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Detection of Benzimidazole Toxins by Molecularly Imprinted Polymer Based On Polyaniline

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ABSTRACT

In present study sensing behavior of molecular imprinting polymer (MIP) based on polyaniline as a monomer in contrast with agronomy fungicides as like as Carbendazim and Tiophanate-methyl as a sample via measurment of electrical conductivity by using a four-probe method was investigated. Molecular imprinting has proved to be an effective technique for the creation of recognition sites on a polymer scaffold. Polyaniline as a conductive polymer has been used in many application like conducting dyes, chemical and gas sensors and it is one of the best choices for this technique. Molecularly imprinted polymers (MIPs) are synthetic materials that mimic antibodies and widely used in pharmaceutical, medical and chemical applications such as chemical sensors. Analyte concentration and polymer response time are the sensing properties studied in this work. The selectivity of molecular imprinting polymer as compared with those of analyte molecular with similar structure was investigated. In all cases, the conductivity of molecular imprinting polymer in comparison with the molecular non-imprinting polymer (NIP) was noticeable. This is due to analyte effect on polymer structure.

Keywords*: Molecular Imprinting Polymer (MIP), Sensor, Polyaniline, Fungicide, Benzimidazole.*

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INTRODUCTION

An analytical sensor is needed to effectively identify the chemical materials in the environment. A sensor should be simple and besides high speed, it should have high sensitivity and selectivity. Natural sensors can use enzyme, antibody, bacteria, tissue, etc as factors of identifying substances, while chemical sensors do not have these factors. These sensors are made of semiconductors, solid electrolytes, insulators, metallic and catalytic materials. Due to the elasticity of polymers, the use of these substances in the identification devices of substances has a great importance. Based on this fact that conductive polymers are sensitive to oxidation and doping gases, applying these polymers as the chemical sensors seems natural. These sensors are able to measure substances at very low concentrations.

One of the most common poisons that are utilized in agriculture is the fungicides. Although, the use of fungicides is beneficial for controlling the pathogenic agents in agriculture, but these pesticides can cause illness and death in humans. This project deals with the production of a sensor based on polyaniline and the use of molecular imprinting method to identify two types of conventional fungicides.

Increasing the selectivity of the respective sensor in accordance with conductometry and based on changes in the electrical conductivity of polymer before and after connecting to the desired toxin, by the use of molecular imprinting method for the preparation of polymers is the proposed method in this research work.

Molecularly imprinted polymers (MIPs) are being utilized in an increasing number of applications, as “tailor-made” separation materials, antibody–receptor binding site mimics in recognition and assay systems, enzyme mimics in catalytic applications, recognition elements in sensors, and in facilitated chemical synthesis(Nor Azah Yusof, Nor Dyana Zakaria, Nor Amirah Mohd Maamor, Abdul Halim Abdullah, Jelas Haron, 2013; Ricardo C, Tarley T, Kubota L.T, 2006; Guney O, Yilmaz Y, Pekcan O, 2002; Kataky R, Morgan E, 2003). To date, their most extensively investigated application has been as separation materials for the analysis of various compounds, including drugs(Weiss R, Molinelli A, Jakusch M, Mizaikoff B, 2001; Walshe M, Garcia E, Howarth J.M, Smyth R, Kelly M.T, 1997), pesticides (Yang G, Liu H, Wang M, Liu S, Chen Y, 2006; Zhang H, Song T, Zong F, Chen T, Pan C, 2008), and amino acids(Shim Y.H, Yilmaz E, Lavielle S, Haupt K, 2004). A highly specific detection technology, MIPs have been used for the separation of isomers and enantiomers(Glad M, Reinholdsson P, Mosbach K, 1995) , solid extraction(Li X, Husson S.M, 2006), in biochemical sensors(Xia Y.Q, Guo T.Y, Song M.D, Zhang B.H, Zhang B.L, 2006) and chemosensors (Tong A, Dong H, Li L, 2002), in simulating enzyme-catalysed pharmaceutical analysis (Owens P.K, Karlsson L, 1999), in sorbents, and in membrane separation technologies(El-Toufaili F.A, Visnjevski A, Brüggemann O, 2004) . They have been prepared in various configurations including polymer beads, monoliths, and membranes—and have numerous advantages, such as physical robustness, high strength, resistance to elevated temperatures and pressures, and inertness towards organic solvents, acids, and bases(Lin Y, Shi Y, Jiang M, Jin Y, Peng Y, Lua B, Dai K, 2008). Further, MIPs are stable, easy to prepare, and inexpensive.

The molecular imprinting method includes the formation of analyte complex (desired poison) with functional monomers in an analyte solution. Analyte will be surrounded by functional

and cross-linking monomers in a non-covalent form. After polymerization, a number of analytes, placed at the surface of the polymer, will be washed out by a suitable solvent and will be removed from the polymer's substrate, as a result, cavities to the size of an analyte and with active sites inside the polymer will be formed. In the following, these cavities can be used for trapping the desired poison and eventually, for identification. The electrical conductivity of the polymer is measured once before connecting to the poison (non- imprinted polymer) and once again after connecting to the poison and forming cavities on it. In recent decades, there has been a rapid expansion for this method because of having an easy synthesis, stability and low cost.

In recent years, conductive polymers carried out applications in this field, and in this case, Polyaniline has potential capabilities. Among all the conducting polymers, polyaniline is known for its unique electrical conductivity which can be controlled by the degree of oxidation of the main chain and protonation. Also, it shows different color changes with respect to the degree of oxidation and the surrounding pH.

Carbendazim ($C_9H_9N_3O_2$), (Figure 1), is a systemic benzimidazole fungicide with molar weight of 191.19 g/mol, against smut of wheat and barley which is used for prevention and treatment. Thiophanate-Methyl ($C_{12}H_{14}N_4O_4S_2$), (Figure 2), is a systemic benzimidazole fungicide with a protective action, molar weight 342.39 g/mol.

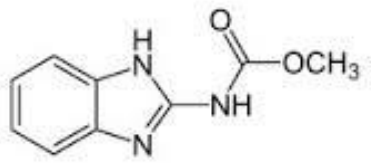

Figure 1: The structure of Carbendazim

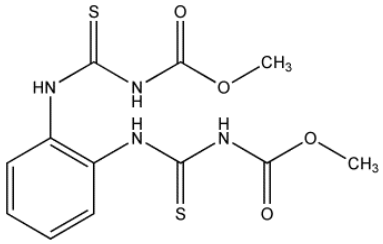

Figure 2: The structure of Thiophanate-Methyl

2. EXPERIMENTALS

2.1 Instrumentals

Scanning electron microscopy (SEM) was performed by a (Hitachi, S-4610) at an operating voltage of 10KV. Prior to scanning, the specimens were coated with a very thin layer of gold. To measure the conductivity a four-point conductivity meter by (WTW, Inolab Cond 7110) was used.

2.2 The preparation of linear polyaniline polymer with Carbendazim

In a beaker, 1 gram Carbendazim was dissolved in 20 ml of distilled water and then, 5.0 ml aniline was added to it and placed in an ice-water bath and then it was placed on the magnetic heater. A primer solution containing 1.19 g of dissolved ammonium per sulfate in 10 ml of distilled water was prepared in a beaker and was added drop by drop to the contents of the first beaker within 10 minutes. Polymerization was continued for 2 hours. Then, the solution became smooth by using a Buchner funnel and was dried in an oven at 70 °C and then, their conductivity was measured.

2.3 The preparation of non-imprinted polymer (NIP) for Carbendazim

Some of the linear polymer from previous step was poured in a beaker and 5 ml of chloroform was added to it. The beaker was covered with a Para-film and was remained in that condition for 2 hours in order to form template on the polymers. Then, the solution became smooth with a Buchner funnel. At first, it was washed with a small amount of chloroform and then with distilled water and was dried in an oven at 70 °C and then, the conductivity was measured.

2.4 The preparation of molecular imprinted polymer (MIP) based on polyaniline for Carbendazim

Firstly, eight standard solutions of 0/01, 0/02, 0/03, 0/04, 0/05, 0/06, 0/07 and 0/08 mol/L were made from the poison of Carbendazim, in 25 ml of chloroform solvent. Then, 0.1 g was added to each separated solution of non-imprinted polymer (NIP) of Carbendazim. Dishes were covered by para-film and were placed in a fixed location for a period of 4 hours. After this term, solutions became smooth with buchner funnel and dried in an oven at 70 °C and their conductivity was measured.

2.5 Investigation of sensing properties of molecularly imprinted polymer based on polyaniline sensitive to Carbendazim

2.5.1 Checking the sensor's response time

At first, from the poison of Carbendazim, a 0.01 mol/L standard solution was prepared in 25 ml of chloroform solvent. Then 5 beakers were taken and each of them was filled with 0.1g of non-imprinted polymer (NIP) of Carbendazim. 3 ml of the prepared standard solution was added to each beaker. Beakers became smooth with a Buchner funnel after 15, 30, 45, 60 and 75

minutes respectively. Then all of the polymers were dried in an oven at 70°C and their conductivity was measured.

2.5.2 Evaluation of the selectivity of the sensor

First of all, a 0.01 mol/L in 25 ml of Chloroform solvent was prepared from three poisons of the Benzimidazole family that includes Thiophanate-methyl, Carbendazim and Iprodion+Carbendazim in order to evaluate the selectivity of the sensor. Then, 0.1g non-imprinted polymer (NIP) of Carbendazim was poured in a beaker and 3 ml of the mixture of three poisons was added to it and placed in a fixed position for 1 hour at room temperature. Then, the solution became smooth with buchner funnel and was dried in an oven at 70 °C and its conductivity was measured.

2.6 The preparation of linear polyaniline polymer with Thiophanate-methyl

In a beaker, 1 gram Thiophanate-methyl was dissolved in 20 ml of distilled water and then, 5.0 ml aniline was added to it and placed in an ice-water bath and then it was placed on the magnetic heater. A primer solution containing 1.19 g of dissolved ammonium per sulfate in 10 ml of distilled water was prepared in a beaker and was added drop by drop to the contents of the first beaker within 10 minutes. Polymerization was continued for 2 hours. Then, the solution became smooth by using a buchner funnel and was dried in an oven at 70 °C and then, their conductivity was measured.

2.7 The preparation of non-imprinted polymer (NIP) for Thiophanate-methyl

Some of the linear polymer from previous step was poured in a beaker and 5 ml of chloroform was added to it. The beaker was covered with a para-film and was remained in that condition for 2 hours in order to form template on the polymers. Then, the solution became smooth with a buchner funnel. At first, it was washed with a small amount of chloroform and then with distilled water and was dried in an oven at 70 °C and then, the conductivity was measured.

2.8 The preparation of molecular imprinted polymer (MIP) based on polyaniline for Thiophanate-methyl

Firstly, eight standard solutions of 0/01, 0/02, 0/03, 0/04, 0/05, 0/06, 0/07 and 0/08 mol/L were made from the poison of Thiophanate-methyl, in 25 ml of chloroform solvent. Then, 0.1 g was added to each separated solution of non-imprinted polymer (NIP) of Thiophanate-methyl. Dishes were covered by Para-film and were placed in a fixed location for a period of 4 hours. After this term, solutions became smooth with buchner funnel and dried in an oven at 70 °C and their conductivity was measured.

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3. RESULTS AND DISCUSSION

3.1 The sensor's response time

The amounts of conductivity and their changes have been reported in diagrams 1 and 2 against the sensor's response time for the concentration of 0/01 mol/l Carbendazim and Thiophanate-methyl at room temperature. It is seen that with the increase in time, the penetration rate of analyte into the polymer cavity will increase. The maximum amount of conductivity is in 75 minutes. Increasing the polymer conductivity and its long response time proves the existence of cavities in the polymer.

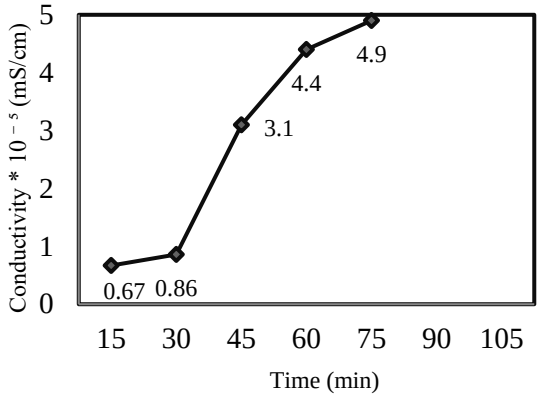

Diagram 1: Conductivity changes based on time increasing for Carbendazim toxin

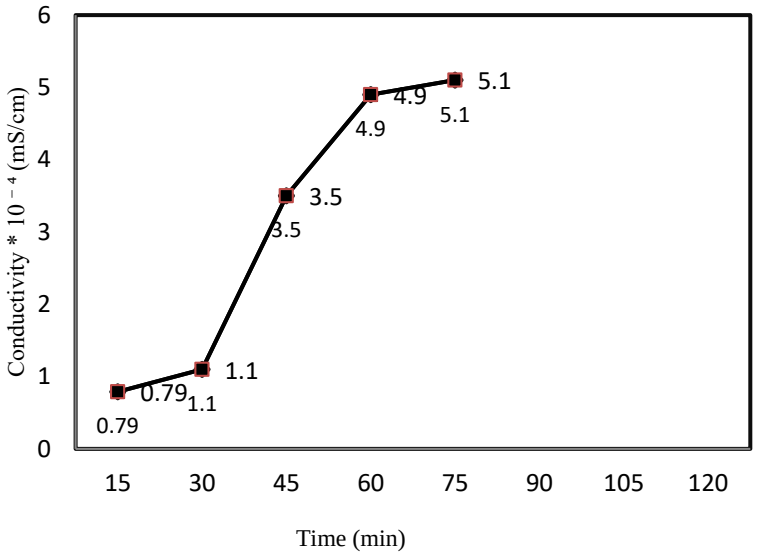

Diagram 2: Conductivity changes based on time increasing for Thiophanate-methyl toxin

3.2 The impact of Analyte concentration

The obtained conductivity values in investigating the impact of analyte concentration and their relationship related to molecular imprinted polymer of Carbendazim and Thiophanate-methyl has been reported in diagrams 3 and 4. At this point, varying concentrations of analyte reacted with the polymer at room temperature for 4 hours. It can be seen that analyte concentration is directly related to the conductivity, this means that with increasing concentration, conductivity will also increase.

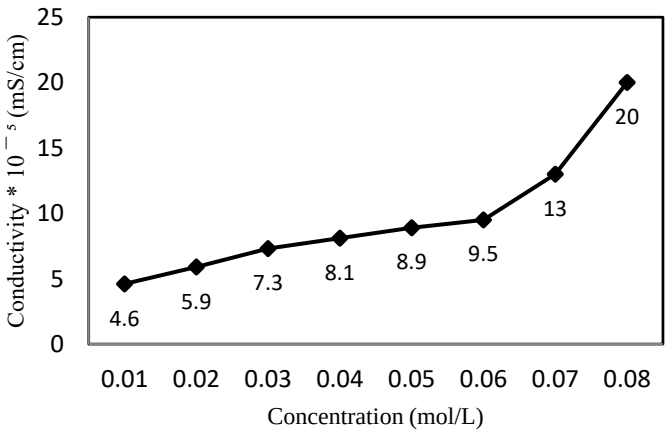

Diagram 3: Conductivity changes based on density increase in Carbendazim

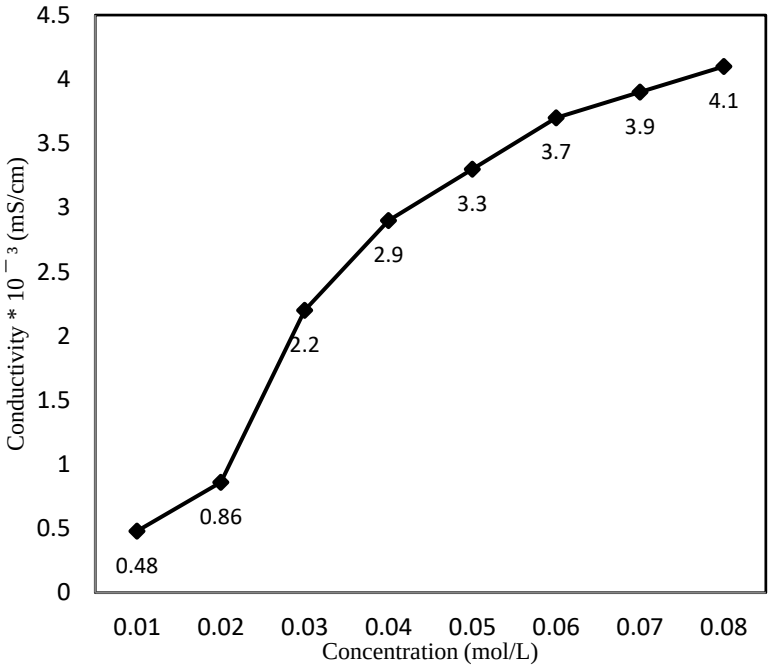

Diagram 4: Conductivity changes based on density increase in Thiophanate-methyl

3.3 The study and comparison of the microscopic properties of molecular imprinted polymers

The image of scanning electron microscope (SEM) for the non-imprinted polymer (NIP) Carbendazim and Thiophanate-methyl is illustrated in figures 3 and 4. The images show a porous surface which is due to the absence of analyte in the polymer. The diameter of emerged cavities is about 200-300 nm.

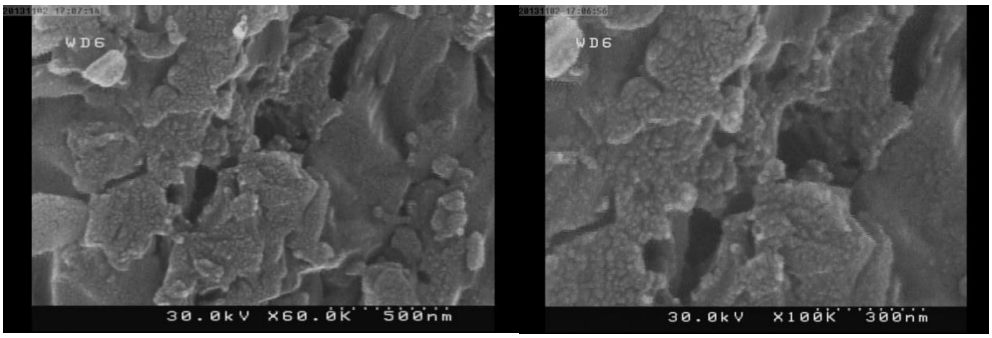

Figure 3: Scanning electron microscopic (SEM) image for non-imprinted polymer (NIP) of Carbendazim toxin

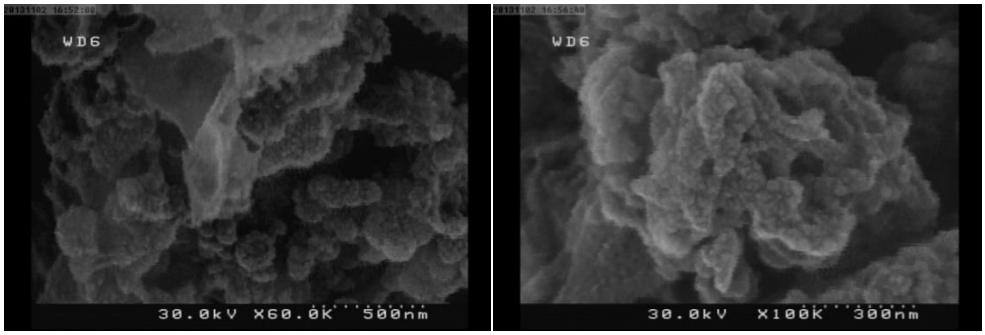

Figure 4: Scanning electron microscopic (SEM) image for non-imprinted polymer (NIP) of Thiophanate-methyl toxin

Figures 5 and 6 show the images of scanning electron microscope (SEM) for the molecular imprinted polymer related to Carbendazim and Thiophanate-methyl after reconnecting analyte in 0.02 mol/L concentrations. The surface of polymer with almost no pores, in comparing with non- imprinted polymer (NIP), shows the presence of analyte. The number of pores is less than NIP, which is due to the presence of molecules that are trapped in the inner cavities.

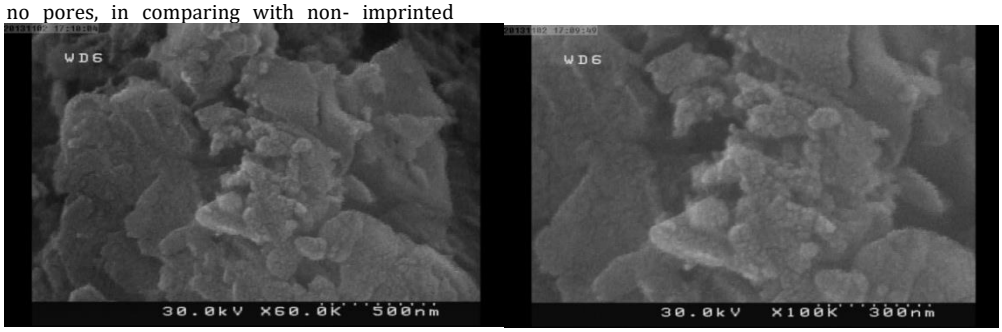

Figure 5: Scanning electron microscopic (SEM) image for imprinted polymer (MIP) of Carbendazim toxin

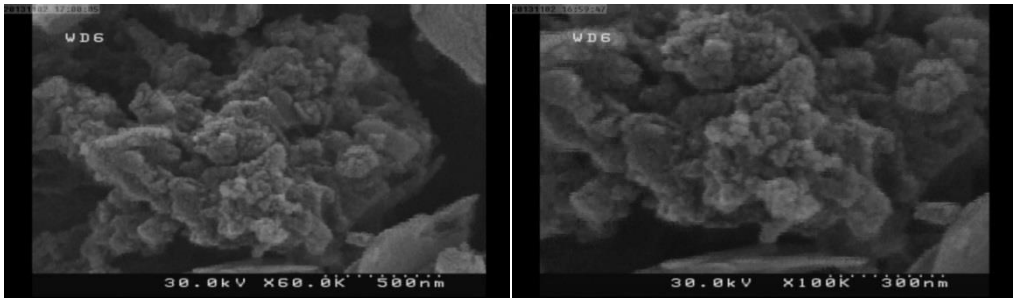

Figure 6: Scanning electron microscopic (SEM) image for imprinted polymer (MIP) of Thiophanate-methyl toxin

CONCLUSION

We have synthesis polyaniline as molecular imprinting polymer for sensing Benzimidazol agronomy fungicides. The sensing behavior of molecular imprinting polymer was measured for molecular imprinting polymer (MIP) and non-imprinting polymer (NIP) by FT-IR and using a four-point probe conductivity meter method. Among sensing properties studied in this work can mention to analyte concentration and polymer response time. Sensing results for Benzimidazols show that molecular imprinted surface in combination with conductometric method and scanning electron microscopic (SEM) are a useful approach for the sensing applications.

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