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Graphen Based Nanocomposites for Glucose and Ethanol Enzymatic Biosensor Fabrication

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Abstract

Recently graphen based nanocomposites are become an emerging research areas for fabrication of enzymatic biosensors due to their property of large surface area, conductivity and biocompatibility. This review summarizes recent research reports of graphen based nanocomposites for the fabrication of glucose and ethanol enzymatic biosensors. The newly fabricated enzyme free Microwave Treated Nitrogen Doped Graphen (MTN-d-GR) had provided highest sensitivity towards glucose and GCE/rGO/AuNPs/ADH composite had provided far highest sensitivity towards ethanol compared to other reported graphen based nanocomposites. The MWCNT/GO/GOx and GCE/ErGO/PTH/ADH nanocomposites had also enhanced wide linear range for glucose and ethanol detection respectively. Generally, graphen based nanocomposite enzymatic biosensors had fast direct electron transfer rate, highest sensitivity and wide linear detection ranges during glucose and ethanol sensing.

Keywords: Glucose • Ethanol • Enzymatic biosensor • Graphen • Nanocomposite

Introduction

Accurate and precise analysis of glucose and ethanol is of great importance in various applications, from industrial and food process control to clinical requirements [1]. Conventional analytical methods like chromatography, photometry, pulse amperometry, spectrometry techniques are widely used till now to detect glucose and ethanol. But these methods are time consuming, labor intensive and not suitable for online monitoring [2-5].

In recent years, enzymatic biosensors are preferable in monitoring glucose and ethanol due to their low cost, ease of manipulation for the intended applications; relatively fast response times and small size [6].

Literature Review

The enzymes normally used in the developed biosensors for glucose and ethanol detection are Glucose Oxidase (GOx) and Alcohol Dehydrogenase (ADH) respectively. GOx catalyzes the conversion of glucose to glucanolactone in the presence of the co-enzyme Flavin Adenine Dinucleotide (FAD) and ADH catalyzes the conversion of ethanol to acetaldehyde in the presence of the cofactor Nicotinamide Adenine Dinucleotide (NAD^+) (Figure 1) [7].

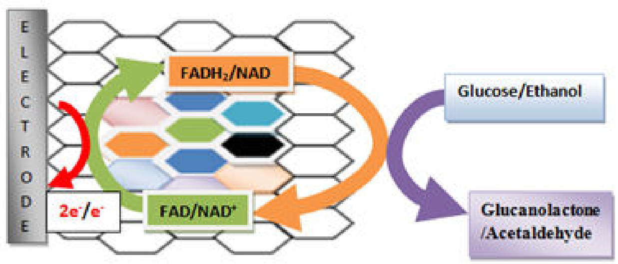

Figure 1. Graphen based glucose/ethanol enzymatic biosensor.

However most enzymatic biosensors have poor direct electron transfer in the enzyme electrode interfaces, due to the enzymes, far redox-active to the electrode and also the enzyme maybe loses its bioactivity when it immobilized directly onto the electrode surface [8,9]. One strategy to solve for aforementioned problem is through modification of electrodes by nano materials/Nano Particles (NPs) composite.

In recent years graphen become promising nano material to fabricate fast electron transfer, high sensitivity and wide linear range analyte det\ector enzymatic biosensors [10]. Graphene is a planar sheet of carbon atoms and which is synthesized from graphite *via* mechanical exfoliation, graphen have large surface area (2630 $m^2 g^{-1}$), which is higher than that of graphite (10 m^2g^{-1}) and CNT (1315 m^2g^{-1}), high mechanical strength (200 times greater than steel), high

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electrical conductivity (which is 60-fold greater than Single Walled CNT (SWCNT), and six times higher than copper), and high elasticity and thermal conductivity [11-16].

This review assesses the recent advances of graphen based nanocomposite for the fabrication of glucose and ethanol enzymatic biosensors.

Graphen based nano composites

Graphen based nano composites are extremely important for developing high performance enzymatic biosensors due to their pretty properties such as much lower particle-particle distance, larger surface/volume ratio, and the ability to create stronger immobilized enzymes [17].

Graphene based nano composites can be made by growing NPs directly on graphene surface or by mixing of graphene and pre-made NPs [18-20].

Nitrogen (N)-doped grapheme: Nitrogen has a comparable atomic size with Carbon, and has five valence electrons. It can form a strong covalent C-N bond. Once the bond is formed, the electronegative N (electronegativity: 3.04) can break the charge neutrality on C (electron negativity: 2.55) in the sp^2carbon lattice. Depending on where N binds to C, N is often categorized as graphitic N, pyridinic N and pyrollic N. Pyridinic nitrogen significantly enhances the electro active sites of graphene surface for selective oxidation (Figure 2 and Table 1).

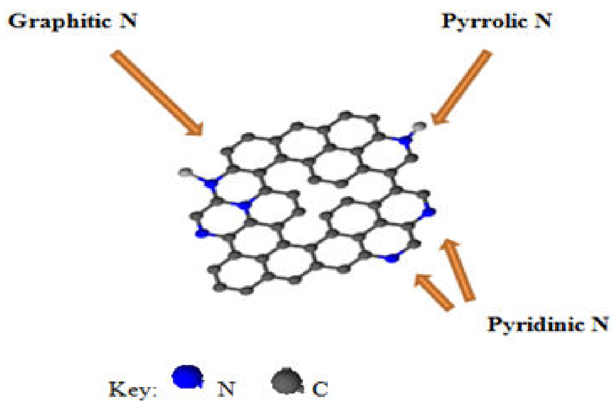

Figure 2. Doping position of N on graphen.

Electrode matrix	Analyte	Eapp(v)	Ks (s^{-1})	Linear range (mM)	LOD (µM)	Sensitivity ($\mu AmM^{-1}cm^{-2}$)
MN-d-GR/GOx	Glucose	0.1	NA	0-10	14.52	122.33
GCE/N-d-GR/ADH	Ethanol	0.49	NA	0-0.000012	0.37	160

Abbreviations: ADH: Alcohol Dehydrogenase, Eapp, applied potential, GCE: Glassy Carbon Electrode, GOx: Glucose Oxidase, LOD: Limit of Detection, MN-d-GR: Microwave Nitrogen doped Graphene, NA: Not Available, N-d-GR-Nitrogen doped Graphen, Pub: Publication, Ref: Reference.

Table 1. Nitrogen doped graphen glucose and ethanol enzymatic biosensors.

For example, a new enzyme free micro wave treated nitrogen doped graphen (MN-d-GR) provided a superior sensitivity of 122.336 $\mu A\ mM^{-1}cm^{-2}$ and lower applied potential (0.1 V) among recently fabricated graphen based glucose biosensors. An electrochemical ethanol biosensor based on glassy also wide linear ethanol detection (0.5 mM-12 mM) compared to recently reported graphen based ethanol enzymatic biosensors.

Graphen based ferrous NPs

Ferrous nanoparticles (Fe_3O_4 NPs) are a type of magnetic materials with high attention due to their low toxicity, paramagnetic property, good biocompatibility, easy preparation, and, more notably, the close contact among the nanoparticles, substrates and enzymes. In recent years graphen based Fe_3O_4 NPs have been commonly utilized for electrode reforming in enzymatic biosensors, because they enhance the electrode surface, electrical conductivity, and electron transfer kinetics among electro active species. For example, the recently fabricated glucose bio sensor based on a self-assembly of Glucose Oxidase (GOx) on reduced Graphene Oxide (rGO) covalently conjugated to magnetic nanoparticles (Fe_3O_4 NPs) modified on a Magnetic Screen-Printed Electrode (MSPE) had provided fast direct electron transfer rate (13.78 s^{-1}) among the recently reported graphen based nanocomposite enzymatic glucose biosensors.

Discussion

Graphen based gold NPs

Gold is an interesting coating material due to its chemical functionality. Gold Nanoparticles (AuNPs) are one of the highest considered nano materials because of their remarkable properties. They have great properties such as large specific surface area, high electrochemical catalytic activity, strong adsorption ability, biocompatibility, well suitability, and high conductivity. Graphen based AuNPs have been employed to synthesize different types of electrochemical sensors successfully. They can strongly interact with enzymes and enhance electron transfer rate and sensitivity of electrochemical biosensors. For example, the immobilization of Glucose Oxidase (GOx) on Graphene-Polyethyleneimine-Gold Nanoparticles Hybrid (GR-PEI-AuNPs) using glutaraldehyde as cross-linking reagent provides good sensitivity 93 ($AmM^{-1}cm^{-2}$) among recently fabricated graphen based gold nano composite glucose biosensors. An electrochemical ethanol biosensor based on Glassy Carbon Electrode (GCE) with Gold nanoparticles (AuNPs) and Reduced Graphene Oxide (rGO) had provided superior sensitivity 916 $AmM^{-1}cm^{-2}$ and fast electron transfer rate (10 s^{-1}) among the recently reported graphen based nanocomposite ethanol enzymatic biosensors (Tables 2 and 3).

Electrode Matrix	Analyte	Eapp (v)	Ks (s^{-1})	Linear range (µM)	LOD (µM)	Sensitivity µAm$M^{-1}cm^{-2}$
rGO/Fe_3O_4/ MSPE/GOx	Glucose	-0.45	13.78	50-1000	0.1	5.9
GCE/Pt/ Fe_3O_4/rGo/ADH	Ethanol	0.5	NA	0.00-1.50	0.005	733
GCE/GR/Fe $(CN)_6$/ADH	Ethanol	0.5	10	10-210	25	10

Abbreviations: ADH: Alcohol Dehydrogenase, Eapp: applied Potential, Fe_3O_4: Ferrous Oxide, $Fe(CN)_6$: Ferri Cyanide, GCE: Glassy Carbon Electrode, GOx: Glucose Oxidase, GR: Graphen, LOD: Limit of Detection, MSPE: Magnetic Screen Printed Electrode, NA: Not Available, Pub: Publication, Pt: Platinum, rGO: Reduced Graphen Oxide, Ref: Reference.

Table 2. Graphen based ferrous nano particle for glucose and ethanol enzymatic biosensors.

Electrode Matrix	Analyte	Eapp(v)	Ks (s^{-1})	Linear range (mM)	LOD (µM)	Sensitivity µAm$M^{-1}cm^{-2}$
GR/PEI/AuNPs/GOx	Glucose	-0.38	5.4	0 – 0.1	0.32	93
GR/MWCNT/ AuNPs/GOx	Glucose	-0.4	3.36	0 – 5.2	4.1	0.61
GCE/rGO/ AuNPs/ADH	Ethanol	0.55	NA	0 – 0.05	0.00113	916
GCE/GR/AuNPs/ADH	Ethanol	0.4	NA	0.02– 0.16	6	10.27
GCE/GR/AuNRs/ADH	Ethanol	0.35	10	0.005– 0.377	1.5	102
GCE/Au-AgNPs/P(L-Cys)-ErGO/ADH/Naf	Ethanol	NA	NA	0.017– 1.845	5	0.177

Abbreviations: ADH: Alcohol Dehydrogenase, AuNPs: Gold Nanoparticles, Au-AgNPs: Gold and Silver Nanoparticles, Eapp: Applied Potential, ErGO: Electrically Reduced Graphen Oxide, GCE: Glassy Carbon Electrode, GOx-Glucose Oxidase, GR: Graphen, LOD: Limit of Detection, MWCNT-Multi Wall Carbon Nano Tube, NA-Not Available, Naf: Nafion, PEI: Polyethylenemine, p(L-Cys)-poly (L-Cysteine), Pub: Publication, rGO: reduced Graphen Oxide, Ref- Reference.

Table 3. Graphen based AuNPs nano composite glucose and ethanol enzymatic biosensors.

Other nanocomposites

Graphen based polymeric materials are one of the most widely used supports in biosensor research. Conducting polymers and organic molecules have charge transfer properties due to the presence of a conjugate system in the polymer chain. It makes them compatible for integration with redox enzymes, permitting electron transfer to the electrode surface. Phthalocyanine (Pc), Nafion (Naf), carboxyl terminated Poly Amido Amine Dendrimer (PAMAM), Polyaniline (PANI), Polythionine (PTH) are some examples of polymers utilized for fabrication of glucose and ethanol enzymatic biosensors. For example, GCE/ErGO/PTH/ADH nanocomposite had provided wider linear detection range (0.01 mM-3.9 mM) of ethanol than the recently fabricated ethanol enzymatic biosensors. It had also good sensitivity (143 mAm$M^{-1}cm^{-2}$) (Table 4).

Electrode Matrix	Analyte	Eapp(v)	Ks (s^{-1})	Linear range (mM)	LOD (µM)	Sensitivity (µAm$M^{-1}cm^{-2}$)
GR/CoPc/GOx	Glucose	0.4	3.57	0.01-14.8	1.6	5.09
ErGO/MWCNT/GOx	Glucose	NA	3.02	0.01-6.5	10	NA
rGO/GOx	Glucose	NA	4.8	0.1-27	NA	NA
rGO/PAMAM/GOx	Glucose	NA	8.59	0.03-1.89	4.5	NA
MWCNT/GO/GOx	Glucose	-0.42	11.22	0.1-23.2	28	1.11

GR/PANI/GOx	Glucose	0.65	NA	0.01-1.48	2.77	22.1
GCE/ErGO/PTH/ADH	Ethanol	0.4	NA	0.01 -3.9	0.1	143
RuO_2-GR/ SPCE/ADH/Naf	Ethanol	0.6	NA	0.001-1.8	0.19	4.5

Abbreviations: CoPc: Cobalt(II) phthalocyanine, Eapp: Applied Potential, ErGO: Electrochemically reduced Graphene Oxide, GCE: Glassy Carbon Electrode, GO: Graphen Oxide, GOx: Glucose Oxidase, GR: Graphene, LOD: Limit of Detection, MWCNT: Multi wall Carbon Nano Tube, Naf: Nafion, PAMAM: Carboxyl terminated poly amido amine dendrimer, PANI: Polyaniline, PTH: Polythionine, Pub: Publication, rGO: reduced Graphen Oxide, Ref: Reference, RuO_2: Ruthenium dioxide, SPCE: Screen-Printed Carbon Electrodes.

Table 4. Graphen based polymeric materials are one of the most widely used supports in biosensor research.

Conclusion

GR based nano composites with metal oxides; metal nanoparticles and polymers are the most studied composites for the fabrication of enzymatic biosensor.

Graphen based Fe_3O_4 NPs nano composite, graphen based AuNPs nano composite and other polymeric nano composites had been commonly utilized for electrode reforming in enzymatic biosensors, because they can strongly interact with enzymes and enhance electron transfer rate and sensitivity of electrochemical biosensors.

The newly fabricated enzyme free microwave treated nitrogen doped graphen (MN-d-GR) had provided highest sensitivity (122.336 μA mM^{-1} cm^{-2}) towards glucose and GCE/rGO/AuNPs/ ADH composite had provided far highest sensitivity (916 μA $mM^{-1}cm^{-2}$) towards ethanol compared to other reported graphen based nanocomposites.

The wide linear detection ranges of glucose (0.1 mM-23.2 mM) and ethanol (0.01 mM-3.9 mM) achieved by MWCNT/GO/GOx and GCE/ErGO/PTH/ADH nanocomposites respectively.

Generally, graphen based nanocomposites had provided desirable analytical performance (fast direct electron transfer rate, highest sensitivity and wide linear detection range). However, the recently fabricated graphen based nano composites had one desirable analytical performance (e.g high sensitivity, poor electron transfer, poor linear detection range, vice versa).

For the future combining nanocomposites that had more desirable analytical performance may be a significant role to enhance the overall performance of enzymatic biosensors towards the detection of glucose and ethanol.

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