*Tạp chí Khoa học và Công nghệ **52** (6) (2014) 777-785*

IMPEDIMETRIC IMMUNOSENSOR FOR ATRAZINE DETECTION BASED ON POLYPYRROLE NANOWIRES

Nguyen Van Anh[1, *], Nguyen Van Quan[1], Nguyen Hai Binh[2], Nguyen Le Huy[1], Le Trong Huyen[1], Tran Dai Lam[2]

[1]*School of Chemical Engineering, Hanoi University of Science and Technology, Số 1, Dai Co Viet Road, Hai Ba Trung, Ha Noi, Vietnam*

[2]*Institute of Materials Science, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road., Cau Giay, Ha Noi, Vietnam*

[*]Email: anh.nguyenvan@hust.edu.vn

Received: 17 January 2014; Accepted for publication: 21 May 2014

ABSTRACT

An impedimetric immunosensor based on polypyrrole (PPy) nanowires was developed and applied for atrazine (ATZ) herbicide detection. In this work, the PPy nanowires were synthesized by an electrochemical free-template method onto an interdigitated platinum micro-electrode (IDµE). The immunosensors were prepared by immobilization of monoclonal anti-atrazine antibody (α-ATZ) onto a PPy surface using glutaraldehyde as a cross-linker. The electrodes were characterized using scanning electron microscopy (SEM), cyclic voltammetry (CV) and Fourier transform infrared spectroscopy (FT-IR) techniques. The ATZ analysis is based on the immunoreaction of ATZ and α-ATZ attached on top of the IDµE area (fingers and inter-digits space) by means of electrochemical impedance spectroscopy (EIS). Thanks to immunoreagent specificity, these sensors exhibit high selectivity and sensitivity for ATZ detection with a threshold of 10 ng/mL.

Keywords: atrazine, polypyrrole nanowires, immunosensors, impedimetric.

1. INTRODUCTION

Pesticides and herbicides are widely used in agriculture and horticulture for a longtime. Atrazine (1-Chloro-3-(ethylamino)-5-(isopropylamino)-2,4,6-triazine; ATZ), a triazine herbicide, is photosystem II (PSII) inhibitors commonly used to control broadleaf and grassy weeds in crops including corn, sorghum and sugarcane. Many methods have been developed for atrazine detection, such as gas chromatography (GC) [1], high-performance liquid chromatography (HPLC) [2]; gas chromatography/mass spectrometry (GC/MS) [3], liquid chromatography/mass spectrometry (LC/MS) [4], and thin layer chromatography (TLC) [5]. These methods are very accurate and low detection limits but require sample pretreatment, expensive equipment and high-purity chemicals for the mobile phases. Moreover, chromatographic methods cannot suitable for on-site analysis. In recent years, electrochemical biosensors have revolutionized the modern analytical chemistry due to their accuracy, easy use, high efficiency, portability and miniaturization. In addition, they offer fast (few seconds) response times, allow a rapid and permanent control, and a direct transduction of the biomolecular recognition event into electronic signals [6]. Electrochemical impedance spectroscopy (EIS) is a sensitive technique based on monitoring the electrical response of a device after application of a periodic small amplitude AC signal in a wide range of frequencies. EIS is able to record directly information on biorecognition events, occurring at the electrode surfaces, inducing capacitance and resistance changes [7]. The analysis of the impedance values measured provides information concerning the electric properties of the sensor–sample interface and the underlying reactions.

The objective of this work has been the development of impedimetric immunosensors for atrazine herbicide analysis. The use of free-template technique for polypyrrole nanowires (PPy NWs) modified interdigitated platinum micro-electrodes (IDµE) improves the conductivity of the sensors, and increases the transduction of the chemical signal to electrical signal.

2. EXPERIMENTALS

2.1. Chemicals and biological

Monomer pyrrole (Py) 99 %, $LiClO_4$, Na_2HPO_4, NaH_2PO_4, and 25 % glutaraldehyde (GA) were purchased from Sigma. 0.1 M and 0.2 M phosphate buffer solutions (PBS) with the molar ratio of $[Na_2HPO_4]/[NaH_2PO4]$ = 3.41 were prepared with deionized water and adjusted to pH 6.8 [8]. Monoclonal anti-atrazine antibody (α-ATZ, Mw = 150 kDa) from Thermo Scientific, USA. Atrazine, 99.5 % pure, atrazine standard solution (1 mg/mL) and diluted solutions were prepared in PBS. All chemicals and solvents were of analytical grade.

2.2. Instrumentation

Electrochemical measurements were carried out using an Autolab PGSTAT30 Electrochemical Analyser (EcoChemie, Netherlands) under the control of GPES and FRA version 4.9 at room temperature. The experiments were carried out using a conventional three-electrode system with interdigitated planar platinum-film microelectrodes (IDµE) as the working electrode (Figure 1A, the detailed fabrication process have been described in [9]), a Pt wire as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode.

The IR spectra of the composites were recorded in Nicolet Impact-410 IR spectrometer in KBr medium at room temperature in the region 4000–450 cm^{-1}. Field-emission scanning electron microscope (FE-SEM) images of the films were recorded with Hitachi S-4800 (Japan).

2.3. Electrodeposition of polypyrrole nanowires onto the interdigitated µ-electrodes

In this work, the template-free electrochemical procedure was performed for the deposition of PPy NWs which is adapted from the work of Zang and coworkers [8]. The deposition was potentiostatically polymerized at 0.85 V for 120s in an aqueous pH 6.8 solution,containing 0.1M Py, 0.07 M $LiClO_4$ and 0.2 M PBS. The electropolymerization solution was deoxygenated before the experiments by purging with nitrogen for 10 min to remove oxygen from the solution. After the polymerization reaction, the electrodes were rinsed with distilled water and storage into a PBS solution. For comparison, a PPy/IDµE was prepared under the conditions: the deposition potential at 0.85 V for 120 s in the aqueous solution with pH 6.8 containing 0.1 M Py, 0.07 M $LiClO_4$ and 0.1 M PBS.

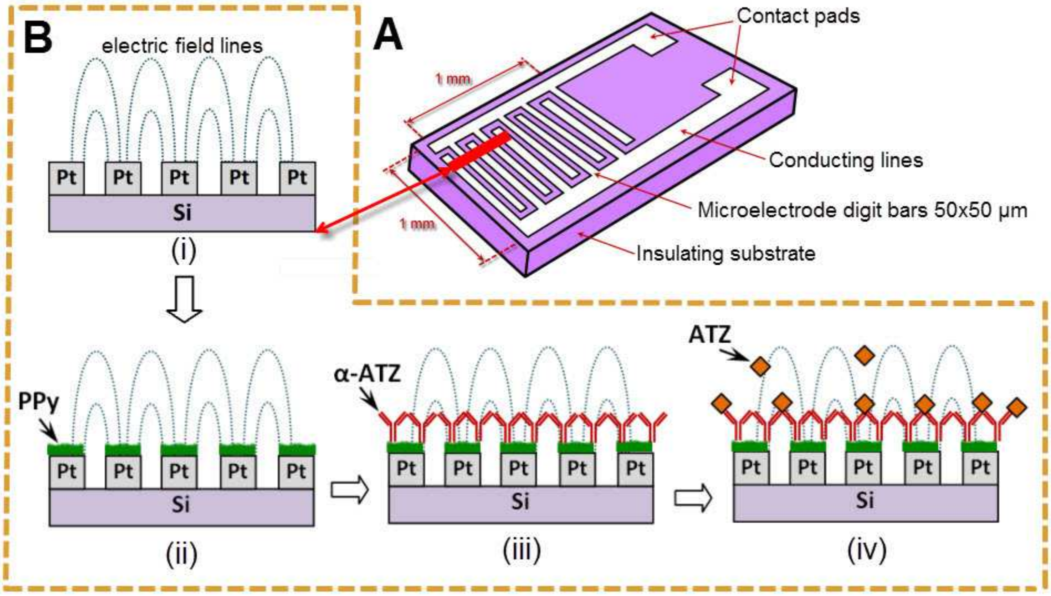

Figure 1. (A) Layout of planar IDμE device and (B) Scheme showing steps used to prepare the immunosensor surfaces and antibody binding.(i) Cross-section of IDμE; (ii) PPy NWs-modified IDμE; (iii) immobilized biomolecules α-ATZ onto PPy NWs/IDμE; (iv) the formation of antibody–antigen interaction in the presence of ATZ.

2.4. Antigen immobilization

Immobilization of α-ATZ onto the surface of the PPy NWs/IDμEs has been done using glutaraldehyde (GA) as a cross-linker. Firstly, the PPy NWs/IDμEs were placed in saturated glutaraldehyde vapor for 60 min then dried in air for 15 min at room temperature. Next, 10 μl of the α-ATZ 0.1 nM solution was spread on the PPy NWs surface using a drop method and left for overnight. The product, the formed α-ATZ/PPy NWs/IDμEs, were rinsed thoroughly with PBS solution and stored at 4 ^{o}C when not in use.

2.5. Immunosensor measurements

Each α-ATZ/PPy NWs/IDμE was placed in a well of a cuvette sample containing the atrazine standards (10 ng/mL ÷ 500 ng/mL). After 30 min of incubation time, they were washed with deionized water. Then, these electrodes were used in impedances measurements. An equimolar ferrocyanide/ferricyanide mixture (5 mM) in PBS solution (pH 6.8) was used as an electrolyte solution. Impedance measurements were recorded in the frequency range 100 kHz and 50 MHz with an AC amplitude of 10 mV. The immunosensors impedance responses were recorded at open circuit potential. The impedance spectra were further analyzed using ZSimpWin ver 3.10.

3. RESULTS AND DISCUSSIONS

3.1. FTIR spectrum of PPy film

The FT-IR spectra of PPy film is shown in Figure 2. The presence of the absorption bands at 1632 cm^{-1} was assigned to the C=C ring stretching of pyrrole. The band at 1401cm^{-1} is due to in-plane deformation of C–H bond and N–H bond of pyrrole ring. The peak at 1118 cm^{-1} is due to C–C stretching. The broad strong bands between 3436 cm^{-1} corresponds to the absorption of N–H stretching of polypyrrole [10]. This result shows that PPy has been deposited onto IDμE by in-situ electropolymerization technique.

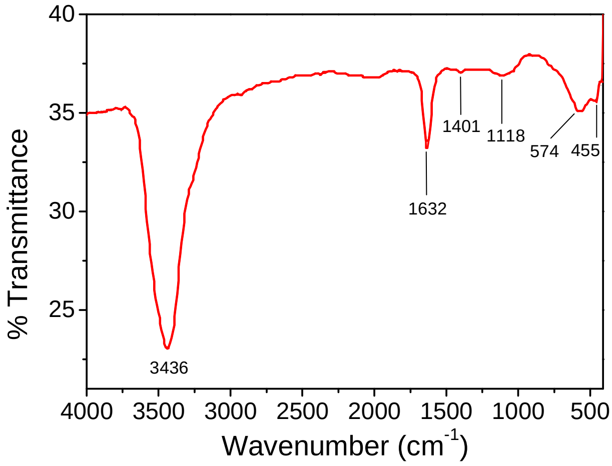

Figure 2. FTIR spectrum of PPy.

3.2. Morphology of synthesized PPy film

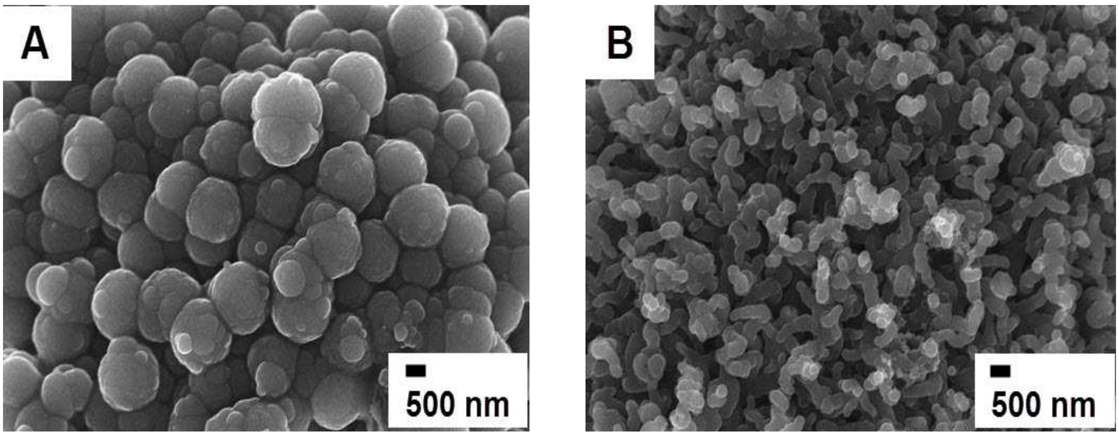

Figure 3. SEM images of PPy cauliflower (A) and PPy nanowires (B).

The morphologies of the PPy nanowires and PPy cauliflower are shown the SEM images in Figure 3. When phosphate (PBS) concentration is 0.2 M, the uniform wire-like PPy nanostructures were obtained (Figure 3B). Meanwhile, the films displayed a cauliflower-like structure of micro-spherical grains when the synthesis at a PBS concentration 0.1 M (Figure 3A). Nanowires, being one-dimensional structures have a higher surface area and shorter diffusion lengths than the analogous bulk materials providing the wires with more attractive properties.

3.3. Electrochemical behavior of PPy nanowires

The electrochemical behaviour of the PPy nanowires and PPy cauliflower were evaluated by cyclic voltammetry in 0.1 M KCl solutions. As shown in the cyclic voltammograms (Figure 4), both samples exhibit electrochemical activity. However, the redox peaks of the PPy nanowires are relatively stronger than those of the cauliflower-like PPy, indicating that the PPy nanowires have a higher electrochemical activity, which may result from the high specific surface area of the nanowires.

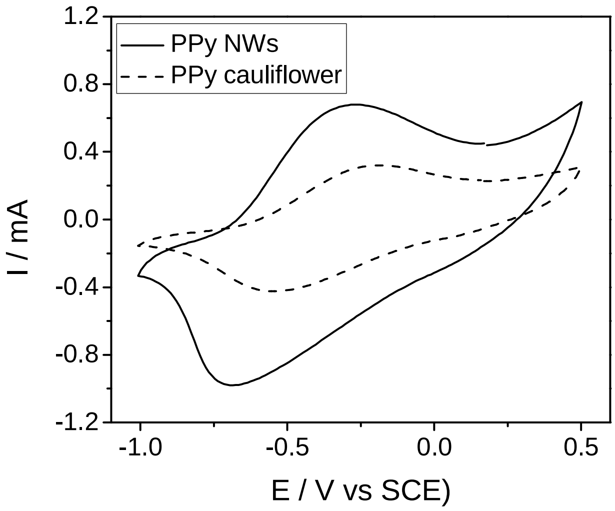

Figure 4. CVs of PPy nanowires and PPy cauliflower in 1M KCl solution at scan rate of 50 mV/s.

3.4. Electrochemical Impedance measurements

According to the literature, IDµE has often performed differential electrochemical measurements (including impedance spectroscopy), which improves the sensitivity thanks to the micro-miniaturization providing a small dielectric gap between the bars of electrode [7, 11, 12]. The formation of antibody–antigen interaction at an electrode surface can change both the charge distribution in the electrical double layer and the resistance of the surface layer; one would anticipate that the impedance could provide information about the association–dissociation kinetics of biological interaction at antibody modified electrode surfaces, as well. Figure 1B illustrates the scheme of this immunosensors. The α-ATZ was first immobilized onto PPy NWs/IDµE. Then, the immuno-electrodes were exposed to ATZ solution. The ATZ interacts with α-ATZ on modified electrode surface as a coating film with the effectively block the charge (electron) transfer and thus amplify impedance signal.

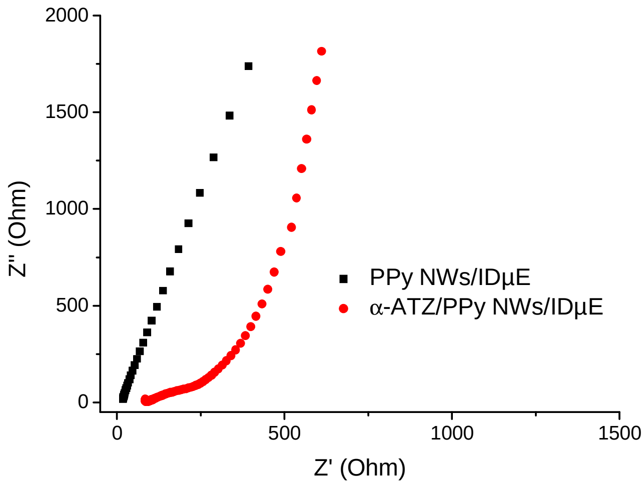

Figure 5. Nyquist plots of EIS of PPy NWs/IDµE (plain squares) and α-ATZ/PPy NWs/IDµE (plain circles) in 5 mM $K_3[Fe(CN)_6]$/ $K_4[Fe(CN)_6]$ (1:1).

Figure 5 shows the Nyquist plots (Z'' *vs.* Z') of the impedance change of the PPyNWs/IDµE before and after immobilization α-ATZ onto surface electrode. As can be seen, the impedance of the film with α-ATZ was greater than that without α-ATZ. This was suggested that α-ATZ is an insulator and, being bounded with PPy via GA cross-linking, may block PPy charge transfer.

For impedimetric ATZ immunosensor, the PPyNWs/IDµE was performed by incubation in the different atrazine concentrations ranging from10 ng/mL up to 500 ng/mL. Then, bioelectrodes capture ATZ in PBS solution outside and transfer electrochemical cell containing a redox probe, $[Fe(CN)_6]^{3-/4-}$. The analysis of EIS was performed via ZSimpWin simulation. The equivalent circuit was shown in Figure 6A (inset) which has proved to fit the experimental measurements. The circuit includes the contact resistance (R_c); capacitance of the electrode (C_e); Ohmic resistance of the electrolyte (R_s); polarization resistance (R_p); Warburg impedance (W); and double-layer capacitance (C_d). The value of R_c controls the origin of the complete impedance spectrum in Z'. The values of R_c and R_s control the end of the second semicircle and the start of the first semicircle in Z'. In this work, the variation of the ohmic resistance of the solution (R_s) from the computer fitting data in relation with atrazine concentration has been selected and analyzed instead the other parameters because the change in the value of R_s was found to be the largest and coherent with all the atrazine concentrations tested. Figure 6B presents the calibration curve of R_s vs. different concentrations of atrazine. A linear range was obtained from 10 to 500 ng/mL of atrazine concentration with a correlation coefficient R = 0.9909.

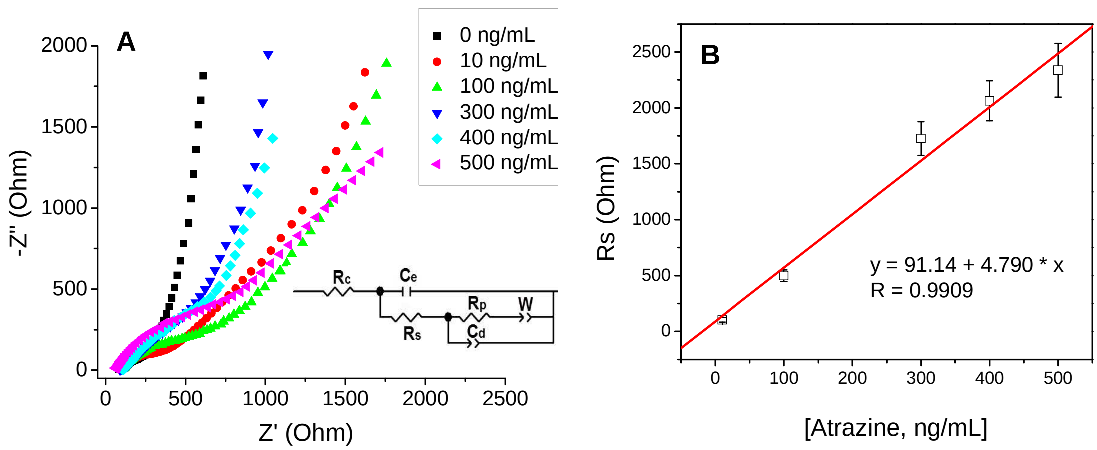

Figure 6. (A) Nyquist plots of impedance spectra corresponding to the immunosensor reaction with different atrazine concentrations and (B) Calibration curve of atrazine immunoassay presented.

4. CONCLUSION

In this work, a template-free technique for the formation of polypyrrole nanowires onto interdigitated platinum micro-electrodes was developed. It promises a concept of immunosensor construction combined with an impedimetric detection of atrazine without reagent and label. Although the experimental measurements were performed only at the laboratory condition it was found that EIS is an impressive technique for detecting the interaction of antigen and antibody. This method can be easily applied for other detection of a broad range of chemical or biological species if the appropriate antibody and competitor are available.

Acknowledgments. This research is funded by Vietnam National Foundation for Science and Technology Development grant (code: 103.02-2012.71).

REFERENCES

1. Amistadi M. K., Hall J. K., Bogus E. R., and Mumma R. O. - Comparison of gas chromatography and immunoassay methods for the detection of atrazine in water and soil, J. Environ. Sci. Health. Part B **32** (6) (1997) 845-860.
2. Beale D. J., Kaserzon S. L., Porter N. A., Roddick F. A., and Carpenter P. D. - Detection of s-triazine pesticides in natural waters by modified large-volume direct injection HPLC, Talanta **82** (2) (2010) 668-674.
3. Ma W. T., Fu K. K., Cai Z., and Jiang G. B. - Gas chromatography/mass spectrometry applied for the analysis of triazine herbicides in environmental waters, Chemosphere **52** (9) (2003) 1627-1632.
4. Di Corcia A., Crescenzi C., Guerriero E., and Samperi R. - Ultratrace Determination of Atrazine and Its Six Major Degradation Products in Water by Solid-Phase Extraction and

Liquid Chromatography, Electrospray/Mass Spectrometry - Environmental Science & Technology **31** (6) (1997) 658-1663.

5. Rezić I. M., Horvat A. J., Babić S., and Kaštelan-Macan M. - Determination of pesticides in honey by ultrasonic solvent extraction and thin-layer chromatography, Ultrasonics Sonochemistry **12** (6) (2005) 477-481.
6. Valera E., Ramón-Azcón J., Sanchez F. J., Marco M. P., and Rodríguez Á. - Conductimetric immunosensor for atrazine detection based on antibodies labelled with gold nanoparticles, Sensors and Actuators B **134** (1) (2008) 95-103.
7. Ramón-Azcón J., Valera E., Rodríguez Á., Barranco A., Alfaro B., Sanchez-Baeza F., and Marco M. P. - An impedimetric immunosensor based on interdigitated microelectrodes (IDμE) for the determination of atrazine residues in food samples, Biosensors and Bioelectronics **23** (9) (2008) 1367-1373.
8. Zang J., Li C. M., Bao S. J., Cui X., Bao Q., and Sun C. Q. - Template-Free Electrochemical Synthesis of Superhydrophilic Polypyrrole Nanofiber Network, Macromolecules **41** (19) (2008) 7053-7057.
9. Trong Huyen L., Ngoc Thang T., Le Huy N., Hai Binh N., Van Anh N., Dai Lam T., and Tuan Dung N. - Electrosynthesis of polyaniline – multiwalled carbon nanotube nanocomposite films in the presence of sodium dodecyl sulfate for glucose biosensing, Adv. Nat. Sci. Nanosci. Nanotechnol. **4** (2) (2013) 025014.
10. Konwer S., Dolui S. K. - Synthesis and characterization of polypyrrole/graphite composites and study of their electrical and electrochemical properties, Mater. Chem. Phys. **124** (1) (2010) 738-743.
11. Sarró E., Lecina M., Fontova A., Solà C., Gòdia F., Cairó J. J., Bragós R. - Electrical impedance spectroscopy measurements using a four-electrode configuration improve on-line monitoring of cell concentration in adherent animal cell cultures, Biosens. Bioelectron. **31** (1) (2012) 258-263.
12. Valera E., Ramón-Azcón J., Rodríguez Á., Castañer L. M., Sánchez F. J., Marco M. P. - Impedimetric immunosensor for atrazine detection using interdigitated μ-electrodes (IDμE's), Sensors and Actuators B. **125** (2) (2007) 526-537.

TÓM TẮT

NGHIÊN CỨU CHẾ TẠO CẢM BIẾN MIỄN DỊCH ĐO TỔNG TRỞ ĐIỆN HÓA XÁC ĐỊNH ATRAZIN TRÊN CƠ SỞ DÂY NANO POLYPYRROL

Nguyễn Vân Anh[1, *], Nguyễn Văn Quân[1], Nguyễn Hải Bình[2], Nguyễn Lê Huy[1], Lê Trọng Huyền[1], Trần Đại Lâm[2]

[1]*Viện Kỹ thuật hóa học, Trường Đại học Bách khoa Hà Nội, Số 1 Đại Cồ Việt, Hai Bà Trưng, Hà Nội, Việt Nam*

[2]*Viện Khoa học vật liệu, Viện Hàn lâm Khoa học và Công nghệ Việt Nam, 18 Hoàng Quốc Việt, Cầu Giấy, Hà Nội, Việt Nam*

[*]Email: anh.nguyenvan@hust.edu.vn

Trong báo cáo này, chúng tôi trình bày những kết quả ban đầu về chế tạo cảm biến miễn dịch đo tổng trở điện hóa trên cơ sở dây nano polypyrrole (PPy) và ứng dụng trong nhận biết thuốc trừ cỏ atrazin (ATZ). Dây nano PPy được trùng hợp trên bề mặt vi điện cực platin cấu trúc răng lược (IDμE) bằng phương pháp điện hóa không dùng khuân. Các kháng thể đơn dòng (α-ATZ) được cố định trên bề mặt PPy thông qua tác nhân tạo liên kết chéo glutarandehit. Nghiên đặc trưng vật liệu được thực hiện bởi phương pháp kính hiển vi điện tử quét (SEM), phương pháp von-ampe vòng tuần hoàn (CV) và phổ hồng ngoại (FT-IR). Tương tác đặc hiệu dựa trên phản ứng miễn dịch giữa ATZ và α-ATZ cố định trên trên điện cực được phát hiện thông qua kĩ thuật tổng trở điện hóa (EIS). Nghiên cứu cho thấy, cảm biến có độ chọn lọc và độ nhạy trong giới hạn rộng, với ngưỡng phát hiện đạt được là 10 ng/mL.

Từ khóa: atrazin, dây nano polypyrrol, cảm biến miễn dịch, tổng trở điện hóa.