Application of Surface Plasmon Resonance Sensor with Polypyrrole Chitosan Graphene Oxide layer to Detect the Napropamide

Amir Reza Sadrolhosseini¹,²,², Suraya Abdul Rashid³, Hassan Soleimani³, Suhaidi Shafie¹,⁴, A.S.M. Noor⁵, Ahmad Mohammadi⁶

¹Functional Devices Laboratory (FDL), Institute of Advanced Technology, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
²Materials Processing and Technology Laboratory, Nanomaterials and Nanotechnology Group, Institute of Advanced Technology, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.
³Department of Fundamental and Applied Science, Universiti Teknologi PETRONAS, 31750 Seri Iskandar, Malaysia,
⁴Department of Electrical and Electronic Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor
⁵Wireless and Photonics Networks Research Center of Excellence (WiPNET), Faculty of Engineering Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
⁶Department of Electrical and Computer Engineering, Buein Zahra Technical University, Buein Zahra, Qazvin, Iran

Corresponding Author’s: amir17984818@gmail.com

Abstract. A polypyrrole Chitosan Graphene Oxide (PPy-Chi/GO) composite layer was prepared using the electro-chemical method. The PPy-Chi/GO layer was used to measure the low concentration of napropamide. In this study, the PPy-Chi/GO composite layer was deposited on the gold layer side of a microscope’s glass slide in different thicknesses. The morphology, thickness, roughness, and refractive index of the layer were obtained using field effect scanning electron microscopy, profilometer, atomic force microscopy, and the surface plasmon resonance technique. The structure of the composite layer was investigated using X-Ray diffraction. The peak that were observed from X-ray diffraction was at 9.9°, corresponding to GO, and the broad peak between 25° and 27° was related to polypyrrole and chitosan. The PPy-Chi/GO layer was used to measure the concentration of napropamide dissolved in hexane in the range of 0.01 to 100 ppm using the surface plasmon resonance technique; the limitation of sensor was 0.1 ppm.
1. **Introduction**

Napropamide is N, N-diethyl-2-(1-naphthoxy) propionamide and a polar herbicide that is readily soluble in water, acetone, chlorobenzene, ethanol, xylene, hexane, and dichloromethane. The empirical formula of napropamid is C17H21NO2. The molecular weight and the specific gravity of napropamide were 271.4 g/ml and 0.584, respectively [1]. Napropamide is used to control broadleaf weeds and annual grasses on numerous agricultural products [2]. Numerous analytical methods are used to measure the amount of napropamide that is used in agriculture, food, and the environment. Gas Chromatography (GC) [3-5], High Performance Liquid Chromatography (HPLC) [6, 7], and UV-visible spectroscopy [2] are the methods that commonly are used to detect and measure the concentration of napropamide in chemical and agricultural laboratories. The disadvantages of the mentioned methods including non-portability, chemical knowledge, standard sample, and the costs of device and the experiment.

The surface plasmon resonance (SPR) technique is a versatile and accurate method for the detection of biomolecules [8, 9]. In order to enhance the sensitivity and selectivity of its, the gold layer should be improved with a sensing layer [8], such as Polypyrrole [10, 11], Polypyrrole -chitosan [12, 13], or Polypyrrole Multi-walled carbon nanotubes [14].

Polypyrrole (PPy) is a high- potential, conducting polymer that is derived from pyrrole. It has conjugated double bonds and good environmental stability and high conductivity [15]. PPy has been used as a biosensor [16, 17] and a gas sensor [18, 19]. The affinity of PPy for organic molecules is based on the intrinsic affinity of the PPy backbone, the affinity of the side groups, and binding to immobilized receptors [20].

Chitosan is poly (b-1-4)-2-amino-2-deoxy-D-glucopyranose, which is derived from chitin. Chitin is a component in the exoskeleton of shellfish. Chitosan has affinity to binding with heavy metal and biomolecules, because the amino (-NH2) and hydroxyl groups are abundant functional groups on chitosan [21, 22], and in acidic medium, the chitosan’s amino groups have positive charges; hence, the chitosan can interact with some biomolecules that have negative charges [23]. The major factor to evaluate the interaction of the chitosan with biomolecules and ions is the degree of deacetylation [24] in the chitosan.

Graphene Oxide (GO) is derived from graphite oxide crystals and it is a single-atomic-layered of all-sp2 hybridized carbon. GO has application in solar cells [25], medicine, biology [26-28], and inorganic optoelectronic devices [29]. The molecular structure of GO includes the hydroxyl (OH−), epoxy (-COO-) groups, and the carboxyl groups, (-COO-) are the main functional group of GO at the molecular structure [30, 31].

In this contribution, the polypyrrole chitosan/Graphene oxide (PPy-Chi/GO) composite layer (sensing layer) was prepared using the electro-chemical method to measure the concentration of napropamide using the surface plasmon resonance technique. The field effect electron microscopy FE-SEM) and X-ray diffraction (XRD) were used to characterize the sensing layer.

2. **Methods**

2.1. **Preparation of Graphene Oxide**

In 2011, Huang et al. [32] reported the preparation of graphene oxide. Briefly, the graphene oxide was obtained from oxidation of graphite. In this process, H2SO4:H3PO4 (320:80 ml), graphite splinter, and KMnO4 (18 g) were mixed using a magnetic stirrer for 3 days to form the GO; the color of the mixture changed to dark brown. Afterward, the H2O2 solution was added to stop the oxidation process. The graphite oxide that formed was washed three times with 1 M of HCl aqueous solution and repeatedly with deionized water until a pH of 4–5 was achieved. The washing process was carried out using a simple decantation of supernatant via a centrifugation technique having a centrifugation force of 10,000 g. During the washing process using deionized water, the graphite oxide experienced exfoliation, which resulted in at thickening of the graphene solution, forming a GO gel. The final concentration of GO was 2 mg/ml, and for this experiment, the final solution was dissolved in deionized water, achieving 0.3 mg/ml.
2.2. Preparation of sensing layer
The gold layer was deposited on microscope glass slide using sputtering coating device (K757 Turbo) at a thickness of 45 nm prior to electro-deposition of sensing layer. The preparation of a sensing layer has been described in ref. [12]. Briefly, the sensing layer was coated on a gold layer by electrochemical deposition using a potentiostat (Model PS 605). The anodic potential of the working electrode was 1.1V relative to a saturated calomel electrode. The polymers were potentiostatically prepared in a solution containing 0.3M pyrrole, 0.1M PTS dopant, and 0.7% w/v of CHI in acetic acid at room temperature, and the concentration of GO was 1 mg/L in final solvent. The morphology and structure of prepared layer on gold side of microscope glass slide were achieved X-Ray diffraction (XRD, Ital Structure, APD 2000) and field effect scanning electron microscopy (FE-SEM, FEI NANOSEM 230).

2.3. SPR Setup
Figure 1 is the SPR setup based on prism in the Kretschmann configuration. It contains a He-Ne laser, a chopper, a polarizer, a pinhole, a precision rotation stage, a high index (ZF52, Focitek), a flow cell, a silicon detector, and a lock-in amplifier. The lock- in amplifier was connected to a computer to register the SPR sensor. The PPy-Chi/GO composite layer (sensing layer) was attached to a high index prism using refractive index matching gel, and the flow cell was attached to the prism. The probe samples flowed into the flow cell separately. The rotation stage was rotated up to 40° in increments of 0.016°. The intensity of the laser beam was registered when the rotation stage was stopped momentarily. The computer program saved the angle of rotation and the intensity of He-Ne laser.

![Figure 1. SPR setup contains a He-Ne laser (633 nm, 2 mW), a Chopper (New focus, Model:3501) , Polarizer, pinhole (Φ=2 mm), precision rotation stage(Sigma KoKi, Model:SGSP-60 YAW-0B), prism (n=1.83956, A=60°) photo-detector (Thorlab, PD A100A ) and Lock in amplifier(Stanford Research, SR530).](image)

The analysis of SPR signals were done based on the Fresnel equation. The transverse mode (TM) was generated using a polarizer for excitation of the surface plasmon. The reflectivity (R=rr’ ) [12] was calculated from the reflective coefficient (r) of the layers based on the matrix method [33]. The resonance angle was obtained with minimizing the equation

$$\Gamma = \sum (R_{\text{Theory}}(n, \theta) - R_{\text{Experiment}}(n, \theta))$$  \hspace{1cm} (1)

2.4. Preparation of solution
In order to prepare the solution of napropamide, 100 mg of C₁₇H₂₁NO₂ was dissolved in 1 liter of hexane at 35°C for producing in 100 ppm. Then, other concentrations (0.1 ppm, 0.5 ppm, 1 ppm, 5 ppm, 10 ppm, 25 ppm, 50 ppm, and 75ppm) were prepared by a systematic dilution of the 100 ppm of C₁₇H₂₁NO₂ in solution.
3. Results and Discussion

3.1. Characterization of PPy-Chi/GO composite layer

Figures 2(a) and 2(b) show the FE-SEM image for the PPy-Chi layer [12, 13, 34-36] and the PPy-Chi/GO composite layer. The PPy-Chi appeared within the GO sheet, which was covered by the PPy-Chi composite.

![FE-SEM image a) PPy-Chi b)PPy-Chi/GO.](image)

Figure 2. The FE-SEM image a) PPy-Chi b)PPy-Chi/GO.

Figure 3 shows the XRD pattern of PPy-Chi/GO. The broad peak about the 25° to 27° is related to PPy-Chi [37] which is match to literature. The peak rise at 9.9° is correspond to GO. This results authenticated the PPy-Chi composite file was formed with graphene oxide (GO).

![XRD Pattern of PPy-Chi/GO layers.](image)

Figure 3. XRD Pattern of PPy-Chi/GO layers.

The electro-deposition time (t) was shifted from 1 to 30 min to determine a different thickness layer. The profilometer measured the thickness of the layers with a limitation of about 5 nm. The thickness of the PPy- GO composite layers was increased from 8 to 60 nm, and Fig. 4 shows the variation of thickness versus electro-deposition time (t). The refractive indices of the layers were measured using the surface plasmon resonance technique. The layers were attached to the prism separately, and they were in contact with water (n=1.3317). Afterward, the SPR signal was registered (Fig. 5) for analysis and calculation of the refractive index of the layer. The resonance angle and the refractive index of each layer were calculated using the matrix method based on the Fresnel equation [9, 12, 33]. Figure 5 shows that the resonance angle was shifted from 54.783° to 70.079° and that it was a function of the refractive index.

![Variation of thickness of layer versus electro-deposition time.](image)

Figure 4. Variation of thickness of layer versus electro-deposition time.
and the thickness (d) of the layers. The real (n) and imaginary (k) parts of the refractive index were shifted from 1.7872 to 1.7504 and 0.164 to 0.218, respectively. Figure 6 depicts that the imaginary part of the refractive index increased monotonically as expected from the Kramers–Kroning formula [38]. The pertinent parameters are listed in Table 1.

![Figure 5](image_url)

**Figure 5.** SPR signal related to different thickness of PPy-Chi/GO composite layer. Dot points are experimental data and solid lines are the reflectance theory fit to the experimental data.

![Figure 6](image_url)

**Figure 6.** Variation of real (n) and imaginary (k) parts of refractive index versus thickness of PPy-Chi/GO composite layers.

| t (min) | d (nm) | Resonance angle (deg) | n       | k       | Reflectance R |
|---------|--------|-----------------------|---------|---------|---------------|
| 1       | 8      | 54.783                | 1.7872  | 0.164   | 0.041         |
| 5       | 14     | 58.892                | 1.7814  | 0.168   | 0.101         |
| 10      | 18     | 58.437                | 1.7763  | 0.176   | 0.152         |
| 15      | 28     | 62.767                | 1.7735  | 0.187   | 0.270         |
| 20      | 35     | 65.512                | 1.7663  | 0.191   | 0.346         |
| 25      | 44     | 68.117                | 1.7589  | 0.204   | 0.443         |
| 30      | 60     | 70.079                | 1.7504  | 0.218   | 0.564         |

**Table 1.** The pertinent parameters of the PPy-Chi/GO layer.

**3.2. Sensing the napropamide**

The pure hexane (n= 1.3724) was loaded in the flow cell, and it contacted to the PPy-Chi/GO sensing layer with 18 nm thickness. The SPR signal was registered to determine the baseline prior to doing the experiments. Figure 7 shows the SPR signal at the baseline, and the angle of resonance was 61.662°.
The napropamide was dissolved in hexane prior to the experiment at concentrations in the range of 0.1 to 100 ppm. The samples were loaded in the flow cell separately, and the SPR signals were registered using a computer program. In order to obtain the sensogram, the experiment was repeated 15 times at room temperature for each concentration of napropamide. The SPR signals were analyzed using the matrix method, and the resonance angles were obtained using Eq. (1). The angle shifts were calculated by subtracting the angle of resonance for each concentration at different times from the angle of resonance at the baseline. Figure 8 shows the sensograms for different concentrations of napropamide. The sensograms indicated the variation of angle shift with time. As a result, variation of the angle shift for 0.5 ppm was larger than for 0.1, and the variation of angle shift was constant after 250 minutes, which was its terminal value.

\[ \Delta \theta = \Delta \theta_{term} (1 - \exp(-k_a t)) \]  

(2)

where \( \Delta \theta \) and \( t \) are the angle shift and response time of the sensor, respectively. The angles shifts at the terminal value are a function of the concentration of napropamaide. Figure 9 shows the variation of
the angle shift at the terminal value versus concentration of napropamide. The data fit well to Langmuir equation [39] well, as follows:

$$\Delta \theta = \frac{K \Delta \theta_{\text{max}} C}{1 + KC}$$  \hspace{1cm} (3)

where $\Delta \theta_{\text{max}}$, $C$, and $K$ are the maximum value of resonance angle shift, concentration of ions, and affinity constant, respectively.

![Fig. 9. Variation of the angle shift at the terminal value versus concentration of napropamide.](image)

4. Conclusion

A polypyrrole-chitosan/graphene oxide layers were prepared using electro-deposition in the range of 8 to 60 nm thickness. The XRD pattern of PPy-Chi/GO layer confirmed the formation of PPy-Chi in the presence of GO. The refractive indices of the layers were measured using the SPR technique. Consequently, the real and imaginary parts of refractive index were in the range of 1.7872 to 1.7504 and 0.164 to 0.218, respectively. Napropamide was dissolved in hexane with different concentration in the range of 0.1 to 100 ppm. PPy-Chi/GO sensing layer could detect the napropamide and the angle shift was in the range of 0.012° to 2.003°. Consequently, PPy-Chi/GO sensing layer can detect the napropamide with the limitation about 0.1ppm.

Acknowledgement

The authors acknowledge Universiti Putra Malaysia for the fund from the Research University Grant Scheme (Putra Grant, vote 941300) and the postdoctoral fellowship under the Institute of Advance Technology (ITMA).

References

[1] Edwards D 2005 Reregistration Eligibility Decision for Napropamide, United States Environmental Protection Agency.
[2] Sadegh-Zadeh F et al 2011 Int. J. of Soi. Sci. 6 199.
[3] Antonious G F and Patterson M A 2005 J. Environ. Sci. Health B. 40 385.
[4] Antonious G F, Patterson M A and Snyder J C 2005 Bull. Environ. Contam. Toxicol. 75 797.
[5] Kim Y-K 2004 J. of Civil Eng. 8 619.
[6] Biswas P K et al 2007 Bull. of Environ. Contam. and Toxicol. 79 566.
[7] Lu J, Wu L, Letey J and Farmer W J (2002) J. Environ. Qual. 31 1234.
[8] Schasfoort R B M and Tudos A J 2008 Handbook of surface plasmon resonance (London, RSC Publishing).
[9] Homola J 2006 Surface plasmon resonance based sensors (Berlin, Springer-Verlag)
[10] Jorn C C et al 2004 Sens. Actuators B 101 236.
[11] Sadrolhosseini A R et al 2012 AIP Conf. Proc., 1482 200.
[12] Sadrolhosseini A R 2011 Optical review 18 331.
[13] M Abdi M et al 2011 PLoS one, 6 e 24578.
[14] Sadrolhosseini A R 2014 PlosOne, 9 e93962.
[15] Chougule M A et al 2011 Soft Nanoscience Letters, 1 6.
[16] Vidal J C, Garcia E and Castillo J R 1999 Analytica Chimica Acta, 385 213.
[17] Campbell T E, Hodgson A J and Wallace G G 1999 Electroanalysis, 11 215.
[18] Kincal D et al 1998 Synthetic Metals 92 53.
[19] Kemp N T et al 1999 Synthetic Metals, 101 434.
[20] Lange U, Roznyatovskaya N V and Mirsky V M 2008 Analytica Chimica Acta 614 1.
[21] Guibal E 2004 Sep. Purif. Technol. 38 43.
[22] Monteiro O A C and Airoldi C J 1999 Colloid Interface Sci. 212 212.
[23] Croisier F and Jerome C 2013 European Polymer 49 780.
[24] Tikhonov V E, Radigina L and Yamskov Y A 1996 Carbohydr. Res. 290 33.
[25] Zhu Y et al 2010 Adv. Mater. 22 3906.
[26] Song Y et al 2010 Adv. Mater. 22 19 2206.
[27] Lv M et al 2012 Nanoscale. 7 3861.
[28] Hu W et al ACS Nano. 4 4317.
[29] Choe M et al Physics Letters 101 031115.
[30] Eda G and Chhowalla M 2010 Adv. Mater. 22(2010) 2392-2415.
[31] Sutar D S, Singh G and Botcha V D 2012 Appl. Phys. Lett. 101 103103.
[32] Huang N M et al 2011 Inter. J. Nanomedicine 6(2011)3443-3448.
[33] Sadrolhosseini A R, Noor A S M and Moksin M M 2009, in Ki Young Kim (Eds) Plasmonics-Principles and Applications; INTECH., 253
[34] Xing S and Zhao G 2006 polym Bull. 57 933.
[35] M Abdi M et al 2009 J. Mater. Sci. 44 3682.
[36] Sadrolhosseini A R, Moksin M M, M Abdi M and Mohammadi M 2012 Int. J of Polym. Mater. 62, 5 284.
[37] M Abdi M et al 2010 Polymer Science Ser. B, 52 662.
[38] Chah S, Yi J and Zare R N 2004 Sens. Actuators B 99 216.
[39] Forzani E S et al 2005 Environ. Sci. Technol. 39 1257.