-enzymatic methods have been developed to fabricate cost efficient, fast, selective and sensitive glucose biosensors [31–33]. Non-enzymatic glucose biosensors suffer from interference from intermediate materials, which show low sensitivity and poor selectivity and the most importantly lack of understanding of the mechanism of glucose oxidation at metals and carbon electrodes that limits effective modification of electrodes [34,35]. In particular, due to their outstanding sensitivity, selectivity, rapid response and low cost, enzymatic glucose biosensors have been drawn to detailed research. [36]. However,
since the enzymatic active site is tightly covered by a thick protein coating in the enzyme, the enzymatic glucose biosensors suffer from poor enzyme immobilization. To overcome these challenges, nanomaterials have been widely used to immobilize the enzyme for accelerating the electron transfer [37,38]. Therefore, the optimized rGO/AuNPs nanocomposite can be used as a template to improve the performance of enzymatic glucose biosensor.

As shown in Figure 4, SPE was first coated with 5 µl of rGO/AuNPs nanocomposite, then 20 µl of crosslinked glucose oxidase enzyme (GOx), and a redox mediator (DHB) matrix immobilize on the SPE to enhance the selective binding to glucose. DHB was used as the redox mediator to transfer the electrons between the enzymatic glucose oxidation and the electrode [39]. Also, gelatin which was used to reduce GO and stabilized AuNPs can also help with the enzyme immobilization [40]. The modified electrode, denoted as rGO/AuNPs-GOx/DHB, was assembled and investigated for glucose biosensor.

### 3.3. Electrochemical response of the biosensor

The rGO/AuNPs-GOx/DHB modified SPE was explored to evaluate the glucose oxidation in 0.1 M of PBS solution (pH 7.0) in potential range of 0.7 to −0.7 V. As shown in Figure 5a CVs for the rGO/AuNPs-GOx/DHB modified SPE were recorded in the presence of different concentration of glucose (0, 1, 2, 3, 4, 6, 8, and 11 mM). After glucose was added into the electrolyte solution (PBS), a significant oxidation peak appeared at around −0.48 V and a reduction peak about 0.22 V due to enzymatic activity of GOx. This response is typical for enzyme catalysis [41]. The calibration curves are presented in Figure 5b. The linear calibration curves in the range 0–11 mM were reported with correlation coefficients (R²) of 0.9983 and 0.9623, for oxidation and reduction peaks, respectively.

The limit of detection (LOD) of the oxidation of rGO/AuNPs-GOx/DHB modified SPE was calculated as 0.63 mM using the following equation (1):

$$ LOD = 3 \times \frac{\sigma}{S} $$

Where $\sigma$ is the standard deviation at the lowest concentration and $S$ represents slope of the calibration curve [42]. The linear concentration range and the limit of detection are comparable to that of enzymatic biosensors reported in the literature, as seen in Table 1 [43–48].
As-prepared SPE electrode were further assessed with respect to their selectivity towards glucose. As shown in Figure 6, a CV test were recorded without adding any analyte, then 4 mM galactose were added into PBS as the interfering molecules of monosaccharides. There was no observable current change, however a sharp response was detected with the addition of 4 mM of glucose. This result demonstrates that the rGO/AuNPs-GOx/DHB modified SPE has high selectivity towards glucose in the presence of interfering species.

4. Conclusion

For the first time, a biopolymer, gelatin, was used as a reducing and stabilizing agent for the optimization of rGO/AuNPs nanocomposite for their best electrochemical response. The optimum experimental conditions were determined based on better electrochemical responses using CVs. The nanocomposite is well characterized by different techniques. Then, as-synthesized nanocomposites were drop casted on SPE and the electrode was further modified by a crosslinked GOx/DHB matrix for the biosensor use. The fabricated enzymatic biosensor showed high sensitivity and selectivity towards glucose. The new method in this study to make rGO/AuNPs nanocomposites offers great advantages including low operational costs, eco-friendly green synthesis, requires very limited laborious task. In addition, this nanocomposite can be utilized as platform substrate for SERS and electrochemical-based biosensors.

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Notes on contributor

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References

1. Sadak O, Sundaramoorthy AK, Gunasekaran S. Facile and green synthesis of highly conducting graphene paper. Carbon N. Y. 2018;138:108–117.
2. Wang W, Sadak O, Guan J, et al. Facile synthesis of graphene paper/polypyrrole nanocomposite as electrode for flexible solid-state supercapacitor. J. Energy Storage. 2020;30:101533.
3. Zhang Y, Shen J, Li H, et al. Recent progress on graphene-based electrochemical biosensors. Chem Rec. 2016;16(1):273–294.
4. Teng Y, Singh CK, Sadak O, et al. Electrochemical detection of mobile zinc ions for early diagnosis of prostate cancer. J. Electroanal. Chem. 2019;833:269–274.
5. Hossain MF, Park JY. A highly sensitive and selective enzymatic glucose sensor based on platinum nanoparticles embedded with acid treated reduced graphene oxide. Proceedings of the IEEE International Conference on Micro Electro Mechanical Systems (MEMS); 2016. Vol. 2016-Febru, pp 278–281.
6. Sadak O, Wang W, Guan J, et al. MnO2 Nanoflowers Deposited on Graphene Paper as Electrode Materials for Supercapacitors. ACS Appl Nano Mater. 2019;2(7):4386–4394.
7. Singh RK, Kumar R, Singh DP. Graphene oxide: strategies for synthesis. RSC Adv. 2016;6(69):64993–65011. pp
8. Yousefi R, Cheraghizade M. Semiconductor/graphene nanocomposites: synthesis, characterization, and applications. In: Applications of Nanomaterials. 2018. p. 23–43.
9. Raji M, Zari N, el Kacem Qaiss A, et al. Chemical preparation and functionalization techniques of graphene and graphene oxide. In: Functionalized Graphene Nanocomposites and Their Derivatives: Synthesis, Processing and Applications. 2018. p. 1–20.
10. Piao Y, Chen B. One-pot synthesis and characterization of reduced graphene oxide–gelatin nanocomposite hydrogels. RSC Adv. 2016;6(8):6171–6181.
11. Muthurasu A, Ganesh V. Glucose oxidase stabilized fluorescent gold nanoparticles as an ideal sensor matrix for dual mode sensing of glucose. RSC Adv. 2016;6(9):7212–7223.
12. Wang YC, Gunasekaran S. Spectroscopic and microscopic investigation of gold nanoparticle nucleation and growth mechanisms using gelatin as a stabilizer. J. Nanoparticle Res. 2012;14:1200.
13. Zhang JJ, Gu MM, Zheng TT, et al. Synthesis of gelatin-stabilized gold nanoparticles and assembly of carboxyl functionalized carbon nanotubes/Au composites for cytosensing and drug uptake. Anal Chem. 2009;81(16):6641–6648.
14. Lim S, Gunasekaran S, Imm JY. Gelatin-templated gold nanoparticles as novel time-temperature indicator. J. Food Sci. 2012;77:77.
15. Turkevich J, Stevenson PC, Hillier J. A study of the nucleation and growth processes in the synthesis of colloidal gold. Discuss Faraday Soc. 1951;11:55–75.
16. Dong J, Carpinone PL, Pyrgiotakis G, et al. Synthesis of precision gold nanoparticles using turkevich method. KONA. 2020;37(0):224–232.
17. Alkilany AM, Bani Yaseen AI, Kailani MH. Synthesis of monodispersed gold nanoparticles with exceptional colloidal stability with grafted polyethylene glycol- g-polyvinyl alcohol. J. Nanomater. 2015;2015:1–9.
18. Ians H, Ians K, Laage L, et al. Poly(acrylic acid)-stabilized colloidal gold nanoparticles: synthesis and properties. Nanotechnology. 2010;21(45):45702.
19. Kholmanov IN, Stoller MD, Edgeworth J, et al. Nanostructured hybrid transparent conductive films with antibacterial properties. ACS Nano. 2012;6(6):5157–5163.
20. Sadak O, Prathap MUA, Gunasekaran S. Facile fabrication of highly ordered polyaniline–exfoliated graphite composite for enhanced charge storage. Carbon N. Y. 2019;144:756–763.
21. Vinodgopal K, Neppolian B, Lightcap IV, et al. Sonolytic design of graphene–Au nanocomposites. Simultaneous and sequential reduction of graphene oxide and Au(III). J Phys Chem Lett. 2010;1(3):1987–1993.
22. Thirumalraj B, Rajkumar C, Chen SM, et al. One-pot green synthesis of graphene nanosheets encapsulated gold nanoparticles for sensitive and selective detection of dopamine. Sci. Rep. 2017;7.
23. Zhang P, Zhang X, Zhang S, et al. One-pot green synthesis, characterization, and biosensor application of self-assembled reduced graphene oxide–gold nanoparticle hybrid membranes. J Mater Chem B. 2013;1(47):6525–6531.
24. Marinou A, Raceanu M, Andrulevicius M, et al. Low-cost preparation method of well dispersed gold nanoparticles on reduced graphene oxide and electrocatalytic stability in PEM fuel cell. Arab. J. Chem. 2020;13(1):3585–3600.
25. Zhang Q, Ren Q, Miao Y, et al. One-step synthesis of graphene/polyallylamine-Au nanocomposites and their electrocatalysis toward oxygen reduction. Talanta. 2012;89:391–395.
26. Dong X, Huang W, Chen P. In situ synthesis of reduced graphene oxide and gold nanocomposites for nanoelectronics and biosensing. Nanoscale Res. Lett. 2011;6(1):1–6.
27. Amanulla B, Palanisamy S, Chen SM, et al. Elective colorimetric detection of nitrite in water using chitosan stabilized gold nanoparticles decorated reduced graphene oxide. Sci. Rep. 2017;7(1).
28. Rafatmah E, Hemmateenjad B. Dendrite gold nanostructures electrodeposited on paper fibers: Application to electrochemical non-enzymatic determination of glucose. Sensors Actuators, B Chem. 2020;304:127335.
29. Toghill KE, Compton RG. Electrochemical non-enzymatic glucose sensors: a perspective and an evaluation. Int. J. Electrochem. Sci. 2010; 5(9):1246–1301.
30. Tian K, Alex S, Siegel G, et al. Enzymatic glucose sensor based on Au nanoparticle and plant-like ZnO film modified electrode. Mater. Sci. Eng. C. 2015;46:548–552.
31. Jeong H, Nguyen DM, Lee MS, et al. N-doped graphene-carbon nanotube hybrid networks attaching with gold nanoparticles for glucose non-enzymatic sensor. Mater. Sci. Eng. C. 2018;90:38–45.
32. Das G, Tran TQN, Yoon HH. Spherulitic copper--copper oxide nanostructure–based highly sensitive nonenzymatic glucose sensor. Int. J. Nanomedicine. 2015; 10:165–178.
33. Anu P, Sun MU, Wei S, et al. Z. J. A novel non-enzymatic lindane sensor based on CuO-MnO$_2$ hierarchical nano-microstructures for enhanced sensitivity. Chem. Commun. 2015; 51(21):4376–4379.
34. Anu P, Kaur MU, Srivastava B. R. Hydrothermal synthesis of CuO micro-/nanostructures and their applications in the oxidative degradation of methylene blue and non-enzymatic sensing of glucose/H$_2$O$_2$. J. Colloid Interface Sci. 2012;370(1):144–154.
35. Rahman MM, Ahammad AJS, Jin JH, et al. A comprehensive review of glucose biosensors based on nanostructured metal-oxides. Sensors (Basel). 2010; 10(5):4855–4886.
36. Wang J. Glucose biosensors: 40 years of advances and challenges. Electroanalysis. 2001;13(12):983–988.
37. Zhu Z, Garcia-Gancedo L, Flewitt AJ, et al. A critical review of glucose biosensors based on carbon nanomaterials: carbon nanotubes and graphene. Sensors (Switzerland). 2012;12(5):5996–6022.
38. Zhou X, Dai X, Li J, et al. A sensitive glucose biosensor based on Ag@C core–shell matrix. Mater. Sci. Eng. C. 2015;49:579–587.
39. Yu CM, Yen MJ, Chen LC. A bioanode based on MWCNT/protein-assisted co-immobilization of glucose oxidase and 2,5-dihydroxybenzaldehyde for glucose fuel cells. Biosens Bioelectron. 2010;25(11):2515–2521.
40. Singh V, Singh D. Glucose Oxidase Immobilization on Guar Gum–Gelatin Dual-Templated Silica Hybrid Xerogel. Ind Eng Chem Res. 2014;53(10):3854–3860.
41. Kang Z, Jiao K, Yu C, et al. Direct electrochemistry and bioelectrocatalysis of glucose oxidase in CS/CNC film and its application in glucose biosensing and biofuel cells. RSC Adv. 2017;7(8):4572–4579.
42. Sadak O, Sundaramoorthy AK, Gunasekaran S. Highly selective colorimetric and electrochemical sensing of iron (III) using Nile red functionalized graphene film. Biosens Bioelectron. 2017;89:430–436.
43. Zhu Z, Song W, Burugapalli K, et al. Nano-yarn carbon nanotube fiber based enzymatic glucose biosensor. Nanotechnology. 2010;21(16):165501.
44. Paul A, Vyas G, Paul P, et al. Gold-nanoparticle-encapsulated ZIF-8 for a mediator-free enzymatic glucose sensor by amperometry. ACS Appl Nano Mater. 2018, 1;1(7):3600–3607.
45. Barathi P, Thirumalraj B, Chen SM, et al. A simple and flexible enzymatic glucose biosensor using chitosan entrapped mesoporous carbon nanocomposite. Microchem. J. 2019;147:848–856.
46. Pang P, Yang W, Huang S, et al. Measurement of glucose concentration in blood plasma based on a wireless magnetoeelastic biosensor. Analytical Lett. 2007;40(5):897–906.
47. Wang C;P, Min; Chen H, Chen DCY, Department of Biomedical Engineering, Zhejiang University, Hangzhou 310027, China. Novel glucose oxidase interlocked prussian blue/polysulfone stereo-structure and its application in amperometric glucose biosensor. Int J Electrochem Sci. 2019;14:8014–8027.
48. Wang Y, Li H, Kong J. Facile preparation of mesocellular graphene foam for direct glucose oxidase electrochemistry and sensitive glucose sensing. Sensors Actuators, B Chem. 2014;193:708–714.