Utilization of graphene oxide electrophoretic deposition for construction of electrochemical sensors and biosensors

Jana Vlachova[1,2*], Jan Labuda[3], David Hynek[1,2], Ondrej Zitka[1,2] and Rene Kizek[1,2]

[1] Laboratory of Metallomics and Nanotechnologies, Department of Chemistry and Biochemistry, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic; E-Mails: vlachova.jana@centrum.cz (V.J.), d.hynek@email.cz (D.H.), zitkao@seznam.cz (O.Z.), kizek@sci.muni.cz (R.K.)

[2] Central European Institute of Technology, Brno University of Technology, Technicka 3058/10, CZ-616 00 Brno, Czech Republic;

[3] Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9 81237, Bratislava, Slovakia; E-Mails: jan.labuda@stuba.sk (J.L.)

* Author to whom correspondence should be addressed; E-Mail: vlachova.jana@centrum.cz

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This review summarizes and discusses electrophoretic methods for the fabrication deposited graphene and graphene-based structures. Graphenes are commonly dispersed in organic solvents or in water. Deposition procedures are performed mostly under constant voltage and deposition time seems to be an important parameter for influence prepared graphene structures. It was shown that electrophoretically deposited graphene layers have excellent properties suitable for electrochemical sensors and biosensors construction, e.g. high electrical conductivity and large surface area. Electrophoretic deposition enables also preparation of material which combines graphene with metal nanoparticles or polymers.

Keywords: graphene oxide, electrophoretic deposition, sensor, biosensor

1. Introduction

Graphene and graphene oxide (GO) has attracted increasing interest of many scientists because of its unique properties which find application across many fields. Enormous number of papers regarding to graphene and graphene oxide are focused on improvement of preparation of these materials, their deposition on substrates and formation of novel graphene structures which enhance detection abilities of electrodes as sensitivity and electron transfer.

This mini-review focuses on the fabrication of GO-based electrodes prepared by electrophoretic deposition (EPD) and their utilization in electrochemical sensors and biosensors.

2. Graphene oxide

Graphene oxide along with carbon nanotubes, nanofibers, fullerens, nanodiamonds and nanocomposites are important carbon-based materials [1] which have been widely used for various applications as a catalyst of chemical reactions [2], luminescent nanomaterial [3,4], nanocomposite [5-8] and drug deliver [4,9]. Especially in electrochemistry, application of graphene oxide is employed in the photovoltaic device [10], chemical sensor and biosensors [11,12] due to wide electrochemical potential window, low charge-transfer resistance and great electrochemical activity [13].

Although the structure of GO was studied by several authors, amorphous character of GO and lack of analytical technique for its characterizing make an obstacles in GO characterization. Thus, despite numerous attempts to propose precise structure of GO, an unambiguous model has not been clarified yet, only the Lerf-Klinowski model is thought to be the most acceptable [14,15]. GO is formed of monolayer of carbon atoms which creates of two-dimensional hexagonal lattice with oxygenated functionalities as a carboxylic acid group, hydroxyl and epoxy groups [15]. The consequences of oxygenated groups on surface are hydrophilicity and solubility of GO in water and other organic solvents [16]. Through these oxygenated groups

and non-covalent binding via π-π stacking, cation-π or van der Waals interactions on the sp2 networks, GO can be easily modified by diverse small molecules and polymers which lead to improvement of electrical, thermal and mechanical properties of graphene oxide [15].

The GO is commonly prepared by oxidation of graphite using various methods. Three most popular methods were proposed by Brodie [17], Staudenmaier [18] and Hummers [19]. The shortest and recently most used method is the Hummers method [20]. Preparation and functionalization of GO-based electrodes can be performed by several methods. Basic approach involves simple evaporation of GO suspension on the electrode surface. Unfortunately, layer formed using this method causes aggregation of GO and nonuniform deposition [21]. Using of spin-coater is more sophisticated method which create thin continuous layer [22]. Langmuir-Blodgett [23] and layer-by-layer [24] method are another alternative approaches mainly useful for sensors fabrication. Interesting method of GO deposition, which creates uniform layer, is vacuum filtration [25]. This method involves filtration of GO sheets suspension through porous membrane where GO sheets lodge and form a film. This film can be transferred onto electrode by gently pressing the film against electrode surface [21]. Direct pattering using for example inject printing of GO also offers opportunity how create layer of GO directly onto substrates [26]. The last approach for modification of electrodes is electrophoretic deposition which is described below.

3. Electrophoretic deposition

Electrophoretic deposition is a two-step process in which charged particles, dispersed or suspended in a liquid medium, move toward conductive substrate of opposite charge through the application of external electric field and then deposit on it [27,28]. According to the charge of deposited particles, there exist two types of EPD. When the particles are negatively charged, application of the electric field causes deposition of particles on positively charge electrode (anode). This process is called anodic EPD. Opposite to this process is EDP of positively charged particles on negatively charged electrode (cathode) which is called cathodic EPD [28].

EPD is simple short-time process which requires cost-efficient equipment and provides the possibility of scaling up to large dimensions [27]. The main factors influencing EPD, among others, are applied voltage, deposition time, distance between electrodes and properties of used solvent. Via alteration of these parameters the thickness and morphology of deposited layer can be easily controlled [28]. Due to EPD, it is possible to modify any shape of conductive surface by GO with diverse kind of molecules as metal ions [29], particles [30], carbon nanotubes [31], biopolymers [32] and so to create a compact thin film as well as complex 3D structures [13,27,28]. A disadvantage is EPD performed in an aqueous medium where the application of voltage higher then 3-4 V leads to the formation of gas bubbles because of water electrolysis. Decomposition of water can be avoided by using alternating current where the main influencing factor is frequency [33,34]. Comparison of different reports shows wide variation of EPD condition. For example, applied voltage ranges from 5 up to 300 V, deposition time ranges from few seconds to half hour and GO is dispersed in water or organic solvents. Worth noticing is that only few papers describe creation of gas bubbles during EPD from water where high voltages were applied while other authors did not mention it [27].

4. Sensors and biosensors

Various structures of pure GO or modified with metal ions, particles or biopolymers prepared by electrophoretic deposition have a wide potential for application in sensors and biosensors. High sensitivity of electrodes modified with GO allows miniaturization of detection systems into portable device. Such sensors could find usage in fields as an food quality control or clinical diagnosis [13].

4.1 Sensors for clinical diagnosis

GO-based electrodes offer a new approach for sensors used in clinical diagnosis. For example reduced GO-$Ni(OH)_2$ composite for nonenzy-

matic glucose sensing was prepared by EPD on gold electrode from mixture of reduced GO (rGO) with $NiCl_2 \cdot 6\ H_2O$ in ethanol at 50 V for 20 seconds. Ni^{2+} ions decorated negatively charged GO sheet and such positively charged rGO-Ni^{2+} sheets in electric field move to cathode and deposit on it (Figure 1). rGO-$Ni(OH)_2$ composite was accomplished by cycling in 0.1 M NaOH. Electrochemical measurement of glucose on rGO-$Ni(OH)_2$ modified electrode was based on electrooxidation of $Ni(OH)_2$ on NiOOH in alkaline solution following oxidation of glucose to gluconic acid while NiOOH reduce back to $Ni(OH)_2$ [35].

Another possibility of utilization of graphene material as graphene nanowalls is for DNA

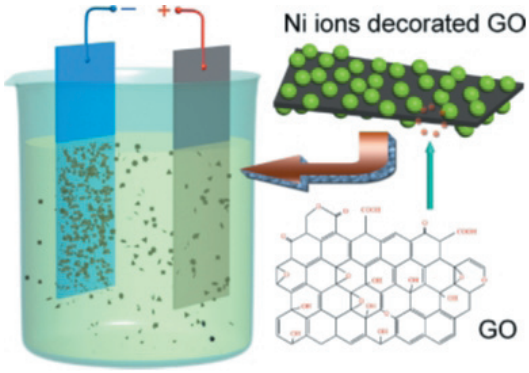

Figure 1: Schema of EPD of GO-Ni^{2+} sheets. Ni^{2+} ions decorated negatively charged GO sheet and such positively charged rGO-Ni^{2+} sheets in electric field move to cathode and deposit on it. (Reprinted with permission from ref 8. Copyright 2013 American Chemical Society.)

xture of GO and $Mg(NO_3)_2 \cdot 6\ H_2O$ on the similar principle as was written above. EPD was carried out in aqueous suspension of GO-Mg^{2+} sheets at 30 V for 10 minutes and such modified electrode was subsequently reduced by hydrazine vapor for 1 hour. An Electrode modified by the graphene nanowalls was able to detect all four free bases simultaneously even bases bounded in ssDNA and dsDNA in femtomolar concentrations. Due to high sensitivity, authors were able to detect single-based mismatch in sequence of DNA [36].

Later this modification was used for the determination of leukemia and normal blood cells based on an electrochemical detection of the guanine oxidation. It was found that the current signal of guanine was significantly higher in the case of leukemia cells than in normal cell and, moreover, the peak position of leukemia cells was observed at lower potential 0.61 V than in normal cell 0.76 V [37]. Previous study of free and bounded guanine showed the position of peaks at 0.57 V and 0.71 V, respectively [36]. The authors concluded that the higher signal and shift of potential to lower values for leukemia cells could be consequence of increased concentration of free guanine in electrolyte. This increased concentration could be caused by penetration of extremely sharped edges of graphene nanowalls into the cells and release of overexpressed free guanine from

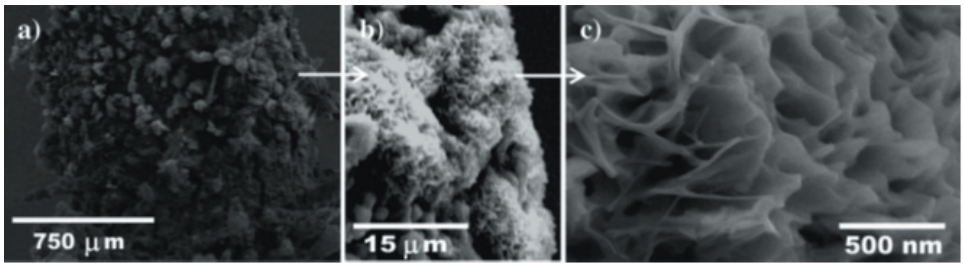

Figure 2: Images of electrode modified by graphene nanowalls obtained using field-emission scanning electron microscopy working at 15 kV. (Reprinted with permission from ref 36. Copyright 2012 American Chemical Society.)

sensors (Figure 2). Graphene nanowalls were created by EPD on graphite electrode from mi-

cancer cells [37]. Possible indicator of renal as well as liver diseases is increased level of

urea in serum, blood or urine. For urea sensing, the mesoporous silica particle embedded GO platform was created. EPD on indium tin oxide (ITO) glass substrate was performed at 120 V for 2 minutes in mixture of SiO_2 particles and GO in acetonitrile and with addition of $Mg(NO_3)_2 \cdot 6 H_2O$ as an electrolyte and to ensure creation of surface charge on the GO. After EPD, the immobilization of urease and glutamate dehydrogenase (GLDH) enzymes was carried out to create biosensor for urea sensing. The electrochemical detection of urea was performed in the presence of nicotinamide adenine dinucleotide (NADH) and α-ketoglutarate using cyclic voltammetry in phosphate buffer saline containing $[Fe(CN)_6]^{3-/4-}$. Increased concentration of urea triggers system lead to the decomposition of urea by urease, then electrons are transfered to α-ketoglutarate which is transformed to 2-L-glutamete by GLDH. Finally, 2-L-glutamete reduces $[Fe(CN)_6]^{3-}$ to $[Fe(CN)_6]^{4-}$ which is oxidized back on the electrode surface (Figure 3) [30].

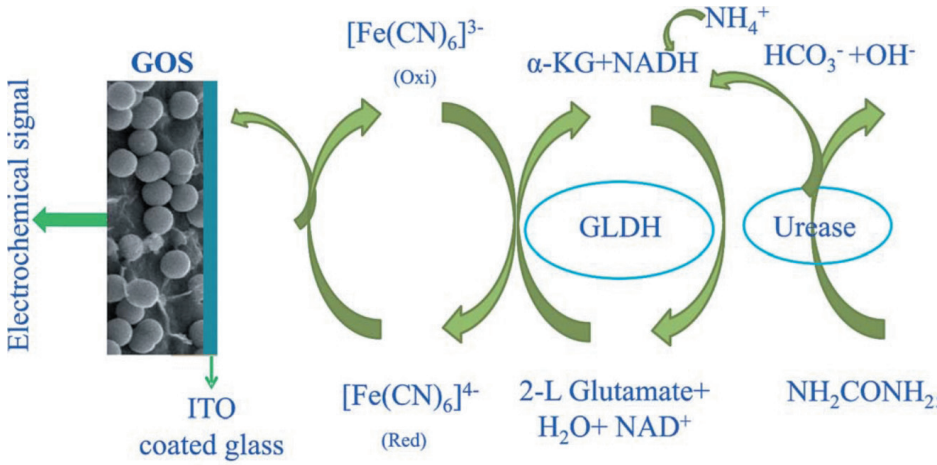

Figure 3: Schema of urea sensing using GO-based bioelectrode. Electrons from urea decomposition are transferred via GLDH, α-ketoglutarate and $[Fe(CN)_6]^{3-}$ to electrode surface. (Reprinted from ref 30.)

4.2 Sensors for food analysis

The graphene-based sensors can be used in food analysis as well as in clinical diagnosis. These sensors can be utilized for detection of nutrients or food toxins. Quercetin is natural flavonoid which plays important role in medical and nutritional science because of its antioxidant activity, cardiovascular protection and anti-inflammatory activity. Due these properties, detection of quercetin has become conscious of increased interest. Such sensor for quercetin was prepared by EPD at 1.7 V from aqueous solution of graphene with addition of KCl on glassy carbon electrode and after modified with β-cyclodextrin by electro-polymerization. Detection of quercetin using such modified electrode was studied in real samples of tea and honeysuckle [32].

For food quality control, the detection of food toxin is very important. Alfatoxin B1 is secondary fungal metabolite produced by Aspergillus flavus and Aspergillus parasiticus which is responsible for human hepatocellular carcinoma. The utilization of graphene oxide could be useful platform for creation of immunosensor for alfatoxin B1 detection. For example, electrode modified with rGO-Mg^{2+} sheets using EPD at 60-70 V for 2 minutes was functionalized with monoclonal antibody for alfatoxin B1 and then utilized for the electrochemical sensing studies of alfatoxin [38,39].

Overview of EPD conditions for modifications of electrode by GO described above is shown in Table 1.

GO modification	solvent	voltage	time	application	reference
rGO-$Ni(OH)_2$	ethanol	50 V	20 s	glucose detection	[35]
rGO-Mg^{2+} sheets	water	30 V	10 min	DNA detection	[36]
rGO-Mg^{2+} sheets	water	30 V	10 min	leukemia cell detection	[37]
rGO	water	150 V	20 s	lysozyme detection	[40]
GO-SiO_2 particles	acetonitrile	120 V	2 min	urea detection	[30]
GR/β-cyclodextrin	water	1.7	–	quercetin detection	[32]
rGO-Mg^{2+} sheets	acetonitrile	60-70 V	2 min	aflatoxin B1 detection	[38,39]

5. Conclusions

This review summarizes the utilization of GO-based electrodes prepared by the electrophoretic deposition for application in sensors and biosensors. The electrodes for such application can be modified by pure graphene oxide, graphene oxide with SiO_2 particles or biopolymers. However, the most often modification of the GO-based electrodes used in sensors and biosensors is the modification by metal ions. Electrophoretic deposition of such GO-M+ sheets leads to the formation of 3D structures of graphene oxide which cause an increase of the active surface of electrode and improve an electron transfer from analyte to the electrode surface which result in significant increase of the sensitivity.

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Conflicts of Interest

State any potential conflicts of interest here or "The authors declare no conflict of interest".

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform requirements" for biomedical papers.

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