Carbon Nanostructures for Electrochemical Sensors

Lina Mikoliunaite, Mingaile Geceviciute, Jaroslav Voronovic, Ieva Paklonskaitė, Jurgis Barkauskas Almira Ramanaviciene, Arunas Ramanavicius
Faculty of Chemistry and Geosciences,
Vilnius University, Vilnius, Lithuania.
lina.mikoliunaite@chf.vu.lt

***Abstract*— Determination of analytes (e.g. glucose) in complex samples (e.g. blood) is one of the challenging task of recent bio-analytical chemistry. For this purpose various kinds of sensors are applied, however, the simplest and cheapest sensors, and thus the most widely exploited, are electrochemical enzymatic biosensors. However, these sensors still has number of limitations to overcome in order to demonstrate their practical applicability. One of them is an effective electron transfer from the active center of the redox enzyme to the electrode surface. In this work carbon-based nanostructures have been applied in the design of the electrodes and the parameters, such as an optimal enzyme concentration, the type of the coating and the electrode selectivity have been evaluated.**

Keywords—biosensor; glucose; carbon nanotubes; nanomaterials; sensor.

I. Introduction

Recently, a lot of efforts has been focused on the development of fast and reliable methods for the determination of glucose concentrations. Such sensors are extremely important in many fields such as clinical diagnostics, food industry and biotechnology [1, 2, 3, 4]. Among many existing detection principles, electrochemical analysis has been widely used due to several advantages: (1) low detection limit, (2) high sensitivity and (3) selectivity [1, 3, 4, 5]. For this kind of analysis enzyme based sensors are applied. The most popular and widely used enzyme for such a sensor is Glucose oxidase (GOx) which converts glucose in the presence of oxygen to hydrogen peroxide and gluconolactone. Later hydrogen peroxide, which is formed during the reaction, could be registered amperometrically. Other zproach is based on application of advanced redox system, which is accepting electrons directly from the redox centre of GOx [4]. However in this kind of biosensors, the immobilization of the enzyme and the construction of highly effective electrical communication among the redox center of enzyme and electrode still remain a challenge [6, 7, 8].

Carbon is very well known for a long time, however, only recently various kind of nanomaterials has been discovered and applied for various applications. Carbon nanomaterials (CNMs) (such as fullerenes, single and multi-walled nanotubes graphite) are also widely used in biosensors as an excellent signal transducer component. They have diverse and robust electronic properties. In addition to this, tunable surface chemistry opportunities are widely expressed and used. The application of CNMs in biosensor design in the form of quantum dots, carbon nanotubes (CNTs), graphene, graphene oxide (GO), and the like has been reported [9]. The electrical activity and increased conductivity of CNMs are well exploited in biosensors. However there is still a necessity to understand more precisely the interaction between the protein and CNM surface.

Graphene, GO and CNTs are the most widely used CNMs in sensor applications. All of these materials has their own advantages and limitations, which are related to their properties and structural characteristics. For example, graphene exhibits potential advantages of low cost and higher purity from heavy metals in the case, when it is produced using GO as a precursor, if to compare with CNTs. Nevertheless, the cost of epitaxial grown graphene is comparable to that or even higher as CNTs. What is more, the defect-free graphene owns an excellent electrical conductivity, which significantly decreases in the case of reduced graphene oxide (rGO), or especially, GO. On the other hand, graphene do not have much of the functional groups on the surface what makes some difficulties for enzyme immobilization. The surface of CNTs is more active, both at the sidewalls and, especially, at the tips. GO and rGO are useful platforms for the enzyme immobilization, if the procedures are adjusted to maintain the enzyme activity.

In this work carbon nanostructures were applied for the design of glucose biosensors in order to increase the surface activity, conductivity and electron transfer to the carbon electrode. For this reason the CNT modified flexible polycarbonate membrane was used and the analytical properties of the system were analyzed.

II. Materials and Methods

A. Chemicals

All chemicals were purchased from global suppliers and of analytical grade. D-(+) glucose, N-methylfenazin methosulphate (FMS), Glutar aldyhyde, enzyme Glucose oxidase (from *Aspergilus Niger*, activity 215,266 U/mg) were obtained from Applichem, Germany. D-(+) galactose, D-(+) xylose and D-(+) mannose were purchased from Roth, Germany.

Acetate/phosphate buffer (A-PBS) was prepared in distilled water, containing 50 mM $Na_2HPO_4 \cdot \times 12H_2O$, 50 mM $NaH_2PO_4 \cdot \times H_2O$ and 50 mM CH_3COONa. pH value was adjusted using CH_3COOH, HCl or NaOH.

978-1-5386-2810-2/17/$31.00 ©2017 IEEE

The 1.0 M solutions of glucose were prepared in distilled water at least 24 h before use to allow glucose to mutarotate and to reach equilibrium of α- and β- forms. 1.0 M solution of galactose, xylose and mannose were prepared in distilled water as well.

40.0 mg/ml and 10.0 mg/ml GOx solution was prepared in acetate-phosphate buffer pH 6.0.

40.0 mg/ml FMS solution was prepared in deionized water.

B. Preparation procedures

Preparation of CNT suspension. 0.05 g of Single-walled carbon nanotubes (SWCNT) powder (Cheap Nanotubes Inc., outer diameter 1 – 2 nm, inner diameter 0.8 – 1.6 nm, length 5 – 30 nm, purity > 90%, electrical conductivity > 0.01 S/cm), 0.1 g of Tween-80 and 25 mL of distilled water was mixed and left for 12 h. Later, the suspension was sonicated for 3 h (a desintegrator VCX 130 PB, SONYCS VibraCell) at 12 % sonication amplitude and afterwards diluted up to 100 mL, and sonicated again for 3 h at 15 % amplitude. Concentration of the prepared SWCNT stock suspension was equal 5.0 10^{-4} g/mL. The concentration of working suspension was 5.0 10^{-5} g/mL.

Preparation of GO suspension. 0.05 g of GO synthesized in the laboratory and 10 mL of distilled water was mixed in a small beaker and left for swelling in a shaker at the RT for 24 h. Later the mixture was sonicated for 1 h. Prepared suspension was diluted up to 100 mL and sonicated again for 1 h. 1.00 mL of prepared suspension was diluted in a measuring flask up to 100 mL, poured into a beaker and sonicated for 1 h. Obtained working suspension of GO (5.0 10^{-6} g/mL) was used for the preparation of SWCNT-rGO coatings.

Preparation of SWCNT-rGO coatings. SWCNT coatings were prepared on the polycarbonate membrane filter (Merck Millipore Ltd., pore diameter 400 nm). The preparation procedure is described in more detail elsewhere [10]. The average thickness of prepared SWCNT coating was equal 400 nm. Then attachment of GO flakes on the top of SWCNT coating was carried out from the aqueous GO solutions using the same filtering procedure. An aliquot of GO working suspension (12 mL) was filtered through the SWCNT coating. The average thickness of GO layer on top of SWCNT coating was obtained equal to 20 nm. SWCNT-GO coatings were annealed up to 160 °C in an oven (SNOL 8,2/1100 AB Utenos elektrotechnika, Lithuania) in order to obtain better adhesion of GO nanoplatelets to the SWCNT layer (SWCNT-rGO coatings).

Preparation of the electrodes. Graphite rod electrode (3.0 mm diameter; 30 mm length, 99.999%, Sigma-Aldrich, Germany) was polished up to shining surface with rough and later with smooth emery paper and finally with paper. Then electrode was covered with SWCNT-rGO coating and sides were isolated with the plastic tube. Several different coating techniques were chosen for the modification: coating was placed as obtained conducting side on top, conducting side inside, and crumbled. Different amount of GOx was dropped on the electrode. Each next drop was added only after the previous one dried. Then the modified electrode was stored for 15 min over a 25% solution of glutaraldehyde in a closed vessel. For comparison electrode without SWCNT-rGO coating was prepared the same, as described previously, only omitting the coting step.

Measurements. For the analysis of biosensor the potentiostat/galvanostat PGSTAT 30 (Autolab, Netherlands) equipped with reference $Ag/AgCl_{3M\ KCl}$ and counter platinum electrodes was used. In the beginning of the measurement the 20ml cell was filled with 4750 µl of A-PBS and 250 µl of FMS. All the measurements were performed at +0.3 V working potential *v.s.* $Ag/AgCl_{3M\ KCl}$. Later, during the measurement, glucose solution was added to the cell. The obtained amperometric signals were interpreted using Michaelis-Menten kinetics.

III. Results and Discussion

In these experiments, firstly the optimal concentration of enzyme glucose oxidase was determined by comparing two different concentrations: 40 mg/ml and 10 mg/ml. Later the effect of SWCNT-rGO coating was evaluated and finally, the selectivity of the composed sensor to various saccharides was evaluated.

A. Enzyme concentration effect

The enzyme concentration effect was evaluated using three electrode cell at +0.3 V working potential v.s. $Ag/AgCl_{3M\ KCl}$ electrode. For this reason, a polished carbon electrode was covered with SWCNT-rGO coating and two different techniques for covering of the electrode surface by the enzyme was evaluated. On the first electrode 3 µl of 40 mg/ml concentration enzyme solution was dropped and allowed to dry. On the second electrode 3 times 3 µl of 10 mg/ml concentration enzyme solution was dropped and after each drop the electrode was left to dry. Later the procedure with GA, described in the Methods part, was the same for both electrodes. The amperometric signal was evaluated outright and after 20 days. Between the measurements electrodes were stored at +4 °C under the A-PBS drop.

The obtained results showed that after the preparation both electrodes had similar parameters (K_M and I_{max})(TABLE I.). However, after 20 days the electrode with smaller concentration of initial enzyme solution possessed a larger Michaelis-Menten constant. This means that the higher concentration of the enzyme initial solution placed on the electrode is less stable, or that it was washed from the surface easier than a smaller concentration. For this reason, for the following experiments the modification of the electrode with smaller initial enzyme concentration was chosen.

TABLE I. KINETIC PARAMETERS OF THE ELECTRODES WITH DIFFERENT AMOUNT OF ENZYME GLUCOSE OXIDASE. THE SIGNAL WAS EVALUATED OUTRIGHT AND AFTER 20 DAYS.

Concentration and measurement time	Parameters		
	$K_{M(tar)}$, mM	I_{max}, µA	R^2
40 mg/ml, 1st day	16,0	143,8	0,9815
10 mg/ml, 1st day	18,4	140,8	0,9758
40 mg/ml, 20th day	15,9	121,0	0,9797
10 mg/ml, 20th day	26,1	136,4	0,9752

B. *Effect of SWCNT-rGO coating*

The polycarbonate membrane modified with SWCNT-rGO has two differently modified sides. One side is heavily covered with SWCNTs and GO on top. This side is called the conductive side and the other side has only few SWCNTs crawled out from the pores of the membrane. This side is called the nonconductive side. A polished carbon electrode was modified with this membrane in two different ways: on the first electrode, the membrane is placed with conductive side on top and nonconductive side in the contact with electrode (GR /SWCNT/GOx). On the second electrode, the membrane was turned halfway leaving the nonconductive part inside and then placed on the electrode (GR/2SWCNT/GOx). All the other modification procedures were the same. For the control measurements, the electrode without the membrane (GR/GOx) and the electrode with the membrane turned halfway and conductive part inside were prepared (GR/NON-SWCNT/GOx).

From the obtained results, it was noticed that GR/NONSWCNT/GOx showed almost no current (Fig. 2). For the other three electrodes, the obtained amperometric values were very similar. The GR/SWCNT/GOx electrode had slightly higher maximal current value (up to 8%) in comparison to the other electrodes and GR/2SWCNT/GOx showed the smallest K_M value, which shows the fast electrode response time. Taking into account the obtained results, for the following measurements the GR/SWCNT/GOx electrode was used.

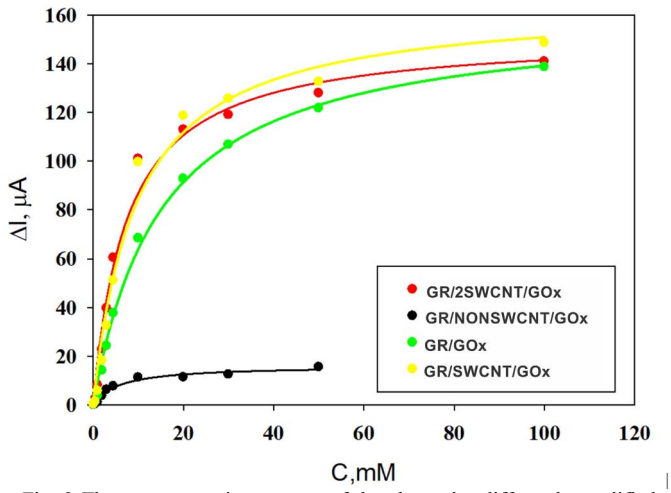

Fig. 2 The amperometric response of the electrodes differently modified with polycarbonate membrane having SWCNT-rGO coating.

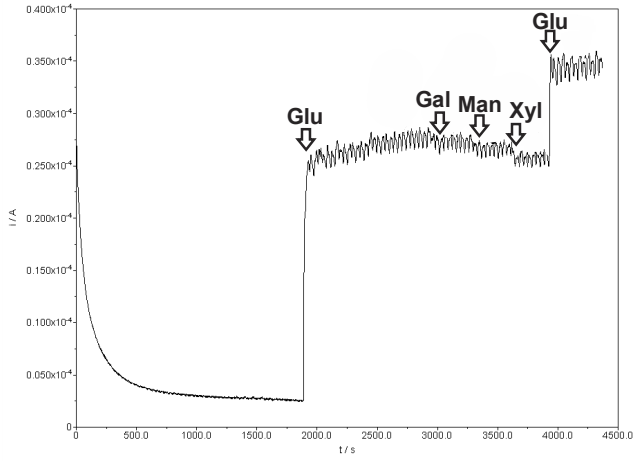

Fig. 1 Selectivity of the electrode to glucose (Glu), mannose (Man), xylose (Xyl) and galactose (Gal).

C. *Selectivity of the electrode*

The selectivity of the electrode was evaluated using several sugars: glucose, galactose, xylose and mannose. Firstly glucose was added to the solution, then galactose, mannose and xylose (40 mM each) and finally again glucose (132 mM). The obtained results demonstrated, that the electrode selectively responded to glucose, but no signal increase was registered after addition of other sugars (Fig. 1).

IV. CONCLUSIONS

In this study it has been showed that the polycarbonate membrane with SWCNT and reduced graphene oxide layer do not reduce the effectiveness of electron transfer from the enzyme active center towards the electrode. The electrode with the membrane and without shows similar electrochemical response to glucose. For this reason it is expected, that such SWCNT-rGO coatings modified with enzymes without carbon black electrode in the future could potentially be used as a flexible glucose biosensor.

ACKNOWLEDGMENT

This research was funded by a grant (No. SEN-21/2015) from the Research Council of Lithuania.

REFERENCES

[1] S. Hui, J. Zhang, X. Chen, H. Xu, D. Ma, Y. Liu, B. Tao, Study of an amperometric glucose sensor based on Pd-Ni/SiNW electrode, Sens. Actuators B Chem. 155 (2011) 592-597.

[2] F. Wang, J. Yao, M. Russel, H. Chen, K. Chen, Y. Zhou, B. Ceccanti, G. Zaray, M.M.F. Choi, Development and analytical application of glucose biosensor based on glucose oxidase/o-(2-hydroxyl) propaly-3-trimethylammonium chitosan chloride nanoparticle-immobilized onion inner epidermis, Biosens. Bioelectron. 25 (2010) 2238-2243.

[3] Y. Oztekin, V. Krikstolaityte, A. Ramanaviciene, Z. Yazicigil, A. Ramanavicius, 1, 10-Phenanthroline derivates as mediators for glucose oxidase, Biosens. Bioelectron. 26 (2010) 267-270.

[4] Y. Oztekin, A. Ramanaviciene, Z. Yazicigil, A.O. Solak, A. Ramanavicius, Direct electron transfer from glucose oxidase immobilized on polyphenanthroline-modified glassy carbon electrode, Biosens. Bioelectron. 26 (2011) 2541–2546.

[5] A. Kausaite, A. Ramanaviciene, A. Ramanavicius, Polyaniline synthesis catalysed by glucose oxidase, Polymer 50 (2009) 1846-1851.

[6] N. German, A. Ramanavicius, J. Voronovic, A. Ramanaviciene, Glucose biosensor based on glucose oxidase and gold nanoparticles of different sizes covered by polypyrrole layer. Colloids and Surfaces A: Physicochemical and Engineering Aspects 413 (2012), 224– 230.

[7] Q. Gao, Y. Guo, J. Liu, X. Yuan, H. Qi, C. Zhang, A biosensor prepared by co-entrapment of a glucose oxidase and a carbon nanotube within an electrochemically deposited redox polymer multilayer, Bioelectrochemistry 81 (2011) 109-111.

[8] U. Bilitewski, Protein-sensing assy formats and devices, Anal. Chim. Acta 568 (2006) 232-247.

[9] F.R. Baptista, S.A. Belhout, S. Giordani, S.J. Quinn, Recent developments in carbon nanomaterial sensors, Chem. Soc. Rev. 44 (2015) 4433.

[10] J. Barkauskas, pH-dependent water penetration through CNT sub-layers arranged on the polycarbonate membrane filters, Carbon 48 (2010) 1858-1865.