Journal of New Materials for Electrochemical Systems 19, 145-150 (2016)

High Sensitivity Electrochemical Multisensors based on Graphene-PANI Nanocomposite for Simultaneous Detection of Glucose and Urea

Meysam Karimi[1], Mohammad Rabiee[1], Mojgan Abdolrahim[1], Mohammadreza Tahriri[1,2,*], Daryoosh Vashayee[3] and Lobat Tayebi[2,4]

[1]Biomaterials Group, Faculty of Biomedical Engineering, Amirkabir University of Technology, Tehran, Iran
[2]Marquette University School of Dentistry, Milwaukee, WI, 53233, USA
[3]Electrical and Computer Engineering Department, North Carolina State University, Raleigh, NC 27606, USA
[4]Department of Engineering Science, University of Oxford, Oxford OX1 3PJ, UK

Received: April 22, 2016, Accepted: July 30, 2016, Available online: September 07, 2016

Abstract: *We present a study of the effect of graphene–PANI nanocomposites on the sensitivity of the urea and glucose multisensory. We used an electroctrochemical multisensor based on two electrodes located in a reservoir with two separate channels. The urease and glucose oxidase (GOD) were employed for detecting the urea and glucose, respectively. We characterized the graphene and graphene-PANI samples with X-ray Diffraction (XRD) analysis and scanning electron microscopy (SEM) observations. We further performed the Cyclic voltammetry and Amperometry tests. The collected experimental results revealed that the intensity of the peak significantly increases with the concentration of the urea and glucose.*

Keywords: Multisensor, Graphene, PANI, Glucose, Urea, Simultaneous

1. INTRODUCTION

The biosensors have been important in analytical science recently. They are used in environmental analysis, clinical diagnostics, and food technology [1-9]. New Biosensors need fewer chemicals than older methods and a preparing of the samples can almost be eliminated. Enzymatic sensors are compact and have small response time and so there are more suitable for fast and simple measurements in the field [10]. Multisensors are attractive and beneficial since they enable measurement of several elements simultaneously in a single sample [11]. Today one of the most occurring causes of death and disability worldwide is diabetes [12]. Therefore, the glucose quantitative monitoring is very important in clinical applications for decreasing the risk of diabetes mellitus which can lead to heart disease, kidney failure, and sometimes blindness [13, 14]. Insulin deficiency causes metabolic disorder, and the blood glucose concentrations higher than the normal range of about 3.9–6.2 (empty stomach) or 3.9–7.8 (2 h after food) mM causes hyperglycemia [15]. Furthermore, another important factor that is vital to be monitored is the Uric Acid (UA). Uric acid is the major end product of purine metabolism. Extraordinary amount of UA are symptoms of several diseases, like hyperuricemia, gout, and Lesch-Nyan disease [16]. Today, biosensors based on optical and electrochemical method have been extensively developed for glucose and Urea detection [17-19]. The first proposed glucose sensor was developed by Clark and Lyons in 1962 [20]. Electrochemical methods, especially amperometric methods, have been widely used in glucose detection. The two main categories of glucose sensing which have been widely investigated and developed are the non-enzymatic and the enzymatic biosensing [21, 22] . In the recent decade, the electrochemical methods for glucose detection have attracted because of their simplicity of fabrication process and application, low cost and excellent performance. In typical electrochemical methods, very small volumes of samples can be used as modern microelectronics allows building microelectrodes; hence, the sensitivity of the sensor is not affected during the measurements. In recent years, many matrix materials were developed for improving the sensitivity and selectivity of glucose biosensors such as cellulose acetate, conductive polymers, as well as carbon materials such as carbon nanotubes (CNTs), graphene, etc.

*To whom correspondence should be addressed:
Email: Mohammadreza.tahriri@marquette.edu
Phone:

2. EXPERIMENTAL PROCEDURE

2.1. Reagent and materials

Ammonia solution (28 wt %), aniline, graphite, ammonium peroxydisulfate (APS), sulfuric acid, hydrochloric acid, ethanol and hydrogen peroxide, hydrazine solution (50 wt %) as reducing agent, potassium permanganate as oxidizing agent and glucose oxidase (GOD) from Aspergillus niger with an activity of 100 U/mg and d-(+)-glucose were obtained from Sigma Aldrich. Phosphate buffer solutions (PBS) were produced by mixing the stock solutions of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4, and then adjusting the pH with H_3PO_4 or NaOH. The glucose stock solution was produced by 0.1 M pH 7.0 PBS and was permitted to mutarotate at ambient temperature overnight before using. All other chemicals were of analytical grade and were employed as received without any purification process. Deionized double-distilled water (18.6 MΩ) (Millipore Co. Ltd.) was employed throughout the experiment.

2.2. Preparation and reduction of graphite oxide (GO)

Graphite oxide (GO) was fabricated by following modified Hummers technique [23]. In this method, graphite (2.0 g) was mixed with 46 mL of H_2SO_4 (95%) and the mixture was stirred for 45 min in an ice bath. Potassium permanganate (6.0 g) was added very slowly in the suspension with vigorous stirring while maintaining a reaction temperature of 20 °C. Subsequently, the ice bath was eliminated, and the reaction mixture was stirred overnight at 35 °C. In the next step, deionized water was added to the pasty solution with constant agitation. Thereafter, the color of the solution changed to yellowish brown. After 2 h of vigorous stirring, 50 mL of 30% H_2O_2 was added and the color turned immediately to yellow. In order to get acid free solution, the mixture was washed several times with 5% HCl and then deionized water. Then the reaction mixture was filtered and dried under vacuum at 65 °C. 0.1 g of GO was dispersed in 50 mL of deionized water for conducting the reduction. Then, 1 mL of hydrazine monohydrate was added to the mixture and heated at 95 °C overnight. After the reaction was completed, the reduced GO was collected by filtration as a black powder. The obtained powder was washed with deionized water several times to eliminate excess hydrazine, and the final powder was dried in a vacuum oven at 75 °C for one day.

2.3. Preparation of Graphene–PANI modified electrodes

The Graphene–PANI nanocomposite was chemically synthesized by oxidative polymerization of aniline using ammonium peroxydisulfate [$(NH_4)_2S_2O_8$)] under controlled conditions. The purified aniline was dissolved in 1M HCl at a concentration of 0.3 M graphene oxide dispersed in the obtained solution by bath-sonicating for 1 h. During vigorous stirring of the solution at ambient temperature, another solution of ammonium peroxydisulfate, with the mole ratio to aniline of 1:4, in 1 M HCl was rapidly poured into that the solution. Ammonium peroxydisulfate (0.025 M) was also dissolved in 20 mL of 1 M HCl solution, and the reaction was continued for one day. The green product of the graphene oxidase–PANI nanocomposites obtained from the reaction vessel was filtered and washed with deionized water, methanol, acetone, and diethyl ether for the removal of the low molecular weight polymer and oligomers. Subsequently, chemical conversion of graphene oxide to reduced graphene oxide was carried out with regard to the reported method. 0.1 mL of hydrazine monohydrate was added and the mixture was heated at 95 °C for 1 h. While the reaction was completed, the color of Graphene-PANI nanocomposite became black. In order to eliminate the excess hydrazine, the obtained product was washed with deionized water several times and eventually it was dried in a vacuum oven at 60 °C.

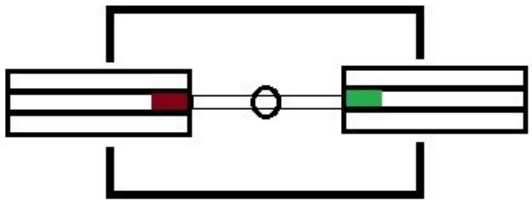

Figure 1. The schematic of two electrodes that is covered with urease (left) and GOD (right)

2.4. Preparation of the amperometric glucose biosensors

In this research, the stable dispersion of graphene-PANI in aqueous solution was fabricated. For this purpose, 4 mg of grapheme-PANI was added into 2 mL of solution to form a homogeneous dispersion through ultrasonication. 50 µL of 4 mg/mL of GOD was mixed in 0.02 M PBS (pH 7.0), afterward, 50 µL of 2 mg/mL of graphene-PANI suspension was added to it, and then the mixture was sonicated vigorously for about 30 min at ambient temperature for GOD adsorption. Au electrode was modified by deposition of 5 µL of the above achieved solution on its center. The PANI/GOD biosensor was fabricated following a similar process but without the presence of graphene.

For the fabrication of the multisensore, we needed to make another electrode. For this purpose, we used 50 µL of 2 mg/mL urease with the same mediator composition at another electrode. In order to increase the accuracy, we made a reservoir with a specific capacity for each electrode. It is worth mentioning that the volume of the reservoir for the two electrodes was equal. A schematic of the two electrodes with their reservoir is shown in Fig. 1.

2.5. Characterization and Electrochemical measurements

In order to find the composition of the prepared samples, X-ray diffraction (XRD) patterns were employed at room temperature using an INEL Equinox3000 (Cu-Ka radiation) operating at 40 kV voltage and and 30 mA current. The prepared samples were evaluated in the 2θ angle range of 10–100◦. The samples were coated with a thin layer of gold by sputtering (EMITECH K450X) and then the microstructure of the samples was inspected using scanning electron microscope (SEM; Tescan Vega 2XMU) operated at an acceleration voltage of 15 kV. The electrochemical measurements were carried out by potentiostat via a conventional two-electrode system. A platinum wire and a saturated calomel electrode (SCE) were used as working counter and reference electrodes, respectively. The working electrode was prepared by deposition of graphene-PANI nanocomposite on the electrode. For the

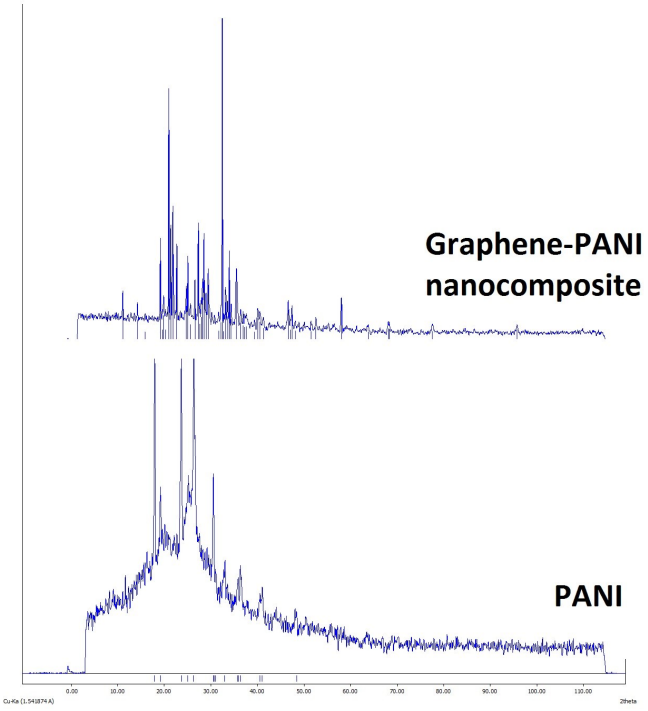

Figure 2. XRD patterns of PANI, grapheme-PANI nanocomposite samples.

amprometric test, we employed two amprometric systems simultaneously.

3. RESULTS

3.1. XRD analysis

Fig. 2 shows the X-ray diffraction patterns of the PANI (2b) and GO/PANI (2a) nanocomposite, respectively. The strongest peak at 2h= 14.1 corresponds to the (0 0 1) diffraction peak of GO, which is a typical layered material (see Fig. 2, trace a). The 2h value corresponds to an interlayer spacing (Ic) of 0.628 nm. Upon intercalation, the (0 0 1) diffraction peak for GO/PANI nanocomposite shifts to a lower angle, and the Ic of GO/PANI nanocomposite increased to 1.11 nm from 0.628 nm of GO (Fig. 2, trace b). The 0.48 nm interlayer expansion is the result of removing one layer of H2O (approximately 0.28 nm) and inserting a single layer of GO/PANI nanocomposite. Therefore, the net expansion due to an intercalated monolayer of PANI corresponds to 0.76 nm and is comparable to that found in most conjugated polymer-intercalated composites, in which parallel polymer chains lie ordered between the host sheets [12, 24]. The XRD result also indicates that the chains of PANI with extended-chain conformation exist in a dimensionally confined environment (1.11 nm), thus GO/PANI composite is a nanocomposite. We hypothesize that the driving force of the reassembling should be the hydrogen bond formation between –NH,@N– and –OCH3 groups of PANI and the oxygen functional groups of the GO layers.

3.2. SEM observations

Fig. 2 shows the SEM images of graphene–PANI nanocomposites comparison to PANI. It can be seen that the morphology of the graphene–PANI nanocomposites differs entirely from that of pristine PANI. The graphene composite has close grained sheet morphology (Fig. 2b). On the other hand, the PANI has a sphere agglomeration appearance (Fig. 2a). These results also indicate that the nanocomposite structure has been formed by our method.

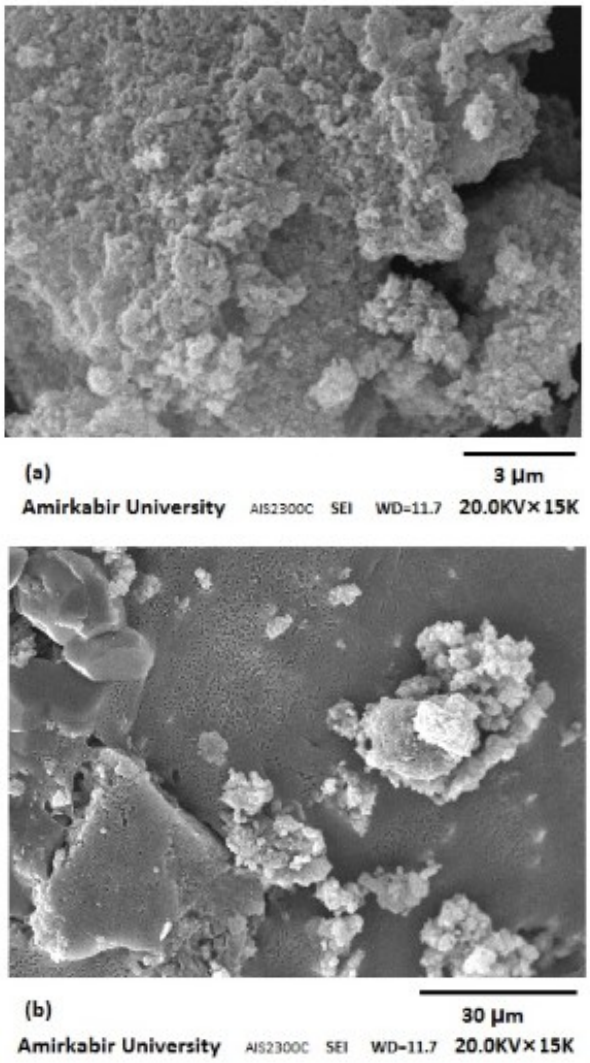

Figure 3. Scanning electron microscopy (SEM) of PANI (a), grapheme-PANI nanocomposite (b) samples

3.3. Amperometric measurements

It is known that the normal range of the urea is changed from 15 to 40 mg/dl and this range for glucose is from 50 to 100 mg/dl. Therefore, we performed our test for two different scenarios: one with a solution of urea 40 mg/dl and glucose 100 mg/dl and the other one for a solution of urea 15 mg/dl and glucose 50 mg/dl. The results are shown at figures 4.

As indicated by these figures, we can see that the intensity of the peak is significantly increased when the concentration of the urea and glucose is increased.

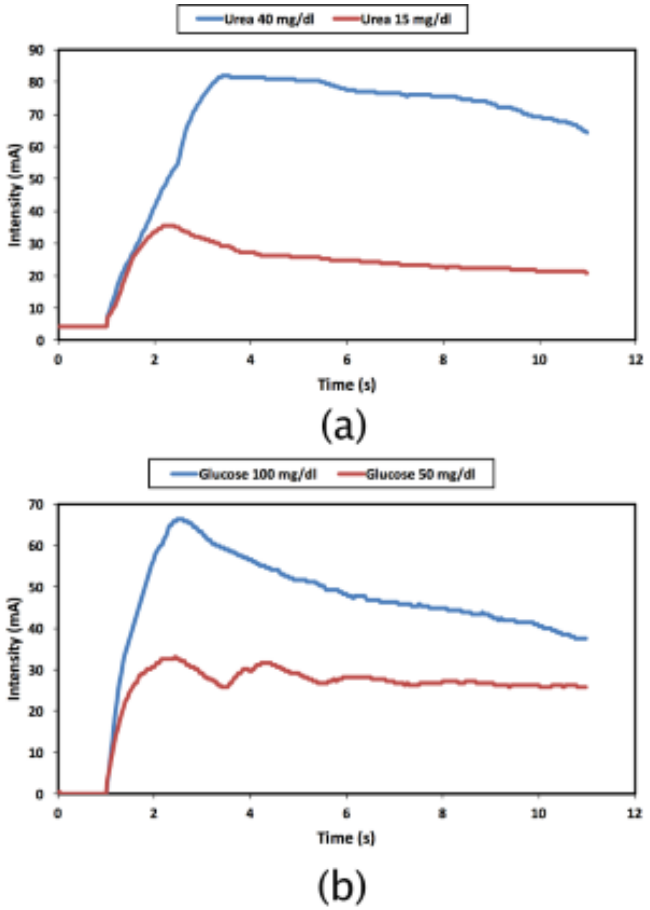

Figure 4. The comparison between amprometric tests of two different solutions; (a) urea 40 mg/dl and urea 15 mg/dl and (b) and glucose 50 mg/dl and glucose 100 mg/dl solution

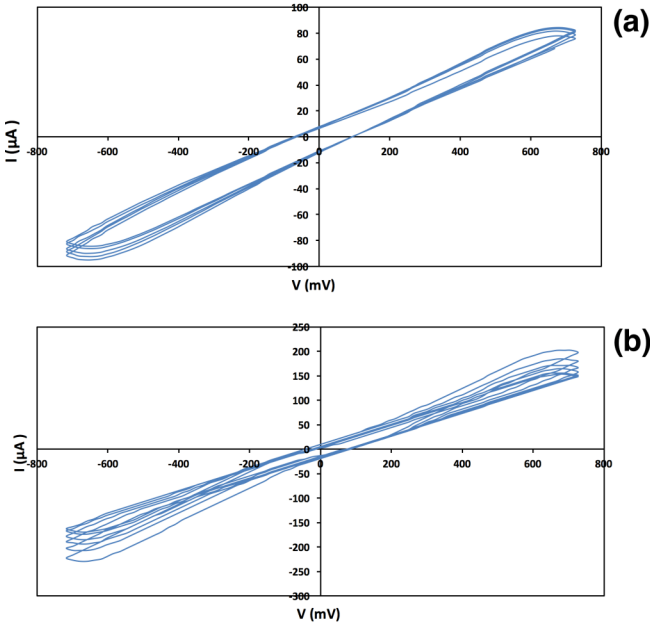

Figure 5. The cyclic voltammetry test of solution of (a) urea15 mg/dl and (b) glucose 50 mg/dl

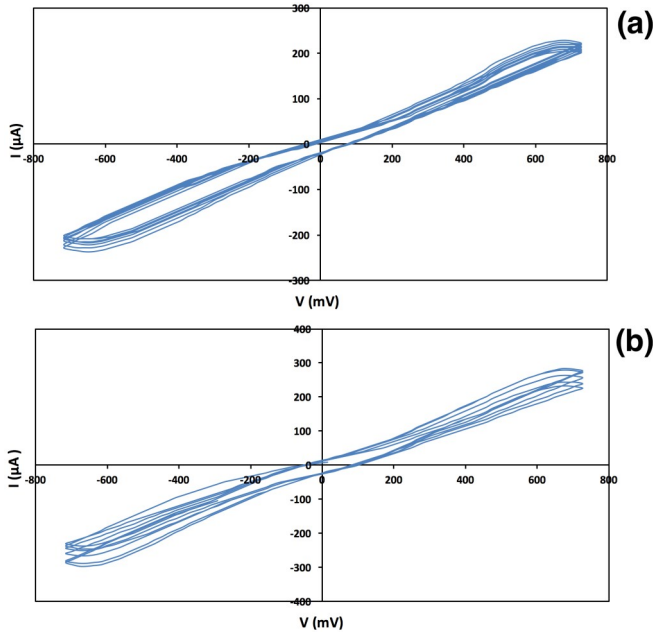

Figure 6. The cyclic voltammetry test of solution of (a) urea 40 mg/dl and (b) glucose 100 mg/dl

3.4. Cyclic voltammetry of the biosensors

We performed the cyclic voltammetry test for the two strips in the reservoir setup similar to the previous test. The results are shown at figures 5 and 6.

It is shown that when we used the higher concentration of urea and glucose, the current intensity is increased. Therefore, it can be concluded that the density of the free electrons as the end product of the interaction between the enzyme and the analyte is intensified.

4. DISCUSSION – GRAPHENE AND PANI APPLICATIONS

Recently, functional nanostructured materials and their applications have received considerable attentions because of several remarkable properties. These have extended their use in broad areas such as catalysts, solar cells, supercapacitors, chemical sensors, biosensors and biomedical fields. In fact, chemical sensors have potential applications in several fields such as environmental monitoring, medical diagnoses, etc. [25].

Owing to the large surface to volume ratios, functional nanostructured materials have been predicted and ascertained to be excellent functional material candidates for solar cells, catalysis and ultrasensitive sensors/biosensors [26, 27]. The mentioned functional materials include inorganic materials, organic materials, and organic-inorganic hybrid materials. Graphene, the two-dimensional monolayer of sp^2-bonded carbon atoms, which is one of the most cited functional materials, has been used in several industries in recent years mainly due to its large specific surface area, low fabri-

cation cost and thermal and high electrical conductivities. As such, it has been employed for applications in preparation of nanoelectronic devices, transparent electrodes, sensors/biosensors, and nanocomposites [28-33]. In particular, the graphene-polymer composites are of scientific and industrial interest due to their synergetic/complementary characteristics. Recently, the research and study on graphene and its composites has been mainly focused on the fabrication, modification and different applications (such as sensors/biosensors, etc) [34-38]. For example, Liu et al. [28] employed graphene sheets as a novel DNA biosensor by using the GO in an array format to identify specific DNA/DNA hybridization interaction. Fang et al. [29] revealed graphene nanosheets that were functionalized with cationic polyelectrolyte poly (diallyldimethyl ammonium chloride) (PDDA) functionalized (GNs) as the building block in the self-assembly of GNs/Au nanoparticle (NP) heterostructure increased the electrochemical catalytic ability, and used the high-loading Au NPs on graphene (GN/Au-NPs) as the product for H_2O_2 electrochemical sensing. Wang et al. [39] evaluated the influence of graphene oxide on the characteristics of its composite with PANI. These results demonstrated the increased specific capacitance and cycling life implying a synergistic effect between the two compounds.

There are many reports on graphene and its composites in the literature. Yan e al. [40] prepared the electrochemically active, free-standing and biocompatible graphene oxide/PANI and graphene/PANI hybrid materials. These hybrid materials showed an outstanding combination of excellent electrochemical performances and biocompatibility, introducing them for new kinds of bioscience applications. Xu et al. [41] reported a facile technique to construct hierarchical nanocomposites by combining 1D conducting PANI nanowires with 2D graphene oxide nanosheets. PANI nanowire arrays are aligned vertically on graphene oxide nanosheets. The morphologies of PANI nanowires could be modulated by adjusting the ratios of aniline to graphene oxide which are related to different nucleation processes. The hierarchical graphene oxide-PANI nanocomposite structures were further confirmed by XRD analysis.

Zhang et al. [35] fabricated chemically modified graphene and PANI nanofiber composites. The obtained graphene oxide-PANI composites with different ratios were decreased to graphene using hydrazine followed by re-oxidation and re-protonation of the decreased PANI to result in the graphene-PANI nanocomposites. They found that the modified graphene and the PANI nanofibers formed a uniform nanocomposite with the PANI fibers absorbed on the graphene surface and/or filled between the graphene sheets. Al-Mashat et al. [33] displayed the synthesis of a graphene-PANI nanocomposite and its application in the development of H_2 gas sensors. It revealed that the graphene-PANI nanocomposite-based material sensitivity is 16.57% toward 1% of H_2 gas, which is much larger than the sensitivities of the sensors based on only graphene sheets and PANI nanofibers. Yan et al. [40] fabricated a graphene-PANI nanocomposite with high specific capacitance.

On the other hand, conducting polymers also have been extremely researched and applied in several organic devices and functional films [42, 43]. In order to boost the performances or extend the functions and applications of the materials, conducting polymers usually have to be nanostructured or modified with different nanomodifiers. Polyaniline (PANI) is a typical conducting polymers with interesting electroactivity, good environmental stability and unusual doping/dedoping chemistry. Nano/microstructured PANI could be prepared via various chemical methods and they have been widely used for producing chemical sensors/biosensors [44-52].

Several references showed graphene/graphene oxide and PANI nanocomposite with some enhanced properties especially electrochemical characteristics [34, 52-57]. It is noticeable that most of the applications are mainly focused on the sensors or biosensors. The utilized conductive polymers (PANI) with graphene, presented in this work and displayed good responses to the mentioned analytes, is another good example for obtaining chemical biosensors with controlled sensitivity and selectivity, such as for glucose and urea.

5. CONCLUSIONS

A multisensor for the determination of glucose and urea was developed. The sensivity of the sensor to urea and glucose was increased by using a graphene–PANI nanocomposite. The graphene–PANI nanocomposite works as an excellent mediator for enhancing the characteristics of the electrochemical sensor by generating a large density of electrons on the surface of the strips. The cyclic voltammetry test emphasized the effect of this nanocomposite on the mulisensors. Compared with a similar macro sensor, the miniaturized multisensor showed an increased current density and two times higher sensitivity for all analytes. This research showed the successful combination of microelectrode materials with the immobilization of biochemical components such as enzymes. Finally, the developed multisensory provided the advantages of a biosensor and microelectrode features. These enhanced results advocate the nanocomposite based multi-sensors for application in variety of biosensors.

REFERENCES

[1] Bahmani, B., et al., Synthetic Metals, 160, 2653 (2010).
[2] Yazdanpanah, S., et al., TrAC Trends in Analytical Chemistry, 72, 53 (2015).
[3] Abdolrahim, M., et al., Analytical biochemistry, 485, 1 (2015).
[4] Bahmani, B., et al., Asian Journal of Chemistry, 21, 923 (2009).
[5] FUNKCIONALIZIRANIH, M.K.S., Materiali in tehnologije, 47, 235 (2013).
[6] Mohagheghpour, E., et al., A new optical bio-sensor: Wet-chemical synthesis and surface treatment of nanocrystalline Zn 1-x S: Mn+ 2 x. in Optical Sensors. 2011. Optical Society of America.
[7] Abdorahim, M., et al., TrAC Trends in Analytical Chemistry, 82, 337 (2016).
[8] Tayebi, M., et al., Journal of Fluorescence, 1 (2016).
[9] Tayebi, M., et al., Colloid and Polymer Science, 1 (2016).
[10] Andreescu, S. and O.A. Sadik, Pure and applied chemistry, 76, 861 (2004).
[11] Shahbazian, E., G. Rogova, and P. Valin, Data fusion for situation monitoring, incident detection, alert and response management. Vol. 198. 2005: IOS Press.
[12] Heller, A. and B. Feldman, Chemical reviews, 108, 2482

(2008).
[13]Ward Muscatello, M.M., L.E. Stunja, and S.A. Asher, Analytical chemistry, 81, 4978 (2009).
[14]Odaci, D., et al., Biomacromolecules, 10, 2928 (2009).
[15]Battelino, T., et al., Diabetes Care, 34, 795 (2011).
[16]Pagon, R., et al., Lesch-Nyhan Syndrome--GeneReviews (®). 2014.
[17]Steiner, M.-S., A. Duerkop, and O.S. Wolfbeis, Chemical Society Reviews, 40, 4805 (2011).
[18]Chen, C., et al., Rsc Advances, 3, 4473 (2013).
[19]So, C.-F., et al., Medical devices, 5, 45 (2012).
[20]Clark, L.C. and C. Lyons, Annals of the New York Academy of sciences, 102, 29 (1962).
[21]Si, P., et al., RSC Advances, 3, 3487 (2013).
[22]Sassolas, A., L.J. Blum, and B.D. Leca-Bouvier, Biotechnology Advances, 30, 489 (2012).
[23]Hummers Jr, W.S. and R.E. Offeman, Journal of the American Chemical Society, 80, 1339 (1958).
[24]Zhao, Y.S., J. Wu, and J. Huang, Journal of the American Chemical Society, 131, 3158 (2009).
[25]Zheng, J., et al., Procedia Engineering, 27, 1478 (2012).
[26]Mohagheghpour, E., et al., IEEE transactions on nanobioscience, 11, 317 (2012).
[27]Mohagheghpour, E., et al., Materials Science and Engineering: C, 29, 1842 (2009).
[28]Liu, F., J.Y. Choi, and T.S. Seo, Biosensors and Bioelectronics, 25, 2361 (2010).
[29]Fang, Y., et al., Langmuir, 26, 11277 (2010).
[30]Zhou, K., et al., Electrochimica Acta, 55, 3055 (2010).
[31]Robinson, J.T., et al., Nano letters, 8, 3137 (2008).
[32]Fowler, J.D., et al., ACS nano, 3, 301 (2009).
[33]Al-Mashat, L., et al., The Journal of Physical Chemistry C, 114, 16168 (2010).
[34]Zhang, L.L., et al., Langmuir, 26, 17624 (2010).
[35]Zhang, K., et al., Chemistry of Materials, 22, 1392 (2010).
[36]Stankovich, S., et al., Nature, 442, 282 (2006).
[37]Ramanathan, T., et al., Nature nanotechnology, 3, 327 (2008).
[38]Xu, Y., et al., Nano Research, 2, 343 (2009).
[39]Wang, H., et al., ACS applied materials & interfaces, 2, 821 (2010).
[40]Yan, X., et al., ACS applied materials & interfaces, 2, 2521 (2010).
[41]Xu, J., et al., ACS nano, 4, 5019 (2010).
[42]Huang, J., et al., Chemistry–A European Journal, 10, 1314 (2004).
[43]Kukla, A., et al., Sensors and Actuators B: Chemical, 135, 541 (2009).
[44]Li, C., H. Bai, and G. Shi, Chemical Society Reviews, 38, 2397 (2009).
[45]Rahman, M.A., et al., Analytical chemistry, 81, 6604 (2009).
[46]Chen, Y. and Y. Luo, Advanced Materials, 21, 2040 (2009).
[47]Tolani, S.B., et al., Analytical and bioanalytical chemistry, 393, 1225 (2009).
[48]Li, W., et al., Sensors and Actuators B: Chemical, 143, 132 (2009).
[49]Ma, X., et al., Materials chemistry and physics, 98, 241 (2006).
[50]Ma, X., et al., Macromolecular Materials and Engineering, 291, 1539 (2006).
[51]Ma, X., et al., Recent patents on nanotechnology, 4, 150 (2010).
[52]Xuan, S., et al., Langmuir, 25, 11835 (2009).
[53]Khan, J.M., R. Kurchania, and V.K. Sethi, Thin Solid Films, 519, 1059 (2010).
[54]Yang, N., et al., Synthetic Metals., 160, 1617 (2010).
[55]Wang, D.-W., et al., ACS nano, 3, 1745 (2009).
[56]Bissessur, R., et al., Langmuir, 22, 1729 (2006).
[57]Wang, H.,ey al., Electrochemistry Communications, 11, 1158 (2009).