Towards Development of Laser Scribed Graphene Transducers for Electrochemical Biosensors

*Adriana Ibarra[1], Eider Aparicio[1], Rocio B. Dominguez[1,2,]**

[1], Department of Engineering and Chemistry of Materials, CIMAV S.C., 31136 Chihuahua, Mexico

[2], CONACyT-CIMAV S.C, 31136 Chihuahua, Mexico

***Abstract*—Development of suitable electrochemical transducers (e.g. electrodes) is a major concern to obtain efficient sensing and biosensing platforms. Graphene has attracted attention as a potential material for electrochemical transduction, but its massive production remains as a challenge. In this work, the development and characterization of graphene electrodes based on thermal reduction by laser action is presented. The method, known as laser scribed graphene, was applied to graphite oxide (GO) obtained by Hummer's method. Then, after several burning cycles, GO was thermally reduced by the action of CD/DVD unit laser. Formation of graphene layers was corroborated by Raman, UV-Vis, XRD and SEM techniques. The electrochemical performance of the material was characterized by EIS and Cyclic voltammetry. Finally, the potential application of developed material as biosensing platform was investigated with glucose oxidase as model enzyme and FTIR. Specter showed distinctive bands, which suggest that enzyme retained its native conformation and activity. The developed material showed enhanced electrochemical properties after laser reduction and is suitable for potential application as biosensing platform.**

Keywords—graphene; laser; transducer; biosensor

I. INTRODUCTION

Plenty of applications have been reported with electrochemical sensing, from biomedical field to environmental detection. Electrochemical sensors are analytical devices usually composed of three main elements: the sensing layer, the transducer and the electronic system. In order to achieve high selectivity, the sensing layer incorporates materials such as ionospheres, metal oxides, conductive polymers and biochemical molecules such as enzymes. In electrochemical sensors, the chemical interaction between the target analyte and the sensing layer creates an electrical signal that is captured by the transducer. Transducers (i.e. electrodes) made from carbonaceous materials such as vitreous carbon, graphite composites, carbon paste, carbon nanotubes and graphene have been used as electrochemical platforms for sensors and biosensors in order to improve the detection of target analytes with high sensitivity, stability and reproducibility. Finally, the electronic system manages the conditioning and processing of the generated signal in order to present the meaningful information (e.g. concentration).

In recent years, graphene has stood out as a suitable material for sensor fabrication mainly due to properties such as transparency, high mobility of charges, mechanical strength, flexibility as well as thermal and electrical conductivity [1]. However, high quality single layer graphene has been mainly obtained by mechanical exfoliation, which is a methodology not suitable for mass production. Thus, different approach for production, such as chemical alternatives, has been applied. In this sense, the Hummer's method has been extensively applied for producing graphite oxide (GO) as precursor, which through a subsequent sonication and reduction process with hydrazine can be turned into chemical reduced graphene oxide (rGOx). The removal of oxygen groups allows the material to move from an isolator in the GO form to a conductive material in the rGO form. However, hydrazine is an extremely toxic compound and the reducing process involves the generation of highly polluting residues.

Recently, graphene thermal reduction assisted by infrared laser devices such as CD/DVD burners and custom laser engraver machines has been reported. The methodology allows the creation of stacked graphene layers over flexible substrates without secondary pollutant residues and with the additional advantage of custom patterning for electrode manufacturing through computer assisted design. The methodology has been mainly reported for the creation of interdigitated electrodes for flexible micro-supercapacitors [3],[4] and only recently was investigated as manufacturing method for disposable electrochemical sensors [5]. Given the importance of optimal transducer performance to achieve suitable electrochemical detection, as well as the importance of novel flexible platforms for electrochemical sensing and biosensing, the present work deals with the application of laser engrave technique to produce electrochemical transducers. The material was obtained from GO as precursor and thermally reduced with a CD/DVD burner into custom electrode patterns. The properties of the material were characterized trough Raman and UV spectroscopy, X-Ray diffraction (XRD), scanning electron microscopy (SEM), cyclic voltammetry and electrochemical impedance. Finally, glucose oxidase enzyme was used as model to investigate the ability of the material a platform for electrochemical biosensors.

978-1-5386-3406-6/17/$31.00 ©2017 IEEE

II. Materials and Methods

A. Materials

Graphite powder (325 mesh) was obtained from Alfa Aesar (USA), $KMnO_4$, $NaNO_3$ and $K_3[Fe(CN)_6]$ were purchased from Fermont (USA). KNO_3 was acquired from Golden Bell (USA) and Sulphuric acid from J.T. Baker

B. Methods

Graphite oxide (GO) was obtained by modified Hummers method, which can be found elsewhere [1]. Later, obtained GO was washed with distilled water until neutrality was reached. Then GO was exfoliated with ultrasonic sound for 30 minutes and dried at 60ºC in order to obtain graphene oxide (GOx). For laser scribing the material, a laser scribe CD was covered with a PET film as substrate **(Fig. 1)**. Later, a solution of 1 mg/mL of GOx was added by drop casting over the PET/CD an allowed to dry for 24 hours. The thermal reduction was performed with a DVD HP LigthScribed unit by repeated exposition to the built-in 780 nm 5 mW infrared laser (burning cycles). Designed custom electrode patterns were created with Corel Draw software and stored for later applications. After a given number of burning cycles PET substrate was removed from CD and reduced graphene oxide patterns were obtained.

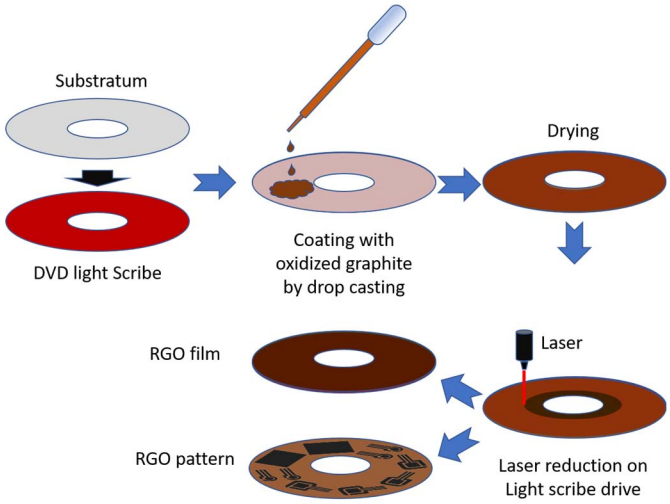

Fig. 1. Schematic diagram of fabrication process of reduced graphene transducers by laser treatment.

C. Instruments

Raman spectroscopy was performed in Micro Raman LabRAM HR Horiba Jobin Yvon with a 630 nm 5 mW laser. X-Ray diffraction was carried out on a Bruker D8 advance diffractometer using Cu Kα irradiation and wavelength of 1.540598 Å. Morphology and microstructure were observed with SEM on a HITACHI SU 3500 microscope. UV-Vis spectroscopy was evaluated using a Cary 5000 UV-Vis-NIR spectrophotometer. Electrochemical measurements were evaluated on a potentiostat/galvanostat Solartron SI1285.

III. Results and Discussions

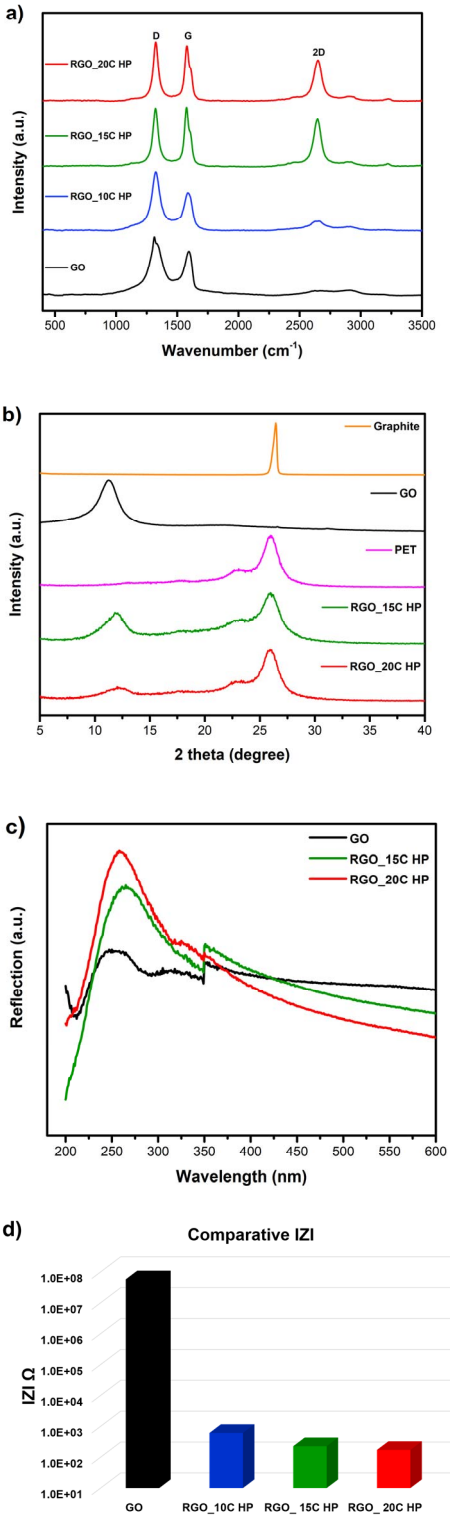

Fig. 2. Characterization of GO and RGO with 10, 15 and 20 laser-scribed times: a) Raman Spectroscopy, b) X-Ray Diffraction, c) UV-Vis Spectroscopy and d) Electrical resistance depending of laser-scribed times by EIS analysis.

A. Raman Spectroscopy

Obtained rGOx were characterized by Raman spectroscopy in order to identify structural changes due to thermic reduction process **(Fig. 2a)**. A high intensity D band at 1330 cm^{-1} was presented by GO precursor due to incorporation of oxygen groups producing a sp^3 hybridization [6]. The G band observed at 1595 cm^{-1} was related to vibration E_{2g} mode of graphitic carbon [7] due to a sp^2 hybridization. Once GOx was reduced with 15 (RGO_15C HP) and 20 (RGO_20C HP) burning cycles, a stretching on D and G bands was produced for both materials. This behavior was attributed to a decrement on defects of the laminar structure. In addition, and increment on intensity of G band as well as a displacement from 1595 cm^{-1} to 1576 cm^{-1} was observed for RGO_15C HP. This was attributed to the reduction of oxygen functionalities causing a partial restoration of some sp^2 domains of graphene sheets [8]; but since full restoration was not achieved D band was still present after laser treatment [9]. Reduction of GOx by laser treatment was also corroborated by the emergence of 2D band (≈2650 cm^{-1}) indicating the formation of few stacked graphene layers. Sharp and defined signals indicated a low number of graphene layers [10]. Intensity of 2D band increased with greater burning cycles, which was attributed to higher reduction of functional oxygen groups and higher material delamination. 2D/G ratio brings information about the number of graphene layers, thus multilayer graphene is expected if 2D/G ratio is lower than unity[11]. The obtained results for 2D/G ratio for 10, 15 and 20 burning cycles were 0.25, 0.80 and 0.74 respectively. These values indicated the formation of few graphene layers, with higher values for RGO_15C HP material.

B. XRD

Precursor graphite and PET substrate were characterized by XRD **(Fig. 2b)**. Graphite diffractogram showed typical peak at 26.5°, corresponding to (002) plane and interlaminar distance of 0.334 nm [14], while peak of GOx showed a displacement at lower 2-theta degrees of 11.25° (001) and interlaminar distance of 0.786nm. The increase on interlaminar distance was attributed to the bond of different oxygen functionalities in basal plane and edges, such as hydroxyl, carboxyl and epoxy groups. GOx presented a base peak broader than graphite, which could be related to decrease on crystallinity of GO due to exfoliation treatment allowing a better delamination of rGOx sheets. RGO_15C HP and RGO_20 C films showed a decrement on characteristic rGOx peak, mainly attributed to loss of oxygen functionalities due to thermal reduction process. However, the presence of GOx after laser treatment indicated remaining oxidized material on the film bottom [8]. Bands at 23° and 26° in rGOx films correspond to PET substrate where rGOx is supported.

C. UV-Vis-NIR

UV-vis specters for GOx film, RGO_15C HP and RGO_20C HP are shown on **Fig. 2c**. GOx specter showed a band at 245 nm, which was related to π–π* transitions of C=C aromatic bonds, while band at 300 nm was related to n- π* transitions of carbonyl group [12]. RGO_15C HP and RGO_20C HP films showed a bathochromic displacement to 265 nm and 260 nm for first and last respectively. This was related to more n- π* transitions suggesting a restoration of the conjugation π network of rGOx layers [6],[13]. In addition, reflectance in rGOx films increased with higher number of burning cycles, which is in good agreement with Raman spectroscopy. The band at 300 nm showed a decreasing after reduction of GOx, especially for RGO_15C HP sample, which also experienced higher bathochromic displacement probably related to a more efficient reduction

D. EIS

GOx electrode as well as RGO_10C HP, RGO_15C HP and RGO_20C HP were characterized with EIS in order to determine the impedance (Z) reduction after laser treatment. |Z| results are shown in **Fig. 2d**, with the highest resistance of 57.4 MΩ for GOx film, which is well-known as a dielectric material[4]. Transducers based on LSG showed a reduction on |Z| of around 5 orders of magnitude. This characteristic was enhanced after higher number of treatments, with rGOx electrode of 20 cycles showing the lower |Z| value of 172 Ω, which is supported by previous studies [9] and support the higher conductivity of rGOx films caused by laser reduction.

E. SEM

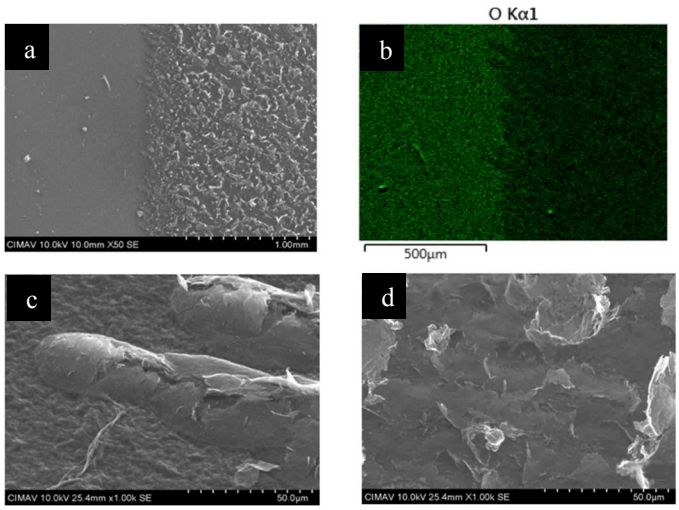

Fig. 3. SEM Microscopy of RGO_20C HP and GO: a) interface between GO (left) and RGO (right) X50, b) EDS Mapping of oxygen between interface GO-RGO c) RGO expansion to X1000 and d) Exfoliation and porous surface of RGO X1000.

SEM characterization was applied to GOx sample and RGO_20C HP sample. **Fig. 3a** showed the interphase between a zone with GOx without laser treatment and rGOx after 20 cycles of laser treatment. Energy dispersive mapping was

applied to the reduced zone and results are shown in **Fig. 3c** with bright green color corresponding to oxygen content. The brighter green color in GOx zone as compared to rGOx zone was due to deoxygenation, with a total quantification of 72.4% for C and 26.3% for O in reduced zone. Expansion and delamination due to thermal reduction is remarkable for rGOx zone, which can be observed closely on figure **Fig. 3c**. A magnification of X1000 showed exfoliation caused by laser treatment and an increment on the superficial area of rGOx sample depicted by a porous surface and considerable number of fissures and edges is shown in **Fig. 3d**.

F. Cyclic Voltammetry

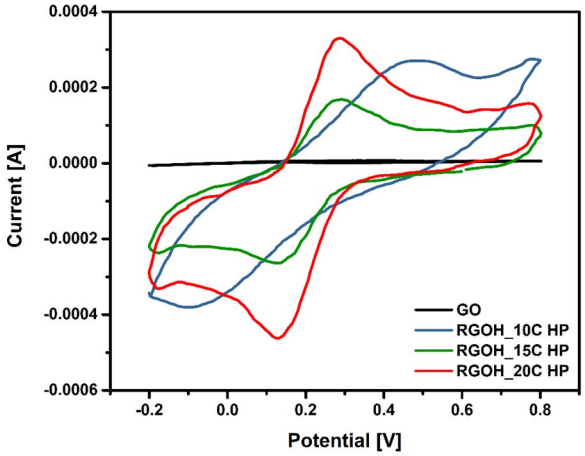

Fig. 4. CV of GO and RGO with 10, 15 and 20 laser-scribed times at scan rate 50 mV/s with potassium ferricyanide

The performance as electrochemical transducers for GOx, RGO_10C HP, RGO_15C HP and RGO_20C HP was evaluated with a redox specie formed by $K_3Fe(CN)_6$ and KNO_3 as supporting electrolyte [15]. The black line on **Fig. 4** corresponds to cyclic voltamperogram for GOx with a scan rate of $50mVs^{-1}$ electrode and null electrochemical behavior. Transducers formed by rGOx samples showed a quasi-reversible electrochemical system, which was improved by the higher cycles of laser treatments [5]. The tendency was supported by ΔEp measurement of 589 mV, 164 mV and 161 mV for 10, 15 y 20 cycles of treatment respectively. Even these values still need further improvement to achieve the 59 mV value of Nerst equation for reversible systems, the application of developed electrodes as potential transducers for electrochemical biosensors was supported by the tendency of laser treated materials to allowed electron transfer given their lower electrical resistance. This was attributed to the high grade of reduction of material, allowing a higher surface contact for a better diffusion of the electroactive species [8].

G. FTIR

Since RGO_20C HP sample obtained the lower ΔEp value, this sample was applied as platform for enzyme immobilization. A solution of 5 mg/mL and 10 mg/mL was incubated for 2 hours on RGO_20C HP electrodes and enzyme bonding was monitored through FTIR. **Fig. 5** showed increased band at 1643 cm^{-1} which can be attributed to C=O bonds present on protein backbone, while 1557 cm^{-1} can be related to a combination of N-H in plane bending and C-N stretching of the peptide groups. Given the similarity of obtained specter for immobilized enzyme compared with native enzyme as well as the appearance of distinctive bands, it can be concluded that enzyme retained its activity and reduced graphene material can be applied as a potential biosensing platform.

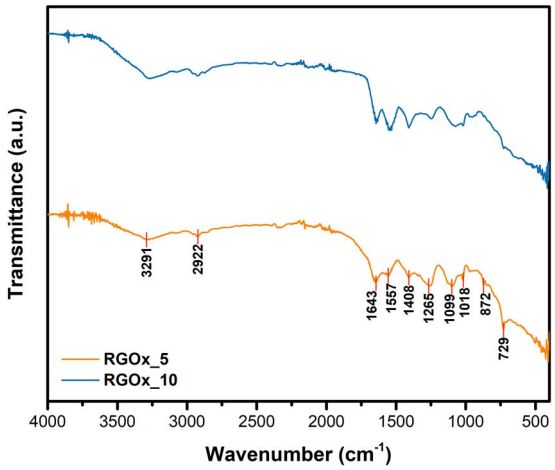

Fig. 5. FTIR for RGO_20C HP sample with glucose oxidase enzyme

IV. CONCLUSIONS

Custom graphene electrodes were manufactured starting from GO as precursor obtained from Hummers method and thermally reduced by CD/DVD laser action. The formation of graphene layers was corroborated by characteristic bands on Raman spectroscopy as well as increased features on UV-Vis spectroscopy. GOx reduction was also supported by lower quantification of O by EDS. Electrochemical features were greatly improved after laser treatment, especially Z. The values revealed a decrement from 57.4 MΩ for GOx to 172 Ω for RGO_20C HP sample, which also showed the lower ΔEp value. Even further improvement is needed to achieve a full behavior as reversible system, the material showed a great potential as electrochemical platform.

ACKNOWLEDGMENT

The authors would like to thank to M.C. Pedro Pizá Ruíz, M.C. Karla Campos Venegas, M.C. Ernesto Guerrero Lestarjette and Dr. Iván Alziri Estrada Moreno for supporting in UV-VIS, SEM, DRX and Raman measurements.

REFERENCES

[1] B. Y. Zhu *et al.*, "Graphene and Graphene Oxide : Synthesis , Properties , and Applications," *Adv. Mater.*, vol. 22, pp. 3906–3924, 2010.

[2] S. Park, J. An, J. R. Potts, A. Velamakanni, S. Murali, and R. S. Ruoff, "Hydrazine-reduction of graphite- and graphene oxide," *Carbon N. Y.*, vol. 49, no. 9, pp. 3019–3023, 2011.

[3] W. Gao *et al.*, "Direct laser writing of micro-supercapacitors on hydrated graphite oxide films," *Nat. Nanotechnol.*, vol. 6, pp. 496–500, 2011.

[4] M. F. El-Kady and R. B. Kaner, "Scalable fabrication of high-power graphene micro-supercapacitors for flexible and on-chip energy storage.," *Nat. Commun.*, vol. 4, p. 1475, 2013.

[5] K. Griffiths, C. Dale, J. Hedley, M. D. Kowal, R. B. Kaner, and N. Keegan, "Laser-scribed graphene presents an opportunity to print a new generation of disposable electrochemical sensors.," *Nanoscale, RSC*, vol. 6, no. 22, pp. 13613–22, 2014.

[6] E. E. Ghadim, N. Rashidi, S. Kimiagar, O. Akhavan, F. Manouchehri, and E. Ghaderi, "Pulsed laser irradiation for environment friendly reduction of graphene oxide suspensions," *Appl. Surf. Sci.*, vol. 301, pp. 183–188, 2014.

[7] P. Nayak, N. Kurra, C. Xia, and H. N. Alshareef, "Highly Efficient Laser Scribed Graphene Electrodes for On-Chip Electrochemical Sensing Applications," *Adv. Electron. Mater.*, vol. 2, no. 10, pp. 1–11, 2016.

[8] V. A. Strong *et al.*, "Patterning and Electronic Tuning of Laser vol. 6, no. 2, pp. 1395–1403, 2012.

[9] H. Tian *et al.*, "Wafer-scale integration of graphene-based electronic, optoelectronic and electroacoustic devices.," *Sci. Rep.*, vol. 4, p. 3598, 2014.

[10] A. C. Ferrari *et al.*, "Raman spectrum of graphene and graphene layers," *Phys. Rev. Lett.*, vol. 97, no. 18, 2006.

[11] H. Tian, M. Ali Mohammad, W.-T. Mi, Y. Yang, and T.-L. Ren, "Laser-Scribing Technology for Wafer-Scale Graphene Devices," in *Advances in Carbon Nanoestructures*, INTECH, 2016, pp. 63–82.

[12] P. K. Ang, S. Wang, Q. Bao, J. T. L. Thong, and K. P. Loh, "High-throughput synthesis of graphene by intercalation-exfoliation of graphite oxide and study of ionic screening in graphene transistor," *ACS Nano*, vol. 3, no. 11, pp. 3587–3594, 2009.

[13] G. Gnana kumar, K. Justice Babu, K. S. Nahm, and Y. J. Hwang, "A facile one-pot green synthesis of reduced graphene oxide and its composites for non-enzymatic hydrogen peroxide sensor applications," *RSC Adv.*, vol. 4, no. 16, p. 7944, 2014.

[14] C. Botas *et al.*, "Graphene materials with different structures prepared from the same graphite by the Hummers and Brodie methods," *Carbon N. Y.*, vol. 65, no. 1, pp. 156–164, 2013.

[15] A. W. Bolt and B. P. Jackson, "Study of Ferricyanide by Cyclic Voltammetry Using the CV-50W," *Curr. Sep.*, vol. 15, no. 1, pp. 25–30, 1996.