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# Development of acetylcholinesterase immobilized CMD (Carboxymethyldextran) chip-based sensor for the detection of nerve agent simulant parathion

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## Abstract

In this study, a carboxymethyldextran chip based sensor system is developed that selectively recognizes and binds nerve agent molecules used in chemical weapons. Nerve agents fall under the group of organophosphorus compounds and irreversibly inhibit the acetylcholinesterase enzyme (AChE). In this study, parathion was used as an organophosphorus compound. The effect of the parathion molecule on enzyme inhibition is similar to nerve agents. The first step to be applied before CMD (Carboxymethyldextran) chip surface enzyme immobilization is the surface activation. After the surface activation was completed, AChE enzyme solution was passed over the chip surface for 40 minutes. In this way, enzyme immobilization was performed on the chip surface and a surface selective to the parathion molecule was obtained. Analysis was performed for parathion samples in different concentrations in the range of $3.43x10^{-8}$-$6.86x10^{-4}$ mol/L. When the analysis results were transferred to the calibration graph, a graphic close to the linear was obtained. The working range of the chip surface developed as a result of the analyzes was calculated as $3.43x10^{-8}$ - $6.86x10^{-4}$, the limit of detection (LOD) value was $3.79x10^{-8}$ and the limit of quantification (LOQ) value was $6.16x10^{-8}$. These results show that samples containing parathion at very low concentrations can be analyzed using the method we have developed.

## Article info

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## 1. Introduction

Molecular structures of both insecticides and nerve agents are based on organophosphorus compounds [1-3]. Organophosphorus compounds show negative effects on the nervous system. Due to their oil solubility properties, they can be absorbed by the skin [4-8]. The most lethal effects of these types of compounds occur when they are taken into the body by inhalation. Apart from inhalation, they can also be absorbed through the mucous membranes, eyes and skin. [9]. This group of chemicals is most commonly used in pesticides, insecticides, acaricides, etc. They are used commercially and can be easily obtained commercially [3,10]. The general chemical structures of organophosphorus compounds are as follows; The center of the molecule has a phosphorus atom, and the phosphorus atom can double bond with oxygen or sulfur. R1-R2 in the general chemical structure represents hydrogen, alkyl (including cyclic structure), aryl, alkoxy, alkylthiole and amino groups [11,12,2]. X represents halogens, cyano and thiol groups and inorganic-organic acids. The general chemical structure of organophosphorus compounds is as shown in Figure 1 [13].

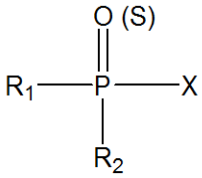


**Figure 1.** Chemical structure of organophosphorus compounds

AChE enzyme is a serine hydrolase which is a member of esterase family. The role of the acetylcholinesterase enzyme in the body is to terminate the signal transmission by hydrolyzing acetylcholine on the post-synaptic membrane. In this way, it ensures the autonomic nervous system to function properly.

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The acetylcholine molecule is the first identified neural stimulating molecule. It is an ester of choline with acetic acid. The acetylcholine molecule is hydrolyzed by AChE, and choline and acetic acid are broken down [1,14]. The breakdown of the acetylcholine molecule in the nerve cells by the enzyme acetylcholinesterase (AChE) is shown in Figure 2. The gene encoding the enzyme acetylcholinesterase is located in the q22 region of the 7th chromosome in the human gene structure. It is a monomer weighing 60,000 daltons. It is a molecule in the form of a dimer or tetramer, consisting of 12 curved, center-mixed beta sheets, surrounded by a 14 alpha helix of 45x60x65 A °, similar to the ellipse. It contains alpha/beta protein with 537 amino acid content. There is aspartic acid (Asp) 1 at the N terminal of the series and 537 at the C terminal [1,14,15].

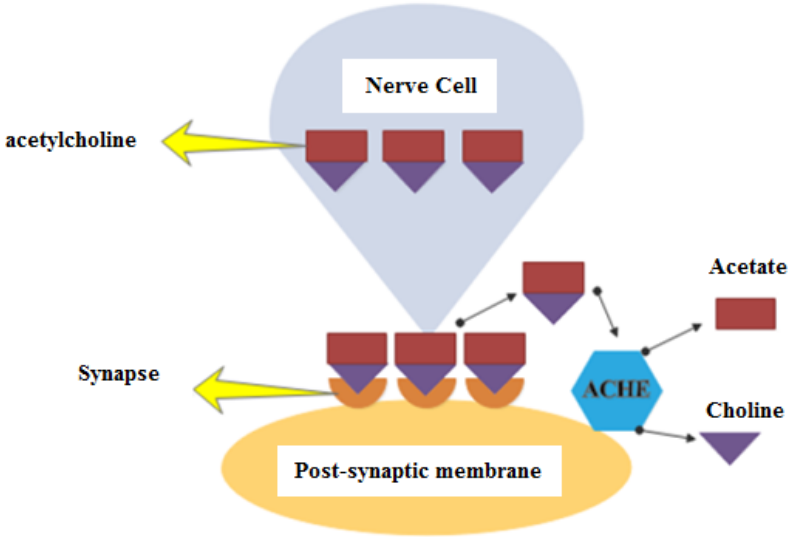


**Figure 2.** The breakdown of the acetylcholine molecule in the nerve cells by the enzyme acetylcholinesterase (AChE) [1]

When a molecule belonging to the organophosphorus group enters the body, acetylcholine is bound to the esterase enzyme. This binding event takes place over the serine amino acid on the enzyme [8]. The interaction of AChE enzyme with an organophosphorus compound takes place in two steps. Reversible inhibition occurs in the first step and irreversible inhibition occurs in the second step. Another name for irreversible inhibition is aging [14-16]. This event is shown in Figure 3.

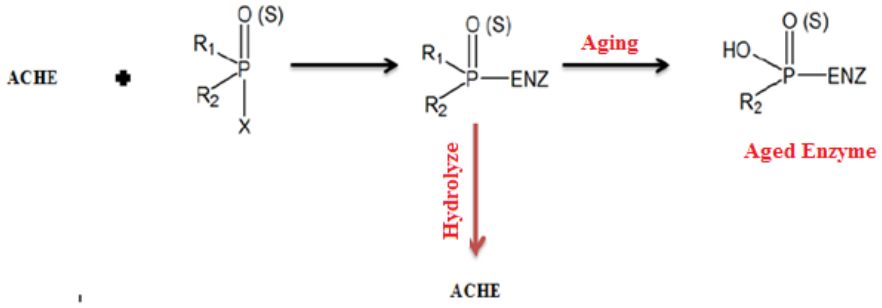


**Figure 3.** Inhibition reaction [1]

Parathion is a widely used, highly toxic organophosphorus insecticide. It was synthesized for the first time in 1940. Its chemical formula is $C_{10}H_{14}NO_5PS$ and its molecular weight is 291.3 g/mol. Parathion affects the nervous system by inhibiting acetylcholinesterase enzyme. The first physical symptoms after exposure to parathion are poor vision, vomiting, abdominal pain, severe diarrhea, loss of consciousness, tremors, shortness of breath.

Paration and similar organophosphorus pesticides cause hundreds of thousands of poisonings per year [23, 24]. The molecular structure of the parathion is given in Figure 4.

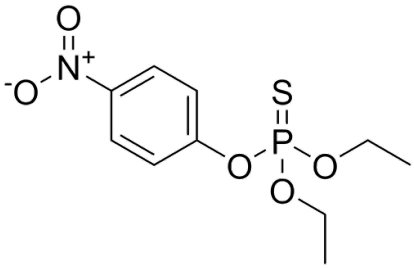


**Figure 4.** Chemical structure of parathion

Reflectometric interference spectroscopy is a method of determination that does not need labeling based on multiple reflections of white light over a thin surface. Biochips used in this method have a glass surface. A typical chip example is given in Figure 5.

**Figure 5.** CMD chip [17]

The glass surface of the chip makes it easier to connect functional groups. When the analyte interacts with the ligand immobilized on the chip surface, the thickness of the chip surface increases, resulting in a change in the spectrum. Antigens, antibodies, proteins, peptides, nucleic acids, aptamers can be immobilized as ligand on the chip surface [17]. Ligand immobilization and interaction with analyte on glass surface is shown in Figure 6.

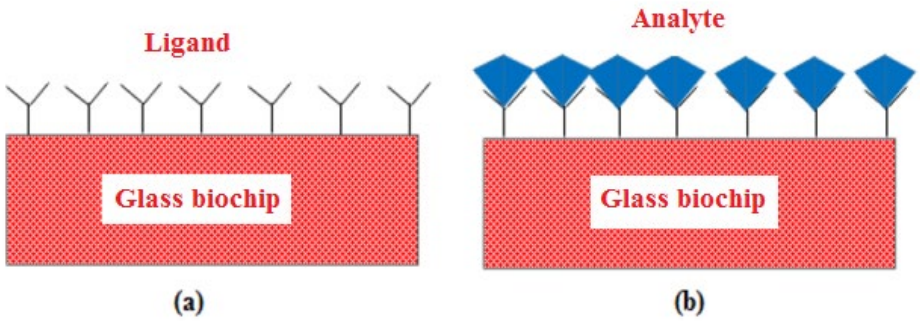


**Figure 6.** The use of glass biochip, (a) ligand immobilized biochip (b) analyte-interacted biochip [17]

After chemical modification to the glass surface, analyte molecules attached to the surface increase the surface thickness and as a result, a change in the refractive index occurs. This situation is shown in Figure 7. The change in the optical thickness causes changes in the interference spectrum. In this way, the binding behavior of the analyte molecule is monitored.

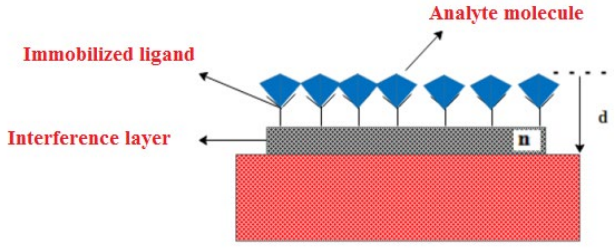


**Figure 7.** The interaction of glass biochip with analyte and its thickness increase [17]

When analyte molecules begin to bind to the surface of the biochip, signal increase occurs in the reflectometric interference spectrometer device. This process, in which the signal increases, is called the process of attachment. After the sample analysis is finished, the step of removing analyte molecules attached to the chip surface is proceeded. This process is called the separation process. Regeneration is called by removing all analyte molecules bound from the chip surface and returning to the state before analysis.

In this study, CMD (carboxymethyldextran) chip-based sensor is developed that selectively recognizes and binds the parathion molecule. The literature has been examined in order to evaluate the performance of the developed sensor. When the previous studies on the analysis of parathion were examined, it was seen that many studies benefited from the hydrolysis of organophosphates and the changes in the result of hydrolysis form the basis of the analysis [18-21]. In our study, the optical changes caused by the binding of the parathion to the surface of the chip were used. With the technique we have developed, analysis of parathion samples at lower concentrations can be performed. In addition, the chip surface is suitable for regeneration, resulting in high repeatability.

## 2. Materials and Methods

### 2.1. Apparatus

All chemicals used in the studies were obtained from Sigma-Aldrich (St. Louis, USA). Thermo Scientific Barnstead ™ Smart2Pure ™ pure water device was used to obtain pure water. The conductivity of the pure water used is 18 megaohmcm$^{-1}$.

### 2.2. CMD (Carboxymethyldextran) chip surface activation

The washing chip was first installed in the RIfS device. The device was washed with distilled water for 10 minutes. Afterwards, the system was washed with buffer for 10 minutes by placing the HEPES ((4- (2-hydroxyethyl) -1-piperazineethanesulfonic acid) buffer prepared instead of pure water [17]. After the washing process with pure water and buffer was completed, the washing chip was removed and the CMD chip to be immobilized was placed. Rate was set to 5 µL/min and HEPES buffer was passed through the chip surface for 10 minutes to pass through two channels. After the buffer transition, measurement was made to determine whether there are air bubbles in the system. After determining there is no problem in the system as a result of the measurement, 70 µL of the solution prepared from 0.1 M N-hydroxysuccinimide (NHS) and 0.1 M 3-dimethyllaminopropyl-N′-ethylcarbodiimide (EDC) in a ratio of 1:1 was placed in the device. Since air bubbles are not desired in the system, it has been paid attention to dip the cable to the bottom of the ependorph. Solution flow was started by setting the flow rate to 5 µL/min. Surface activation is performed by passing the NHS/EDC solution through the chip surface. Schematic representation of the surface activation is presented in Figure 8.

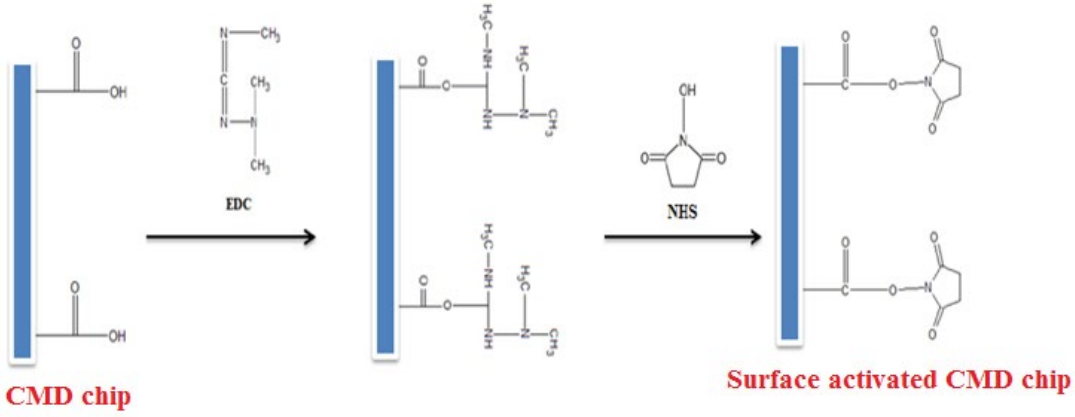


**Figure 8.** Schematic representation of the surface activation of the CMD chip [17]

### 2.3. AChE immobilization to the CMD chip surface

After surface activation was completed, 100 µL of 1 ppm AChE enzyme was prepared. The prepared solution was taken to the ependorph and placed in the inlet no 2 in the RIfS device. Flow rate was set at 5 µL/min and volume at 200 µL. The solution flow was made to pass through a single channel. Thus, the enzyme is not immobilized to a part of the chip surface, thus providing reference surface formation. Under these operating conditions, the enzyme solution was passed over the chip surface for 40 minutes. After completion of the enzyme passage, 70 µL of ethanolamine solution was taken and placed in the inlet 3. The solution was passed through the chip surface by adjusting the flow rate to 5 µL/min and the volume to 70 µL. After the end of the immobilization process, it was decided whether the immobilization was realized by examining the change in the basic signal.

### 2.4. Application of Parathion solutions to the chip surface

After AChE enzyme immobilization was performed on the chip surface, analysis of parathion solutions at different concentrations was made. The enzyme immobilized chip was inserted into the device in the

measuring position before the measurement was started. The HEPES buffer was passed over the chip surface at a flow rate of 10 µL / min for 10 minutes. The system was switched from manual mode to automatic mode by adjusting the buffer flow rate to 10 µL / min. 200 µL of parathion solution was placed at the inlet 1. 50 µL glycine was placed in the inlet 2 and 50 µL HCl solution was placed in the inlet 3. Thus regeneration was made. In this way, measurements were taken for different analyte concentrations.

## 3. Results and Discussion

### 3.1. Taking sensogram of AChE immobilized CMD chip

When the AChE enzyme is bound to the chip surface, an increase in the sensogram obtained is expected as there will be a change in the refractive index of the surface. The increase will show that the immobilization process has taken place. Therefore, the sensogram of the CMD chip was taken during the immobilization of the AChE enzyme. The sensogram obtained is as shown in Figure 9.

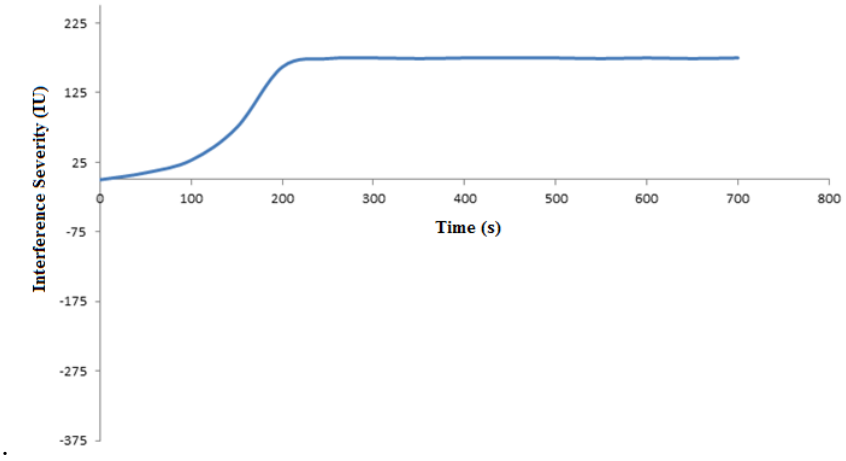


**Figure 9.** Sensogram obtained by AChE enzyme immobilization

The increase in the sensogram obtained showed that enzyme immobilization to the chip surface was successfully performed.

### 3.2. Spectrums of parathion solutions in different concentrations

Electrostatic interactions between chemical species vary depending on the pH of the aqueous solution. In the extraction experiments, the interaction between the analyte and the selected chemical medium should be high.

Different concentrations of parathion solutions were applied to the prepared AChE immobilized CMD chip surface. The sensograms obtained after applying 0.01-200 ppm parathion solutions to the chip surface is shown in Figure 10-18.

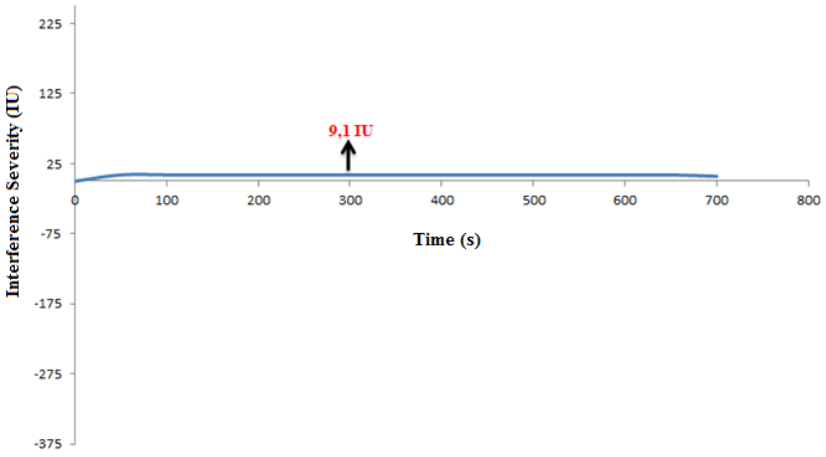


**Figure 10.** Sensogram of 0.01 ppm parathion solution

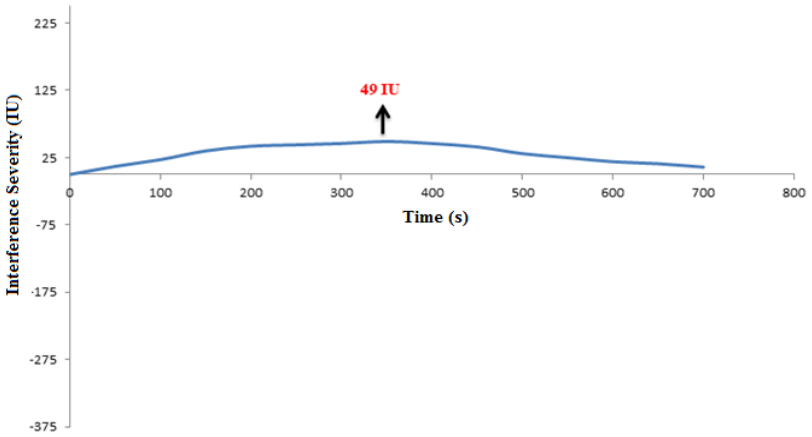


**Figure 11.** Sensogram of 0.1 ppm parathion solution

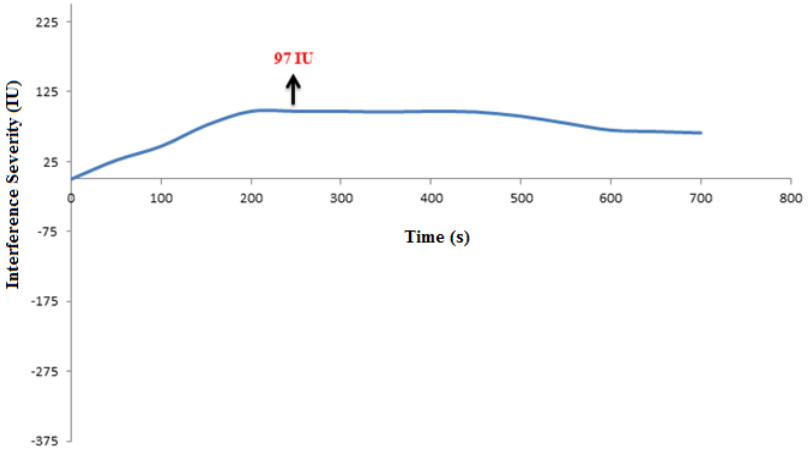


**Figure 12.** Sensogram of 1 ppm parathion solution

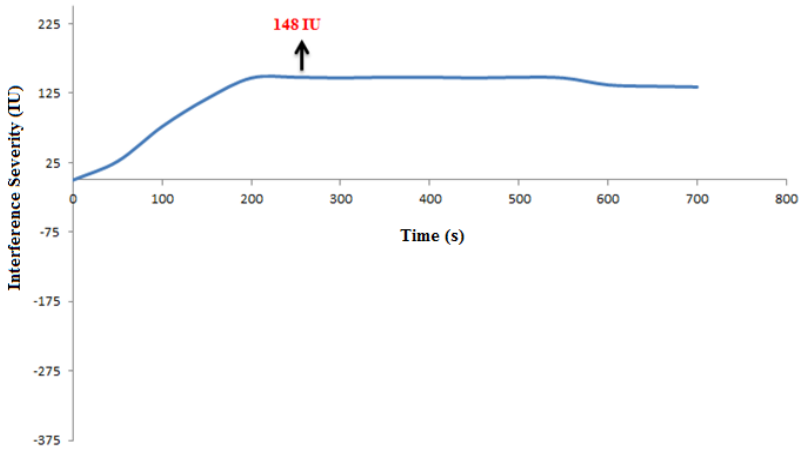


**Figure 13.** Sensogram of 10 ppm parathion solution

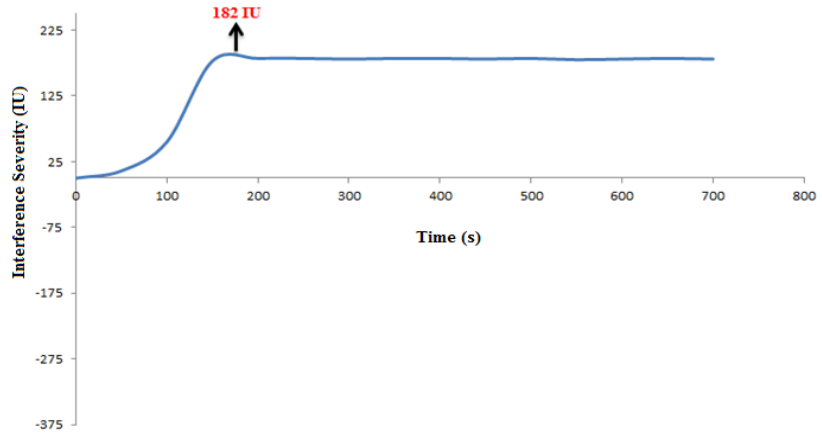


**Figure 14.** Sensogram of 50 ppm parathion solution

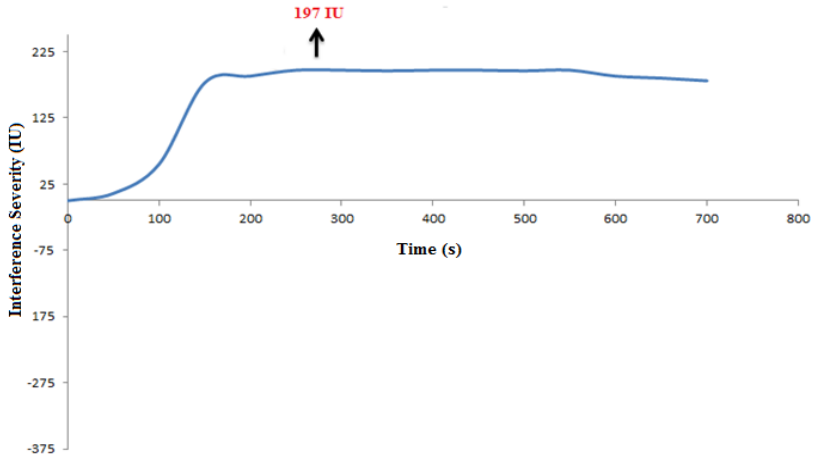


**Figure 15.** Sensogram of 75 ppm parathion solution

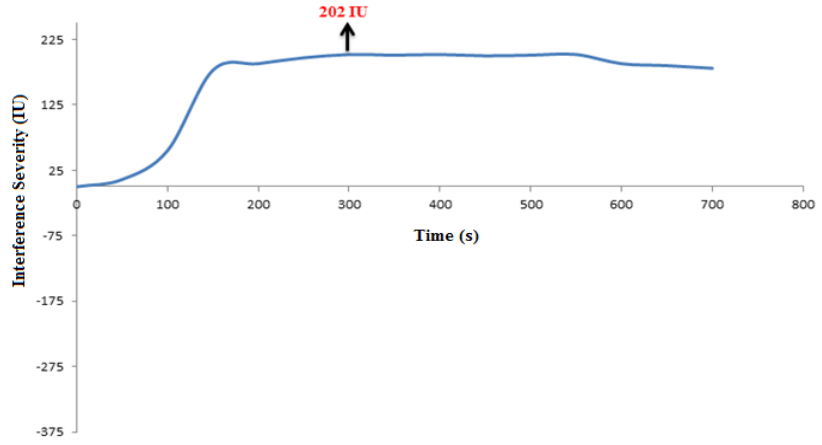


**Figure 16.** Sensogram of 100 ppm parathion solution

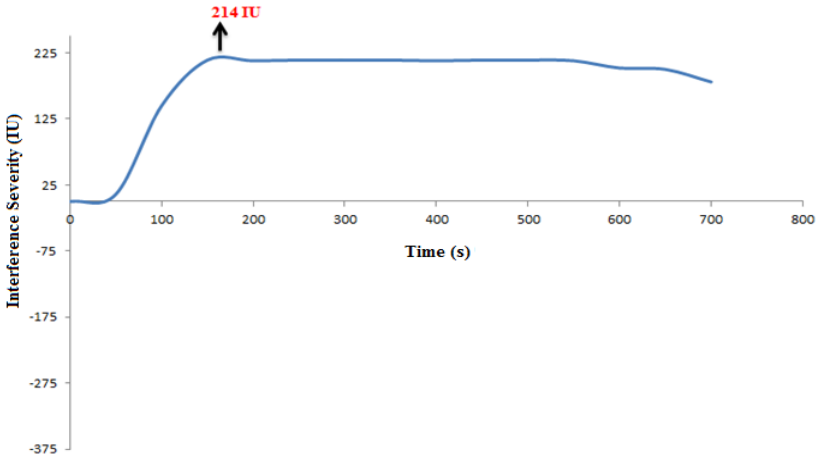


**Figure 17.** Sensogram of 150 ppm parathion solution

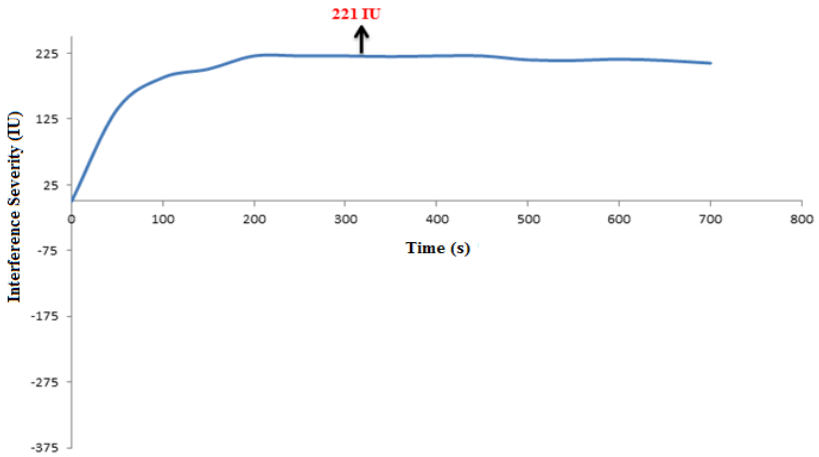


**Figure 18.** Sensogram of 200 ppm parathion solution

### 3.3. Plotting the calibration graph of parathion as a result of RIfS measurements

The measurement results obtained for the samples of Parathiona in different concentrations are shown in Table 1 collectively.

**Table 1.** Measurement results for Parathion solutions

| ppm(mg/L) | Concentration (mol/L) | -log (C) | IU |
|---|---|---|---|
| 0.01 | $3.43x10^{-8}$ | 7,46 | 9,1 |
| 0.1 | $3.43x10^{-7}$ | 6,46 | 49 |
| 1 | $3.43x10^{-6}$ | 5,46 | 97 |
| 10 | $3.43x10^{-5}$ | 4,46 | 148 |
| 50 | $1.72x10^{-4}$ | 3,76 | 182 |
| 75 | $2.6x10^{-4}$ | 3,58 | 197 |
| 100 | $3.43x10^{-4}$ | 3,46 | 202 |
| 150 | $5.14x10^{-4}$ | 3,29 | 214 |
| 200 | $6.86x10^{-4}$ | 3,16 | 221 |

The calibration plot drawn according to these measurement results is given in Figure 19.

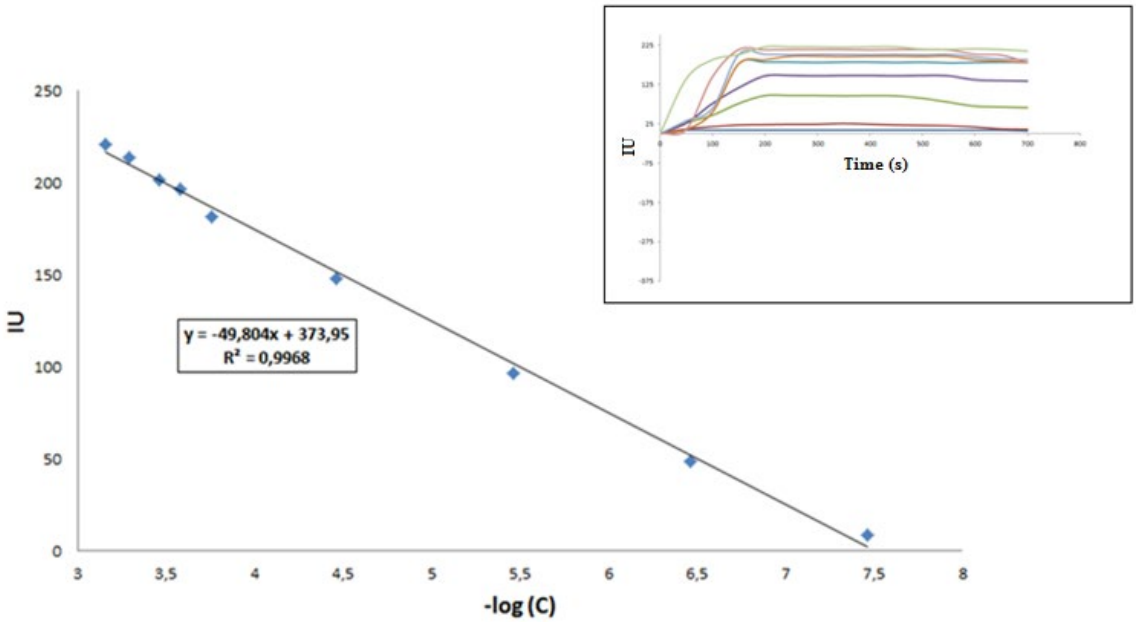


**Figure 19.** Calibration graph of parathion samples in different concentrations

A linear relationship was observed in the reflectometric interference spectroscopy up to $6.86x10^{-4}$ mol/L, the highest concentration of parathion studied for the calibration line. The correlation coefficient was found to be 0.9968. In order to calculate LOD and LOQ, blank solutions were prepared and 5 different measurements were taken. The average of the measured results was calculated as 6 and the standard deviation as 1.45. LOD and LOQ values were used by using the standard deviation value found. As a result of the calculations, the LOD value was found to be $3.79x10^{-8}$ M and the LOQ value was $6.16x10^{-8}$ M.

When Table 2 is examined, it is seen that the samples containing much lower amounts of parathion can be analyzed with the CMD chip based sensor we developed.

**Table 2.** Comparison of different methods with our sensor

| Method | LOD (molL−1) |
|---|---|
| Amperometric proton selective sensor [19] | $5x10^{-5}$ |
| Organophosphorus hydrolase enzyme immobilized glassy carbon electrode based sensor [20] | $4x10^{-7}$ |
| Amperometric sensor based on carbon paste electrode [21] | $1x10^{-4}$ |
| Sensor based on pH change [22] | $2x10^{-6}$ |
| Our Study | $3.79x10^{-8}$ |

## 4. Conclusions

In this study, CMD chip based sensor is developed which selectively recognizes and binds the parathion molecule. Nerve agents fall under the group of organophosphorus compounds and irreversibly inhibit the acetylcholinesterase enzyme (AChE). In this study, parathion was used as an organophosphorus compound. The effect of the parathion molecule on enzyme inhibition is similar to nerve agents. The first step to be applied before CMD (Carboxymethyldextran) chip surface enzyme immobilization is the surface activation. After the surface activation was completed, AChE enzyme solution was passed over the chip surface for 40 minutes. In this way, enzyme immobilization was performed on the chip surface and a surface selective to the parathion molecule was obtained. Analysis was

performed for parathion samples in different concentrations in the range of $3.43x10^{-8}$-$6.86x10^{-4}$ mol/L. The measurement results obtained were graphed and the calibration graph was obtained. A linear correlation was observed in the reflectometric interference spectroscopy up to $6.86x10^{-4}$ mol/L, the highest concentration of parathion studied for the calibration line. Regression coefficient was found to be 0.9968. In order to calculate LOD and LOQ, blank solutions are prepared and measured. As a result of the calculations, the LOD value was found $3.79x10^{-8}$ M, the LOQ value was $6.16x10^{-8}$ M. These results show us that with the developed method, very low amounts of parathion samples can be analyzed.

## Conflicts of interest

The authors state that did not have conflict of interests.

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