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ORIGINAL ARTICLE **OPEN ACCESS**

Study the Sensory Effects of Toxic Compounds of Benzimidazoles by Conductometry

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ABSTRACT
Sensors have different kind. The sensors based on electrical conductivity are considered as one of chemical sensors. Lack of selectivity is among deficiencies of these sensors. This feature has limited the application of these sensors in different industries. The proposed method in this research work is to increase the selectivity of sensor based on the electrical conductivity by molecular molding in order to produce polymer. In recent years, conductive polymers carried out applications in this field and in this case, polyaniline has potential capabilities. In the current research, the sensing effects of Benzimidazole compounds were studied with conductometry by using molecularly imprinting method. 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.
KEYWORDS: *Sensor, Conductometry, Molecular Imprinted Polymer (MIP), Polyaniline, Benzimidazoles.*

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INTRODUCTION

Contamination of food products is an important issue which there is a lot of negligence about it. Primary and secondary contaminations of these products cause such diseases that overtly and covertly lead to death in humans. Micro-organisms and pesticides cause much short-term and long-term intoxication. Short-term intoxication has visible traces, therefore, prevention and rapid recovery is possible, but long-term intoxication resulted from pesticides are associated with serious problems and illnesses.

Unfortunately, a much more important contamination which is found abundantly in food products does not show any research consideration, due to its hidden nature. The contamination is the existing toxins in the agricultural products and indirectly in the protein products. Pesticides which are used by farmers to destroy pests and food products diseases are far more lethal and more problematic than the pathogenic microorganisms.

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 three 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.

Molecular imprinting is the most applicable method for the introduction of molecular recognition properties in synthetic polymers in response of the presence of template species during formation of the three-dimensional structure of the highly cross-linked polymer [1], [2]. However, new MIP formats are being developed to avoid the limitations of the traditional approach: long preparation times, mechanical deformation of the binding sites during grinding of bulk polymers, and a time-consuming sieving procedure [3].

The molecular imprinting method includes the formation of analyte complex (desired poison) with functional monomers in an analyte solution [4]. 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 [5], [6]. 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 [7].

Carbendazim is a systemic Benzimidazole fungicide with molar weight of 191.19, against smut of wheat and barley which is used for prevention and treatment. Thiophanate-Methyl is a systemic Benzimidazole fungicide with a protective action, molar weight 342.39. Iprodion+Carbendazim are a systemic and contact fungicide with molar weight of 594.19 and from the group of de Carbamate+Benzimidazole with wide range of effects for prevention and treatment.

Conductometry is an electrolyte solution containing electrical current and according to equation (1), the Ohm's law can be used for it. Current (I) is a rate of flow and is measured in amps, E is the applied potential difference in volts and R is the resistance of the solution in ohms. Electrical conductivity of the solution is defined as the reverse of the resistance and is shown with a C.

$$E = IR \tag{1}$$

There are two standard methods for measuring the resistivity of semiconductor samples: by the use of two-point technique and a four-point technique.

The four-point technique shows a rapid action in measuring the conductivity and there is no need of having a sample with dimensions and fully certain cross sections. This method is mainly used for samples which have irregular shapes, and only a small flat area of sample is required to contact the probe. Four-point method is just used for those samples which their thickness and the distance of each probe to the nearest edge are at least four times bigger than the distance between probes. The geometric correction can be done for further accuracy of obtained samples.

In this method, four probes are placed on a straight line, on a flat surface of a semiconductor solid sample. A direct current has been passed through the sample by two external pins and the potential difference between them is measured by a voltmeter with the use of two internal needles. The resistance of the sample is calculated by using input current and applied potential difference and the geometrical appropriate factors. The resistance is measured by the formula (2) for circular cross-sections in the four-point technique.

$$P = 2\pi S \frac{V}{I} \tag{2}$$

P is the resistance of the example; S is the distance between the needles and V is the potential difference and I is the current strength. The conductivity of the sample is obtained by reversing the P value.

Polymeric samples can be pulverized in a mortar to come in powder form. Then, about 0.3 grams of this powder is used to form a round compressed tablet by machines pressing at a pressure of 6 tons. After the thermal equilibrium, we measure the amount of conductivity for polymeric tablets by using the four-point system and then, with the use of equation (2), the amount of conductivity of the prepared polymers can be measured. Needles or probes are at the distance of 0.1 cm from each other.

MATERIAL AND METHODS

Instrumentals

Fourier Transform Infrared spectra (FT-IR) of the samples in KBr pellets were recorded on an (Perklin-Elmer RX1) spectrometer. To measure the conductivity a four-point conductivity meter by (WTW, **Inolab Cond 7110)** was used.

The preparation of linear polyaniline polymer with toxin

In a beaker separately for each toxin, 1 gram toxin 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.

The preparation of non-imprinted polymer (NIP)

Some of the linear polymer from previous step was poured in a beaker separately for each toxin 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.

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

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 toxins separately, in 25 ml of chloroform solvent. Then, 0.1 g was added to each separated solution of non-imprinted polymer (NIP) of toxins. 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.

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

Evaluation of the selectivity of the sensors

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 of each toxin was poured in a beaker separatelu 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.

Checking the sensor's response time

At first, from the toxins, a 0.01 mol/L standard solution was prepared in 25 ml of chloroform solvent separately. Then for each toxin 5 beakers were taken and each of them was filled with 0.1g of non-imprinted polymer (NIP) of each toxin. 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.

RESULTS AND DISCUSSION

Molecular imprinted polymer sensitive to carbendazim based on polyaniline

The amount of polymer's conductivity before and after extraction of analyte is as follows:

Polymer conductivity before extraction = 3.2×10^{-4} ms/cm

Polymer conductivity after extraction = 4.3×10^{-5} ms/cm

It can be seen that the conductivity, before being washed with chloroform, is much more than the amount of conductivity after the washing process and this is due to the removal of some carbendazim molecules from the surface of the polymer, in other words, the leaching of molecules that have been absorbed is the reason of this increase.

Investigating the selectivity of the sensor Carbendazim

The polymer's ability in detecting analytes from invasive species, which are structurally similar to analyte (selectivity), is very important for sensor applications. The conductivity of the molecular imprinted polymer of Carbendazim against Iprodion+Carbendazim and Thiophanate-methyl, which is structurally similar to Carbendazim molecule, was 4.6×10^{-5} mS/cm. This conductivity is close to the conductivity of the obtained molecular imprinted polymer after the extraction of analyte, and this result shows the sensitivity and selectivity of the MIP Carbendazim towards this poison.

Molecular imprinted polymer sensitive to Thiophanate-methyl based on polyaniline

The amount of polymer's conductivity before and after extraction of analyte is as follows:

Polymer conductivity before extraction = 2.6×10^{-2} ms/cm

Polymer conductivity after extraction = 2.8×10^{-4} ms/cm

It can be seen that the conductivity, before being washed with chloroform, is much more than the amount of conductivity after the washing process and this is due to the removal of some Thiophanate-methyl molecules from the surface of the polymer, in other words, the leaching of molecules that have been absorbed is the reason of this increase.

Investigating the selectivity of the sensor Thiophanate-methyl

The polymer's ability in detecting analytes from invasive species, which are structurally similar to analyte (selectivity), is very important for sensor applications. The conductivity of the molecular imprinted polymer of Thiophanate-methyl against Iprodion+Carbendazim and Carbendazim, which is structurally similar to Thiophanate-methyl molecule, was 2.9×10^{-4} mS/cm. This conductivity is close to the conductivity of the obtained molecular imprinted polymer after the extraction of analyte, and this result shows the sensitivity and selectivity of the MIP Thiophanate-methyl towards this poison.

Molecular imprinted polymer sensitive to Iprodion+Carbendazim based on polyaniline

The amount of polymer's conductivity before and after extraction of analyte is as follows:
Polymer conductivity before extraction = 8.6×10^{-3} ms/cm
Polymer conductivity after extraction = 2.4×10^{-5} ms/cm
It can be seen that the conductivity, before being washed with chloroform, is much more than the amount of conductivity after the washing process and this is due to the removal of some Iprodion+Carbendazim molecules from the surface of the polymer, in other words, the leaching of molecules that have been absorbed is the reason of this increase.

Investigating the selectivity of the sensor Iprodion+Carbendazim

The polymer's ability in detecting analytes from invasive species, which are structurally similar to analyte (selectivity), is very important for sensor applications. The conductivity of the molecular imprinted polymer of Iprodion+Carbendazim against Thiophanate-methyl and Carbendazim, which is structurally similar to Iprodion+Carbendazim molecule, was 2.3×10^{-5} mS/cm. This conductivity is close to the conductivity of the obtained molecular imprinted polymer after the extraction of analyte, and this result shows the sensitivity and selectivity of the MIP Iprodion+Carbendazim towards this poison.

The sensor's response time

The amounts of conductivity and their changes have been reported in diagram 1, 2 and 3 against the sensor's response time for the concentration of 0.01 mol/L Carbendazim, Thiophanate-methyl and Iprodion+Carbendazim 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.

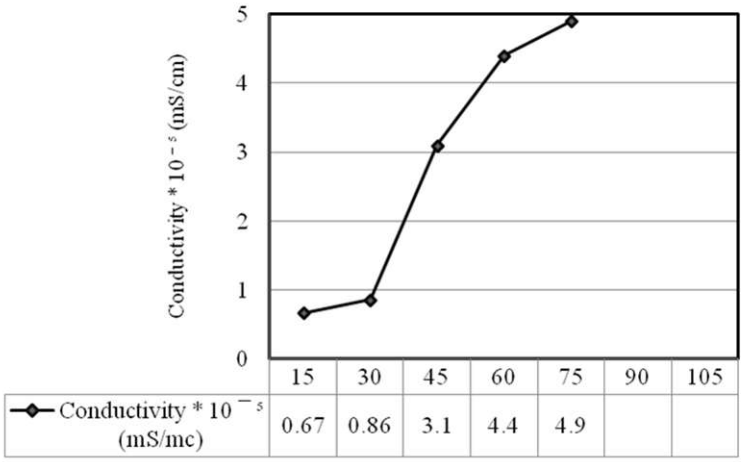


Diagram 1: Conductivity changes based on time increasing for Carbendazim toxin

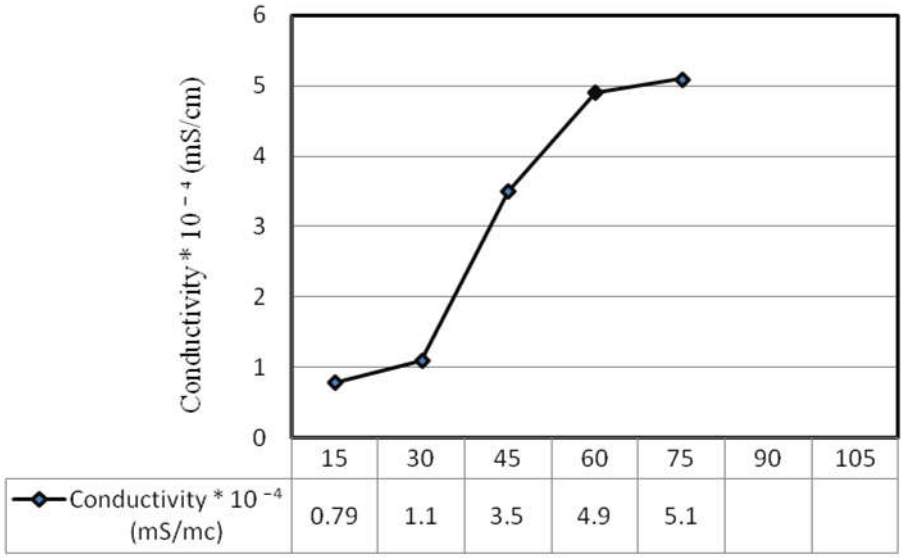
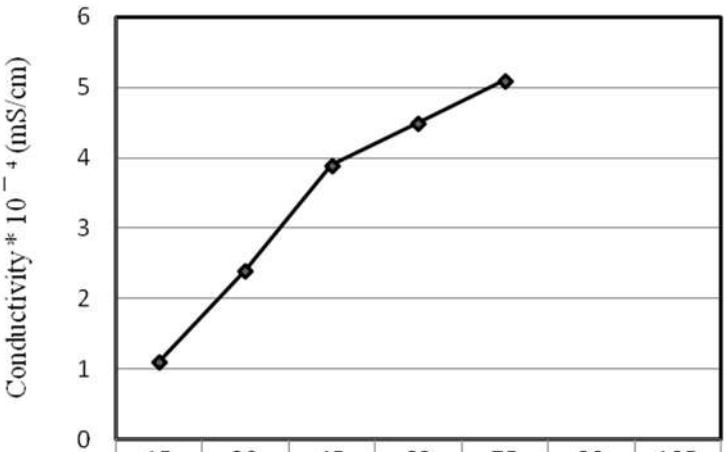


Diagram 2: Conductivity changes based on time increasing for Thiophanate-methyl toxin

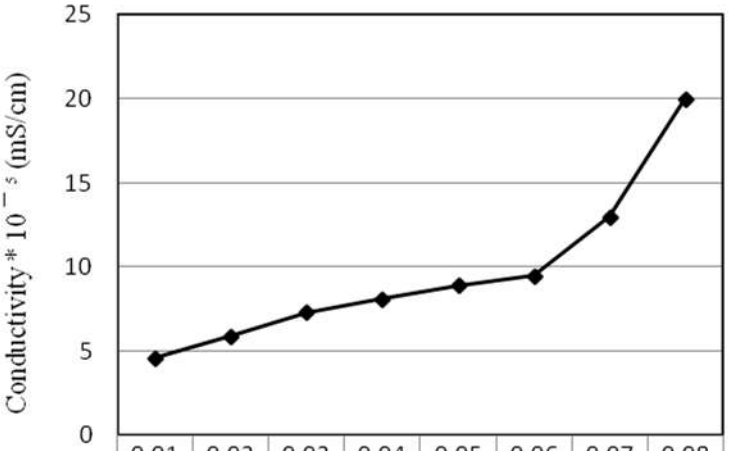


	15	30	45	60	75	90	105
Conductivity * 10 $^{-4}$ (mS/mc)	1.1	2.4	3.9	4.5	5.1		

Diagram 3: Conductivity changes based on time increasing for Iprodion+Carbendazim toxin

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 toxins has been reported in diagram 4, 5 and 6. 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.



	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08
Conductivity * 10 $^{-5}$ (mS/mc)	4.6	5.9	7.3	8.1	8.9	9.5	13	20

Diagram 4: Conductivity changes based on density increase in Carbendazim

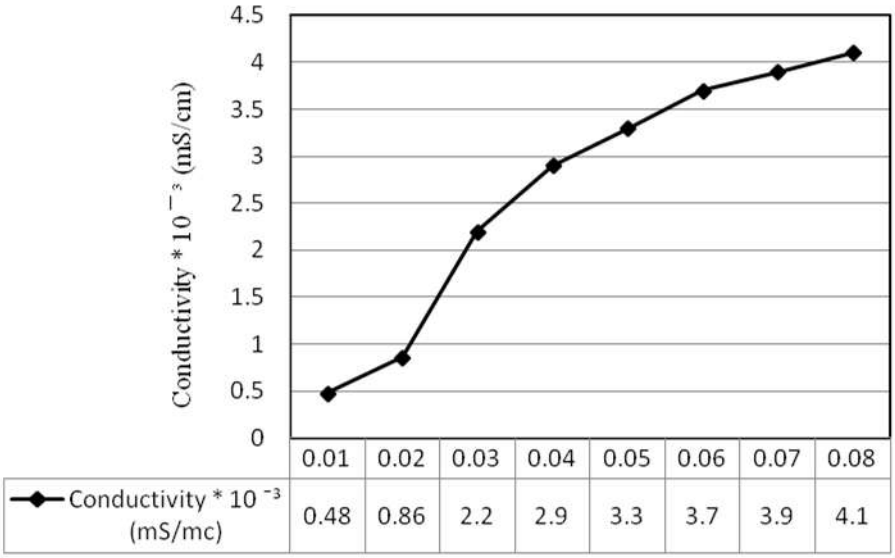


Diagram 5: Conductivity changes based on density increase in Thiophanate-methyl

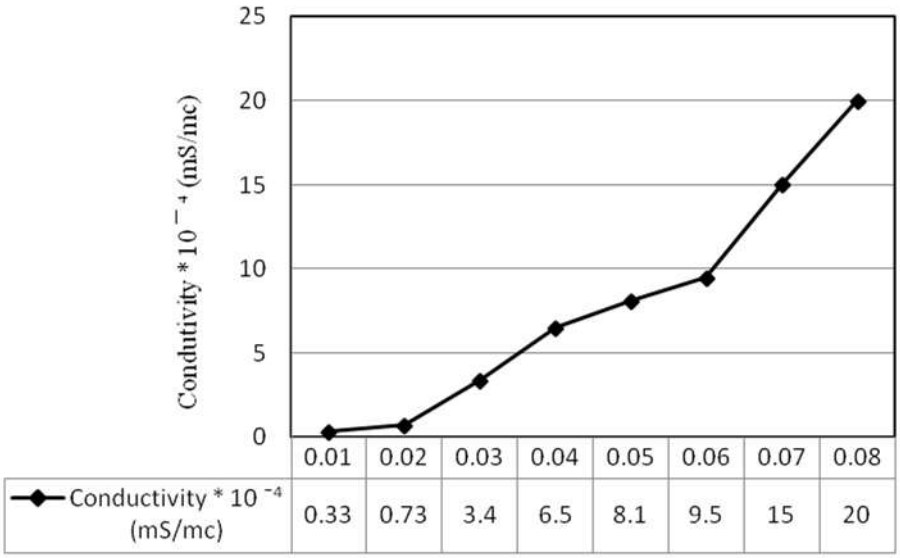


Diagram 6: Conductivity changes based on density increase in Iprodion+Carbendazim

FT-IR Spectroscopy

Spectroscopy has many applications in determining the purity and assessing the structural features and identifying quantification and qualification of compounds. In the following section, some of absorbing frequencies of factorial groups would be described in the ester compounds.

Stretching absorptions of C = C bond will emerge in aromatic rings in pairs (at 3 and 4). The tensile C—N bond in amines will appear at district 5. The tensile N—H bond in secondary aromatic amines has a strong band in the region 6, and its flexural absorption at the secondary amine, will absorb near region 7. C-O stretching, adjacent to the ester carbonyl group, is one of the strongest and most broad bonds in the spectrum. The emergence of a C=C bond, adjacent to the carbonyl group, resulted in the decentralization of π electrons in the bonds of C=O and C=C. This effect increases the simple character of the C = O bond and will decrease its Constant force; as a result, the carbonyl absorption frequency will be reduced. Strong stretching absorptions of C=O and C-O are two manifest characteristics in the spectrum of an ester which appear in ranges of 1 and 2, respectively.

Study the FT-IR spectrum of Carbendazim

Figures 1 and 2 show spectrums of the molecular imprinted polymer related to Carbendazim, which the peaks of its index include: 1287 cm^{-1} (stretching vibration of amine C-N) , 1096 cm^{-1} (C-O ester), 2890 cm^{-1} (C-H bond), 3210 cm^{-1} (flexural bond of N-H) , 1488 and 1596 cm^{-1} (aromatic C=C).

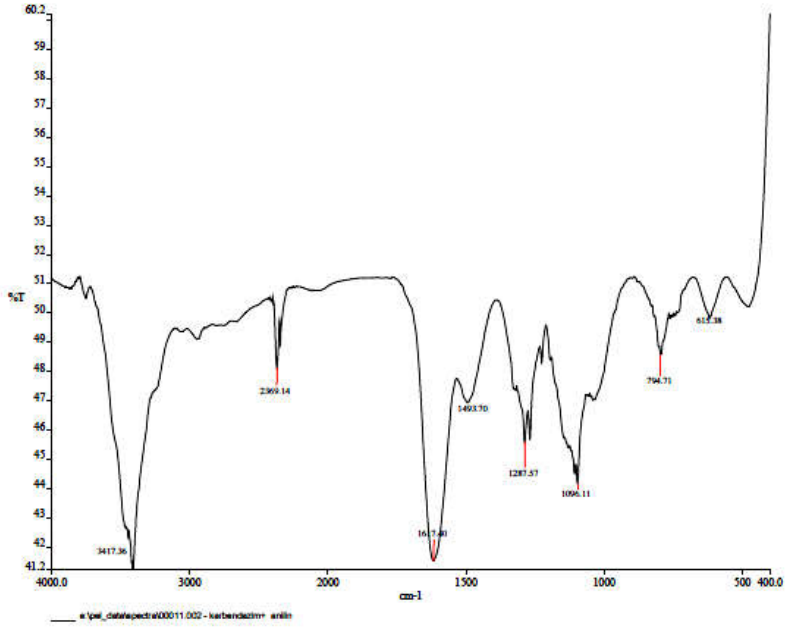


Figure 1: FT-IR spectrum related to the linear polyaniline polymer with Carbendazim

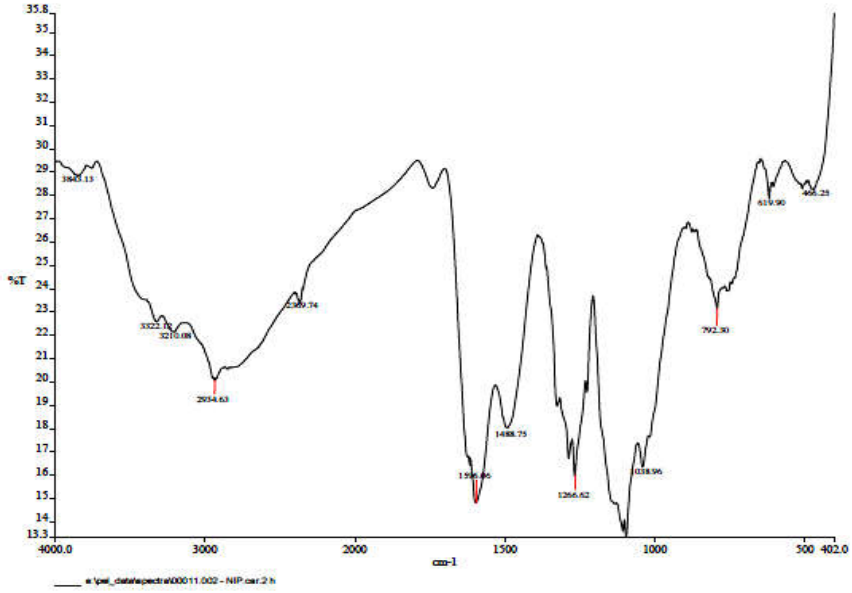


Figure 2: FT-IR spectrum related to MIP polymer of Carbendazim

Study the FT-IR spectrum of Thiophanate-methyl

Figures 3 and 4 show spectrums of the molecular imprinted polymer related to Thiophanate-methyl, which the peaks of its index include: 1338 and 1253 cm^{-1} (C-N bond), 1709 cm^{-1} (C=O bond), 2929 and 3140 cm^{-1} (C-H bond), 3186 cm^{-1} (aromatic N-H), 1492 and 1576 cm^{-1} (aromatic C=C), 1160 and 1144 cm^{-1} (C-O ester).

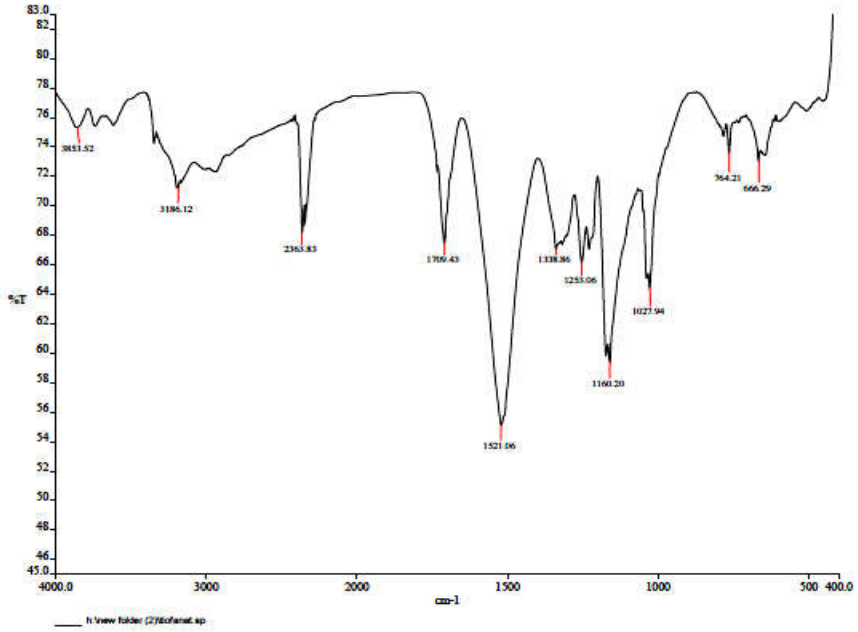


Figure 3: FT-IR spectrum related to the linear polyaniline polymer with Thiophanate-methyl

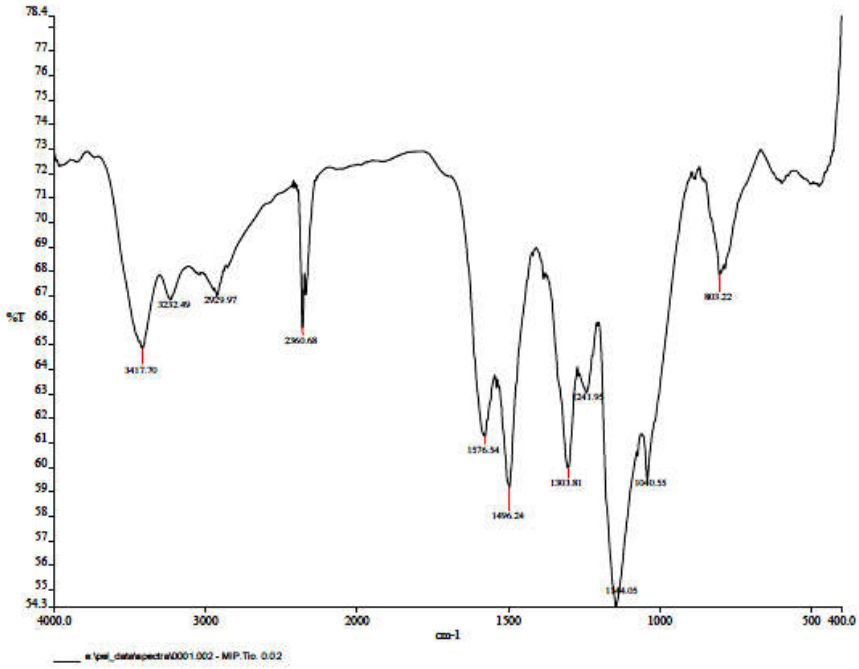


Figure 4: FT-IR spectrum related to MIP polymer of Thiophanate-methyl

Study the FT-IR spectrum of Iprodion+Carbendazim

Figures 5 and 6 show spectrums of the molecular imprinted polymer related to Iprodion+Carbendazim, which the peaks of its index include: 127 cm^{-1} (stretching vibration of amine C-N) , 1788 cm^{-1} (C=O bond), 3180 cm^{-1} (C-H bond), 3210 cm^{-1} (flexural bond of N-H) , 1620 and 1478 cm^{-1} (aromatic C=C) , 667 cm^{-1} (stretching vibration of C-Cl).

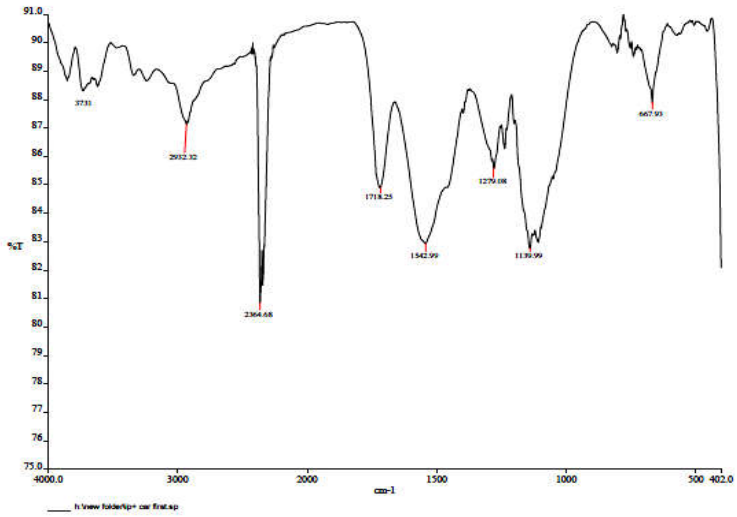


Figure 5: FT-IR spectrum related to the linear polyaniline polymer with Iprodion+Carbendazim

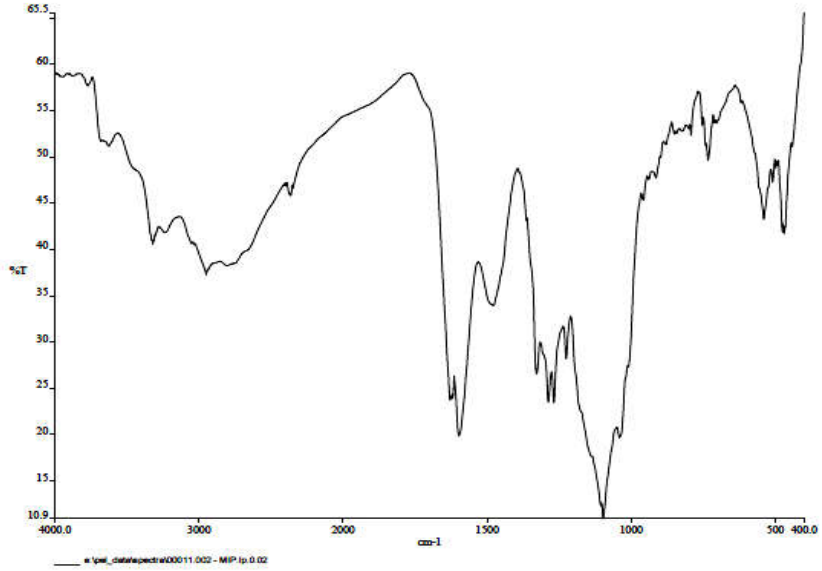


Figure 6: FT-IR spectrum related to MIP polymer of Iprodion+Carbendazim

CONCLUSION

The conductivity of molecular imprinted polymer solutions was tested in 6 periods of 15, 30, 45, 60 and 75 minutes. Investigating results obtained from the conductivity meter, for all three combinations indicate that as the sensor's response time increase, the conductivity will also increase.

The relationship between analyte concentration and electrical conductivity changes were examined by measuring the conductivity of molecular imprinted polymer solutions in 0/01, 0/02, 0/03, 0/04, 0/05, 0/06, 0/07 and 0/08 mol/L concentrations. The investigations based on obtained conductivities indicate that in all three combinations, electrical conductivity will increase with increasing the concentration of studied analyte. About all three cases, the amount of this increase has a significant leap between 0.02 and 0.03 mol/L concentrations.

The results obtained from investigating the sensory effects of toxic compounds of Benzimidazole family indicate that Thiophanate-methyl has the highest electrical conductivity between three examined compounds in all cases of experiment and Iprodion+Carbendazim and then Carbendazim are in the second and third place respectively.

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