

Detection of Endocrine Disruptors Using Nanomaterial Modified Electrode

Janani Viswanathan^{1*}, Gopu Govindasamy²

¹*Assistant Professors, Department of Biomedical Engineering, Sri Ramakrishna Engineering College, Coimbatore.*

²*Professor and Head, Department of Biomedical Engineering, Sri Ramakrishna Engineering College, Coimbatore.*

**Email: janani.bme@srec.ac.in*

Abstract— In past decades, 2D nanomaterial plays a significant role in electrochemical sensing techniques due to their excellent electron transfer and catalytic properties. This work reports on the graphene-based voltammetric sensor for the detection of bisphenol-A. A simple electrochemical sensing platform is developed to determine bisphenol-A (BPA), an endocrine disrupting compound (EDC), which potentially can cause adverse health effects. Graphene oxide (GO) nanomaterial was synthesized by a modified Hummers method. Scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier transform infrared (FT-IR) and Ultraviolet spectroscopy (UV-Spectroscopy) were used for the characterization of the synthesized graphene. SEM confirms the presence of few-layered GO with 50 nm average thickness. XRD confirmed the amorphous structure rather than a pristine structure with d spacing 0.7084 nm. FT-IR confirms the presence of functional groups -OH and -COOH groups due to incomplete reduction. Cyclic voltammetry was used to study the electrochemical properties of the prepared graphene/GCE potassium ferricyanide as a redox probe. The developed Gr/GCE exhibited more facile electron kinetics and enhanced current. This modified electrode was used for sensing of BPA. Under the optimum conditions, the oxidation peak current of bisphenol-A varied linearly with concentration over a wide range of 5 μM to 100 μM and the detection limit of 0.3 μM using differential pulse voltammetry (DPV). The modified electrode exhibits excellent reproducibility and good stability. Thus the developed sensing platform is expected to be good choices for sensors involving good sensor characteristics.

Keywords—graphene; endocrine disrupting compound; electrochemical detection; Bisphenol-A; Differential pulse voltammetry.

I. INTRODUCTION

There is an increasing concern about endocrine-disrupting chemicals released into the environment due to rapid/ethnic disparities in health. Thus, Bisphenol-A (BPA), 2, 2-bis (4-hydroxyphenyl) propane, has estrogenic activity and is acutely toxic [1, 2]. BPA is mainly used as raw material for the production of polycarbonate plastics, epoxy resins, and thermoplastics, and is found in food packages, beverage containers, food can linings and plastic bottles which are in direct contact with food that is to be consumed. It is generally exposed to the environment due to incomplete polymerization and degradation under high temperatures. Consistently, infant mortality, low birth weight rates, cardiovascular-related problems, dysfunctions, endometrial hyperplasia, recurrent miscarriages, abnormal karyotypes, and polycystic ovarian syndrome and diabetes can also be outstretched as an outcome [3-5]. Although numerous risk factors have been identified, the basic biological mechanisms underlying these disparities remain unknown [6]. It is also suspected to be hormone-receptor interaction of bisphenol-A which mimics like the natural female reproductive hormone estrogen, with its receptors: Estrogenic activity by prevaricating normal function of the endocrine system. Thus BPA is called endocrine disrupting compound (EDC). However, effects of BPA have been of continuous conflicts [7, 8]. Therefore, potential concerns on health have led to increased need for monitoring the trace amount of BPA.

Among the techniques established in the literature to determine BPA concentration include high-performance liquid chromatography, liquid chromatography-mass spectrometry, flow injection analysis, and resonance light scattering [9-11]. While they give accurate measurements, these techniques are expensive and can involve cumbersome protocols. Electrochemical determination of various analytes is an approach that is highly attractive due to its simplicity, rapidity, versatility, accuracy, reproducibility, and cost-effectiveness. The key factor in the performance of a voltammetric electrochemical sensor is the sensing element, i.e., the electrode material. Its high electroactive area, its ability to selectively catalyze the redox reaction involving the analyte over a wide range of concentrations and its stability in both the analyte-containing extracts/solutions and ambient conditions are critical for better sensor performance measured by high sensitivity, low detection limit, good selectivity over other interferants, wide linear range, stability, reproducibility, and use in practical applications. This work examines the performance of graphene as an electrochemical sensing element for the detection of BPA. Graphene is a two-dimensional material which has the high electroactive surface area, good electrical conductivity, and high chemical stability. It is a layered compound that is recently being studied for its applications in sensor and energy storage. It is a zero-band-gap semiconductor which consists of a two-dimensional sheet of carbon atoms arranged in six-member rings (hexagonal configuration) with atoms connected by sp^2 bonds. These bonds and this electron configuration provide the excellent

properties such as a very large surface area (2630 m²/g), good electron transfer rate, and absorptivity [13]. This material acts as an excellent anodic functional material because the 2D e - e correlations assisting in enriching electronic transport

properties. A typical electrochemical determination of BPA involves electrooxidation of it.

TABLE I. DETECTION OF BPA ELECTROCHEMICAL TECHNIQUES- A SUMMARY OF STUDIES IN THE LITERATURE

SENSORS	ANALYTICAL TECHNIQUES	LINEAR RANGE	LIMIT OF DETECTION	REFERENCES
Au/CyS based peptide sequence	CV	1-500nM	0.7nM	14
rGo/Cys based peptide sequence	EIS	10fM-10nM	5fM	15
SGNF/AUNP/GCE	LSV	0.08-250µM	3.5x10 ⁻⁸ M	16
GNCPE	CV	9 x10 ⁻⁸ -2.5 x10 ⁻⁴ M	55 nM	17
GNP/GR/GCE	CV	0.01µM - 10 µM	5nM	18
Arg-G/GCE	LSV	5.0x10 ⁻⁹ - 4.0x10 ⁻⁵ M	1.1 x10 ⁻⁹ M	19
GR/AU-TYR-CS/GCE	CV	2.5x10 ⁻³ - 3 µM	1nM	20
GR/GCE	CV	5.0 x10 ⁻⁸ -1.0 x10 ⁻⁶ M	4.689 x10 ⁻⁸ M	21
MIP-GR/ABPE.	CV	8nM- µM	6nM	22
Thionine modified CPE	AMPEROMETRY	1.5x10 ⁻⁷ -4.5x10 ⁻⁵	1.5x10 ⁻⁷	23
AUNP/MoS₂	CV	0.05 -100 µM	5x10 ⁻⁹ M	24
MoS₂-SPAN/GCE;	DPV	1.0 nM to 1.0 µM	0.6 nM.	25

CV: cyclic voltammetry, DPV: Differential pulse voltammetry, EIS: electrochemical impedance spectroscopy, LSV: linear sweep voltammetry, Au- gold, Cys: cysteine, rGo- reduced graphene oxide, SGNF: AUNP: gold nanoparticles,

Table 1 provides a summary of recent studies concerning the development of electrochemical sensors to detect BPA. This paper reports, a Graphene –oxide-based non-enzymatic sensing platform for voltammetric determination of BPA. A graphene-oxide modified glassy carbon electrode (GO/GCE) was used for determination of BPA using differential pulse voltammetry (DPV). It was observed that GO/GCE showed enhanced performance compared to bare GCE and that the electrode could detect BPA over reasonably wide linear range with good sensitivity, reproducibility, and stability. A schematic depiction of the overall work carried out is presented in Figure 1.

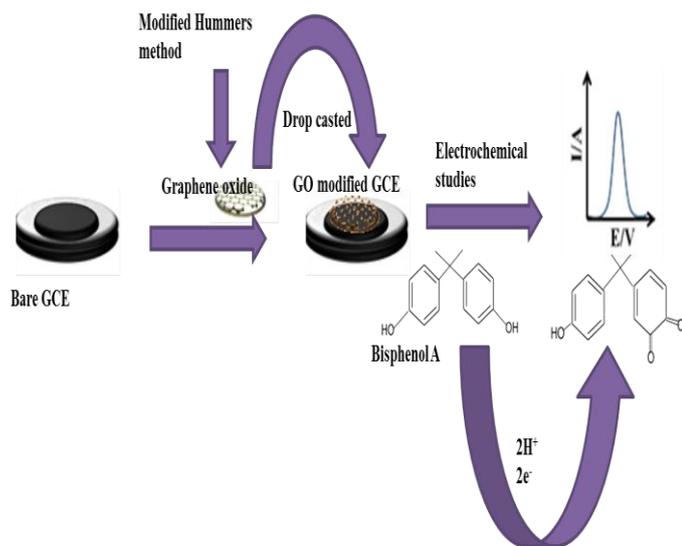


Fig. 1. Schematic depiction of overall working methodology

Experimental details

All chemicals used were of analytical reagent grade. All solutions used throughout the experiment were prepared using double distilled water. The three electrode system was constructed using a glassy carbon working electrode, Ag/AgCl as a reference electrode and a platinum wire as counter electrode. The anodic material was drop casted and made to air dry for using it in experiments.

II. METHODOLOGY

Graphene oxide was synthesized in-house using a modified Hummers method and was characterized to confirm the presence and study the properties of the synthesized material. With the prepared material, the further electrochemical studies are carried out to evaluate the sensor performance for detection of BPA.

A. Preparation of graphene oxide

Graphene oxide (GO) was synthesized by a modified-Hummers method. Graphite powder of 4g was mixed with 100 mL of 95% sulphuric acid in 500 mL container and it was made to stir for 30 minutes in an ice bath. 12 g of KMnO_4 was added at many time intervals provided with vigorous stirring for 2 hours. All these setups have to be maintained below 20°C . the mixture was subsequently stirred for 8 hours at room temperature. Then 100 mL of distilled water was added slowly during which the temperature of the solution increases with brisk effervescence with a color change to yellow. This has to stir for other 12 hours at 98°C . then 40 mL of 30% H_2O_2 was added, and the mixture was washed by rinsing and centrifuging with 5% HCL, followed by washing with distilled

water until the pH is neutral and then dried at 50°C . Finally a brown color, powdered GO flakes are obtained.

B. Preparation of Go-modified electrode

1mg of synthesized GO was dissolved in 1 mL of distilled water and ultrasonicated for 10 minutes. A well surface polished GCE was modified by drop casting an aliquot of 10 μL from 1 mL followed by air drying.

III. RESULTS AND DISCUSSIONS

A. Characterisation of synthesized material

From the figure 2a, the SEM results confirm the presence of layered arrangement with average thickness 50 nm and average width $800\ \mu\text{m}$. Figure 2b graphs the XRD pattern of GO. Generally, the interlayer spacing for materials with XRD is proportional to the degree of oxidation. Diffraction peak becomes broader in the enlarged pattern of Graphite oxide at 11.24° , the significant increase in spacing due to the oxygen functional groups intercalate in the interlayer of graphite. There is also a very weak diffraction peak about 42.95° , which is may be due to the incomplete oxidation. The interlayer distance obtained was 0.7084nm ($2\theta = 11.241$) in GO which is larger when compared to that of graphite (the major peak from (0 0 2) at $2\theta = 26.4$). The grain size of graphene oxide (D) formed is approximately $4.206\ \text{nm}$ by the Scherrer equation. This large interlayer spacing has been ascribed to the formation of different functional groups such as hydroxyl, epoxy, carboxyl, and carbonyl, which is again confirmed by IR spectroscopy results in figure 3. FTIR spectra analysis was performed to investigate the structure and functional groups of the materials. The Graphene Oxide sample showed apparent adsorption bands for the carboxyl $\text{C}=\text{O}$ (1723cm^{-1}), aromatic $\text{C}=\text{C}$ (1619cm^{-1}), epoxy $\text{C}-\text{O}$ ($1383\ \text{cm}^{-1}$) and hydroxyl OH (3396cm^{-1}) groups. Figure 2d shows the UV spectrum for prepared GO. It exhibits a strong absorption band at 231nm and a shoulder at 303nm . The bigger hump near the margin shows the $\pi-\pi^*$ transition and the small shoulder further down through the curve shows the $n-\pi^*$ transition. The $\pi-\pi^*$ transition takes place due to the sample containing conjugation and the $n-\pi^*$ transition takes place due to the sample contains highly electronegative groups like Oxygenated groups. This makes the above-mentioned transitions to happen, which may result in a UV-vis spectrum with two

humps.

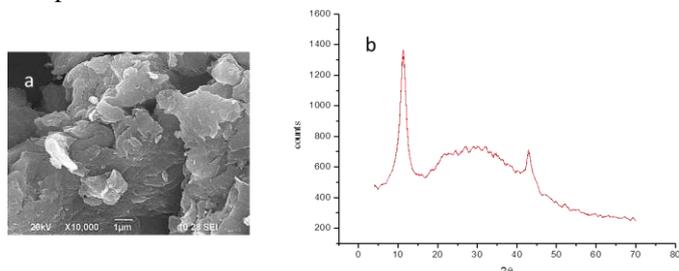


Fig. 2. a) SEM images of GO, b) XRD pattern of GO

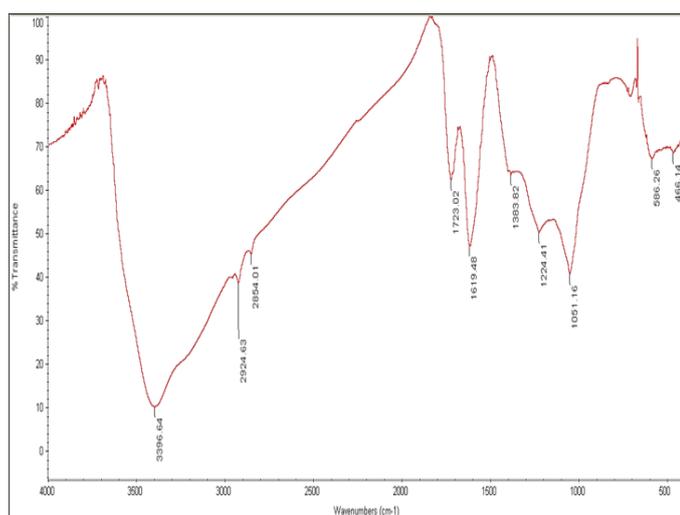


Fig. 3. The FT-IR spectrum of graphene oxide (GO)

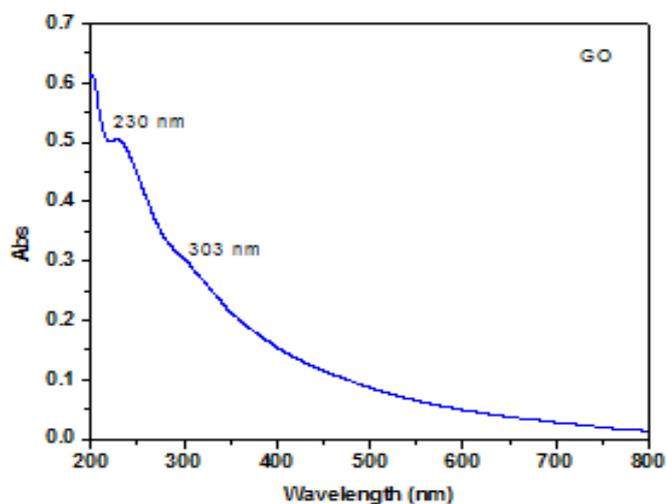


Fig. 4. The UV-visible spectrum of graphene oxide (GO)

Figure 5 shows the electrochemical behavior of bare GCE and GO/GCE. These electrochemical studies are performed using redox probes as the analyte, in order to study electron transfer characteristics of oxidation and reduction reaction. 0.5 mmol of potassium ferricyanide $[Fe(CN)_6]^{3-/4-}$. From the obtained cyclic voltammetry plot which is performed at 50 mV/s scan rate. We can clearly conclude that the prepared material has good transfer rate and better catalytic property than that of bare GCE which enhances sensor performance like the sensitivity of the glassy carbon electrode. The linear dependence of oxidation and reduction peak currents on the square root of the scan rate showed that the process is diffusion controlled as shown in Figure 3. A correlation of 0.984 was obtained from the linear line equation. This result exhibits good linearity with respect to scan rate.

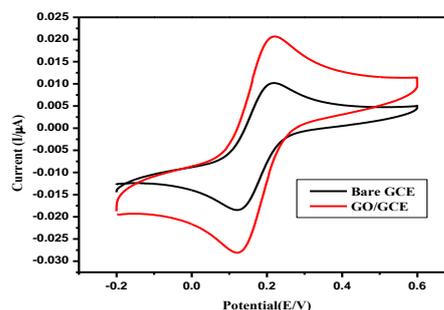


Fig. 5. CV for bare and GO/GCE in 500 μM BPA at 50 mV/s scan rate.

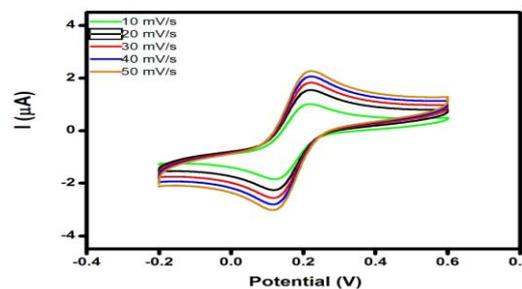


Fig. 6. CV for GO/GCE for different scan rate.

B. Voltammetric detection of BPA and Sensor performance

The electrochemical behavior of BPA in a solution containing 500 μM BPA with pH 7.45 (phosphate buffer) are studied using cyclic voltammetry techniques. Figure 4 shows the oxidation peak of BPA at 0.3 V. the reaction is a

single step electron transfer process. Figure 5 shows the differential pulse voltammetry results in varying concentration of BPA. Since DPV is known for better sensitivity, it was chosen for voltammetric determination of BPA. The response of different concentration of BPA is studied and plotted. The developed sensing platform exhibits a linear response from 5 to 100 μM after which the current deviated from the linear behavior with sensitivity 0.0069 μA . The calibration equations for obtained linear range $y=0.0673x+70.902$; $R^2 = 0.9917$.

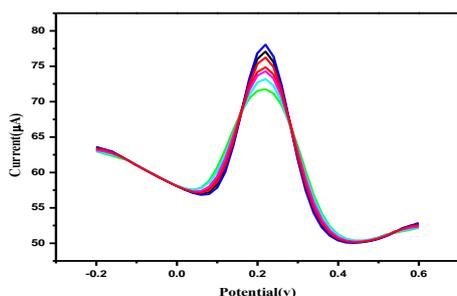


Fig. 7. Differential pulse voltammograms for BPA with concentrations

from a) 5 μM to 200 μM in phosphate buffer (pH 7.45)

A statistical method was employed to calculate the detection limit of the sensing platform GO/GCE. This was done with the standard protocol of running voltammetry studies in blank and with the analyte for seven times and performing a one-sided t-test to find lower detection limit (smallest significant difference from that of blank). It was calculated as 0.3 μM .

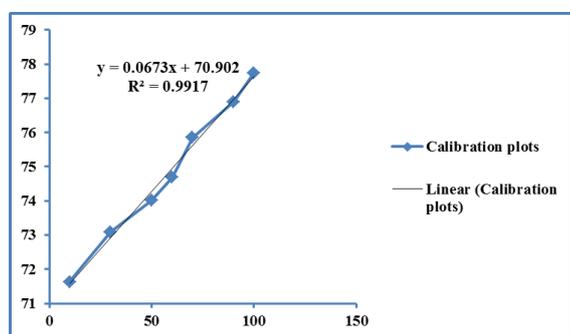


Fig. 8. Calibration plot for figure 7, BPA varying concentrations.

Further, the ability of GO/GCE to reproducibly and stability determine BPA was studied by experimenting

DPV with 500 μM BPA as an analyte. The relative standard deviation as calculated from the obtained peak values and it was found to be 3.4% which indicated the sensor could sense in a reproducible manner. For the stability test, the experimental cycle was carried out at 10th, 7th and 3rd day and peak current was calculated. There was only approximately 2% current drop from the 10 days. As this adds that the sensor is stable for long-term.

IV Conclusion

In this study, the electrocatalytic behavior of bisphenol-A has been studied on graphene oxide modified glassy carbon electrodes. GO was prepared using modified Hummers method which showed an interlayer spacing of 0.78 nm, a layered arrangement of GO sheets with average thickness 50 nm and average width 800 nm with functional groups added to it which was confirmed using IR spectroscopy. The developed sensor showed a good sensitivity 0.0069 μA linear range of 5 to 100 μM and a detection limit of 0.3 μM . These electrodes also showed good reproducibility and stability. These preliminary results indicate that the GO/GCE has good potential to determine BPA.

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References

- [1] Kleinman JJC, Kessel SSS. Racial differences in low birth weight. Trends and risk factors. N Engl J Med 1987; 317: 749–753.
- [2] Centers for Disease Control and Prevention. Infant mortality and low birth weight among black and white infants— the United States, 1980-2000. MMWR Morb Mortal Wkly Rep 2002; 51: 589–592.
- [3] Silbergeld EK, Patrick TE. Environmental exposures, toxicologic mechanisms, and adverse pregnancy outcomes. Am J Obstet Gynecol 2005; 192: S11–S21.
- [4] Morello-Frosch R, Shenassa ED. The environmental riskscape and social inequality: implications for explaining maternal and child health disparities. Environ Health Perspect 2006; 114: 1150–1153.
- [5] Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years [see comment]. Br Med J 1992; 305: 609–613.
- [6] Bergström R, Adami HO, Mo'nhner M, Zatonski W, Storm H, Ekblom A et al. Increase in testicular cancer incidence in six European countries: a birth cohort phenomenon. J Natl Cancer Inst 1996; 88: 727–733.

- [7] Andersen AG, Jensen TK, Carlsen E, Jørgensen N, Andersson AM, Krarup T et al. High frequency of sub-optimal semen quality in an unselected population of young men. *Hum Reprod* 2000; 15: 366–372.
- [8] Toppari J, Kaleva M, Virtanen HE. Trends in the incidence of cryptorchidism and hypospadias, and methodological limitations of registry-based data. *Hum Reprod Update* 2001; 7: 282–286. J. Clerk Maxwell, A Treatise on Electricity and Magnetism, 3rd ed., vol. 2. Oxford: Clarendon, 1892, pp.68–73.
- [9] Wang, X., Zeng, H., Zhao, L. & Lin, J.-M. Selective determination of bisphenol A (BPA) in water by a reversible fluorescence sensor using pyrene/dimethyl- β -cyclodextrin complex. *Anal. Chim. Acta.* 556, 313–318 (2006).
- [10] Hegnerova, K. & Homola, J. Surface plasmon resonance sensor for detection of bisphenol A in drinking water. *Sensor. Actuat. B-Chem.* 151, 177–179 (2010).
- [11] Ragavan, K. V., Selvakumar, L. S. & Thakur, M. S. Functionalized aptamers as nano-bioprobes for ultrasensitive detection of bisphenol-A. *Chem. Commun.* 49, 5960–5962 (2013).
- [12] Molina-García, L., Luisa Fernández-de Córdoba, M. & Ruiz-Medina, A. Analysis of bisphenol A in milk by using a multi-commuted fluorimetric sensor. *Talanta* 96, 95–201 (2012). M. Young, *The Technical Writer's Handbook*. Mill Valley, CA: University Science, 1989.
- [13] Ballesteros, O., Zafra, A., Navalon, A. & Vilchez, J L. Sensitive gas Chromatographic–mass spectrometric method for the determination of phthalate esters, alkylphenols, bisphenol A and their chlorinated derivatives in wastewater samples. *J. Chromatogr. A.* 1121, 154–162 (2006).
- [14] Jiao Yang, Sung-Eun Kim, Misuk Cha, IK-Keun Yoo, Woo-Seok Choe, Youngkwan Lee, Highly sensitive and selective determination of Bisphenol-A using peptide-modified gold electrode, *Biosensors and Bioelectronics* 61 (2014) 38–44.
- [15] Kwang Su Kim, Ji ryang Jang, Woo-Seok Choe, Pil J. Yoo, Electrochemical detection of Bisphenol A with high sensitivity and selectivity using recombinant protein-immobilized graphene electrodes, *Biosensors and Bioelectronics* 71 (2015) 214–221.
- [16] Xiuli Niu, Wu Yanga, Guoying Wang, Jie Ren, Hao Guo, Jinzhang Gao, A novel electrochemical sensor of bisphenol A based on stacked graphene nanofibers/gold nanoparticles composite modified glassy carbon electrode, *Electrochimica Acta* 98 (2013) 167–175.
- [17] Hadi Beitollahi, Somayeh Tajik, Construction of a nanostructure-based electrochemical sensor for voltammetric determination of bisphenol A, *Environ Monit Assess* (2015) 187: 257.
- [18] Ling Zhou, Jianping Wang, Dajuan Li, Yanbin Li, An electrochemical aptamer sensor based on gold nanoparticles dotted graphene modified glassy carbon electrode for label-free detection of bisphenol A in milk samples, *Food Chemistry* 162 (2014) 34–40.
- [19] Y. Zhang, L.T. Wang, D.B. Lu, X.Z. Shi, C.M. Wang, X.J. Duan, Sensitive determination of bisphenol A base on arginine functionalized nanocomposite graphene film, *Electrochimica Acta* 80 (2012) 77.
- [20] Daodong Pan, Yuanyuan Gu, Hangzhen Lan, Yangying Sun, Huiju Gao, a Functional graphene-gold nanocomposite fabricated electrochemical biosensor for direct and rapid detection of bisphenol A, *Analytica Chimica Acta* 853 (2015) 297–302.
- [21] B.Ntsendwana, B.B. Mamba, S. Sampath, O.A. Arotiba, Electrochemical detection of bisphenol A using graphene-modified glassy carbon electrode, *International Journal of Electrochemical Science* 7 (2012) 350.
- [22] Peihong Deng, Zhifeng Xu, Yunfei Kuang, Electrochemical determination of bisphenol A in plastic bottled drinking water and canned beverages using a molecularly imprinted chitosan–graphene composite film modified electrode, *Food Chemistry* 157 (2014) 490–497.
- [23] A.J. Bard, L.R. Faulkner, *Electrochemical Methods Fundamentals Applications*, 2nd ed., Wiley, New York, 1980.
- [24] Jing Huang, Yu-Jie Liu, Yan-Ming Liu, Ling-Ling Wang, Molybdenum disulfide nanoflower-chitosan-Au nanoparticles composites based electrochemical sensing platform for bisphenol A determination, *Journal of Hazardous Materials* 276 (2014) 207–215.
- [25] Tao Yang, Huaiyin Chen, Ruirui Yang, Yuhang Jiang, Weihua Li, Kui Jiao, A glassy carbon electrode modified with a nanocomposite consisting of molybdenum disulfide intercalated into self-doped polyaniline for the detection of bisphenol A, *Microchim Acta* August 2015.