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# PARTIAL PURIFICATION OF THYROID PEROXIDASE TPO FROM BLOOD SERUM AND STUDY IT'S KINETIC PROPERTIES AND MOLECULAR WEIGHT

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## Abstract

This study was done to partially purification of thyroid peroxidase TPO from blood serum using Gel filtration technique, by using Sephadex G100 gel. A single peak in fraction four has been obtained, and the degree of purification (4.28) fold, enzyme yield (25.5%) and specific activity (0.00036 ng/mg). Kinetics studies for the partial purified enzyme were carried out which showed optimal concentration of substrate which was (0.1ng/ml), Michael's - Menten constant (Km=4 mM) and maximum velocity (Vmax=0.0435 IU/L), while optimum Temperature was (37 C°) and optimum pH was (7.4). The molecular weight of the partial purified enzyme has been determined by gel electrophoresis method, in presence of polyacrylamide gel and sodium dodecyl sulphate (SDS-PAGE) which showed that the approximated molecular weight was (31.5 kD).

**Keywords:** Thyroid peroxidase TPO, COVID-19 , kinetics, molecular weight of enzyme

## 1- Introduction

Thyroid peroxidase (TPO) is an enzyme that plays a crucial role in the synthesis of thyroid hormones. Purification of TPO from human serum can provide valuable insights into the structure and function of the enzyme, as well as its potential use as a therapeutic target. There have been several methods reported for the purification of TPO from human serum, including ammonium sulfate precipitation, ion exchange chromatography, and affinity chromatography using antibodies specific to TPO. A commonly used method involves a combination of ammonium sulfate precipitation, anion exchange chromatography, and gel filtration chromatography.

One study published in the Journal of Endocrinology and Metabolism reported the purification of TPO from human serum using a combination of ammonium sulfate precipitation, DEAE-cellulose ion exchange chromatography, and Sephadex G-200 gel filtration chromatography. The resulting purified TPO had a specific activity of 46 U/mg protein and a yield of 24%. [1]

Another study published in the Journal of Biological Chemistry reported the use of an anti-TPO monoclonal antibody coupled to Sepharose beads for affinity chromatography purification of TPO from human serum. The resulting purified TPO had a specific activity of 497 U/mg protein and a yield of 60%. [2]

In addition to the methods mentioned in my previous response, there are other techniques that have been used for the purification of TPO from human serum, such as hydrophobic interaction chromatography and immunoaffinity chromatography using anti-TPO antibodies. Hydrophobic interaction chromatography (HIC) separates proteins based on their hydrophobicity.

A study published in the Journal of Chromatography B reported the purification of TPO from human serum using HIC with phenyl sepharose CL-4B resin. The resulting purified TPO had a specific activity of 33.1 U/mg protein and a yield of 13.5%. [3]

Immunoaffinity chromatography (IAC) uses antibodies specific to the target protein to capture and purify it from a complex mixture. A study published in the Journal of Clinical Endocrinology and Metabolism reported the purification of TPO from human serum using IAC with anti-TPO monoclonal antibodies coupled to Sepharose beads. The resulting purified TPO had a specific activity of 460 U/mg protein and a yield of 20%. [4]

The choice of purification method depends on factors such as the availability of specific antibodies, cost, and desired purity and yield. The purified TPO can be used for various applications, including biochemical and biophysical studies, development of diagnostic assays, and drug discovery research.

## 2- Experimental

### 2.1.Collection of sample:

The total number, of these, samples was (4) samples, serum samples were collected from healthy person for both sexes. Blood was drawn from the vein using a 5 ml plastic syringe with one use. The blood was placed in clean and free anticoagulant tubes, and left to coagulate at room temperature. The blood serum was then separated by centrifuge at a velocity of 6000 rpm for 30 minutes to ensure adequate serum red blood cell extraction. The effectiveness of the enzyme was measured directly and the study was done outside the body (in vitro).

### 2.2.Separation and purification of Thyroid peroxidase TPO from serum of blood.

Thyroid peroxidase was purified from the serum of blood using the following steps: 1-Addition ammonium sulphate (80%) 2-Dialysis 3- Gel Filtration Chromatography (using Sephadex G100)

### 2.3.Kinetics of thyroid peroxidase TPO

The kinetics of thyroid peroxidase were studied after its separation and partially purified from serum of blood by gel filtration. These included:

1- Effect of substrate concentration: by using different concentrations of substrate (33 ,16.50,8.25, 4.13, 2.06, 1.03 mMol)

2- Effect of temperature: using to measure the effectiveness of thyroid peroxidase. The reaction was conducted at different temperatures (7,17,27,37,47 and 57 C°)

3- Effect of pH: The pH effect of the thyroid peroxidase reaction. Different pH solutions ( 2, 4, 6, 8, 10, 12) were used with thyroid peroxidase at 0.1ng and 37 C° .

### 2.4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis to Measurement molecular weight of Purified Enzyme (SDS-PAGE)

Followed the way to the researcher Laemmli to prepare polyacrylamide gel with some modifications.

## 3- Results and Discussion

### 3.1.Partially purification of thyroid peroxidase from serum of blood.

The basic principle is to equalize the charges on the surface of the protein(enzyme) and the degradation of the water layer surrounding the protein and reduce the degree of watering, solubility, of the protein and, sedimentation [5]. Therefore separation and purification process of thyroid peroxidase was made from serum of blood by steps where in the first stage of purification the enzyme was precipitated using ammonium sulphate salts $(NH_4)_2SO_4$ to concentrated enzyme and the access of the resulting salts was removed by using dialysis technique using phosphate buffer solution 1Mm Tris-HCl pH 7.4, wherase the degree of purification of enzyme was (4.28)folds and yield of enzyme 25.5% , specific activity 0.00036 ng/ml showing the result in table (1), the stages of purification were complete by using gel filtration using Sephadex G100 which showed a single peak in fraction nine with the degree of purification was (1.2) fold and enzyme yield was(108.2%) while specific activity was (0.189ng/mg).

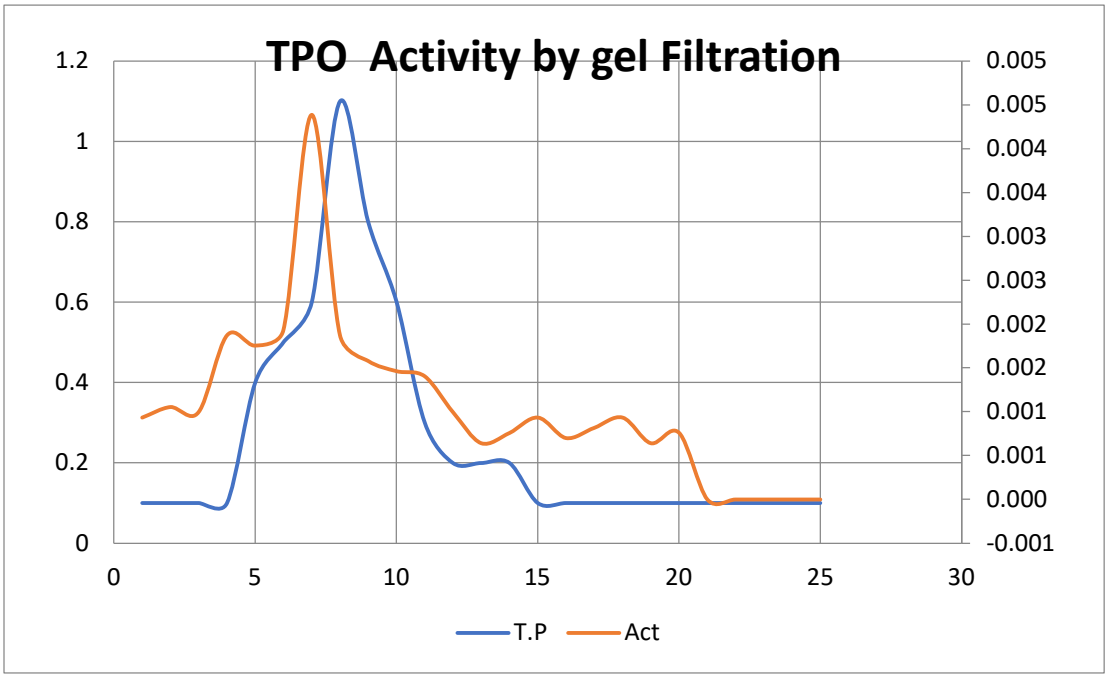



**Figure (1) Gel filtration chromatography of TPO by Sephadex G100.**

**Table (1): Steps of TPO purification from human Serum.**

| Steps of purifications | Volume ml | Activity IU/ml | Total Activity IU | Protein Conc. mg/ml | Specific activity U/mg | Yield % | Folds of Purify. | Total protein mg |
|---|---|---|---|---|---|---|---|---|
| Crude serum TPO | 14 | 0.0056 | 0.0784 | 66 | 0.000084 | 100 | 1 | 924 |
| precipitation | 11 | 0.0068 | 0.0748 | 57 | 0.00011 | 95.4 | 1.3 | 627 |
| Dialysis | 13 | 0.0025 | 0.0325 | 19 | 0.000131 | 41.4 | 1.55 | 247 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Ion exchange (DAEA Cellulose)** | **5** | **0.0030** | **0.015** | **17** | **0.00017** | **19.1** | **2.02** | **85** |
| **Gel filtration Sephadex G-100** | **5** | **0.0040** | **0.02** | **11** | **0.00036** | **25.5** | **4.28** | **55** |

### 3.2.Detemination of molecular weight of thyroid peroxidase using polyacrylamide gel and SDS (SDS-PAGE)

Molecular weight of the enzyme determined by Polyacrylamide gel electrophoresis(PAGE), describes a technique widely used in biochemistry, forensics, genetics, molecular biology. (SDS-PAGE) is a technique for separating proteins based on their ability to move within an electrical current, which is a function of the length of their polypeptide chains or of their molecular weight. This is achieved by adding sodium dodecyl sulphate (SDS) detergent to remove secondary and tertiary protein structures and to maintain the proteins as polypeptide chains. The SDS coats the proteins, mostly proportional to their molecular weight, and confers the same negative electrical charge across all proteins in the sample [6]. figure (2) showed the band of thyroid peroxidase compared with standard solutions bands which known it's molecular weight to found molecular weight of enzyme which was(31.5 kD).

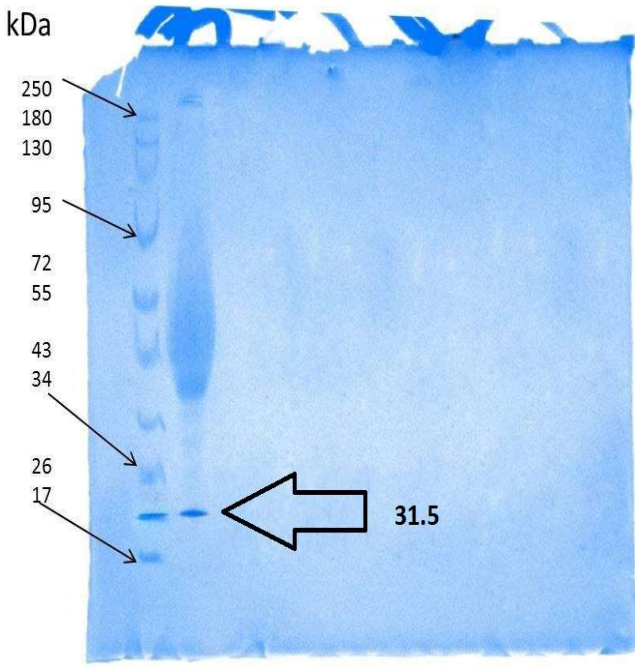


**Figure (2) electrophoresis of thyroid peroxidase TPO to determined molecular weight compared with standard protein.**

### 3.3. Kinetic Study of optimum conditions for TPO

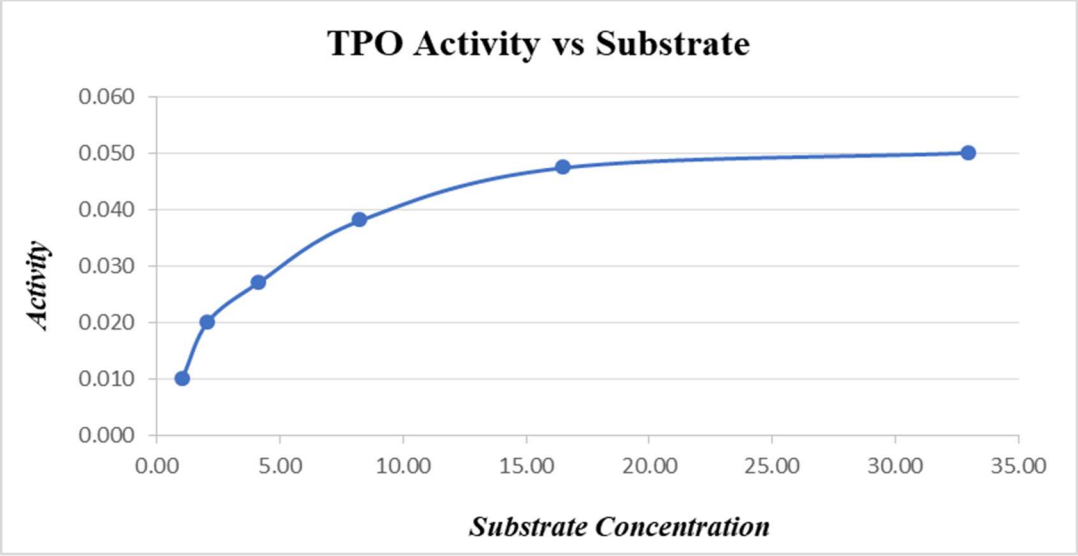
### 3.3.1 Effect of Different Substrate Concentrations on Enzyme Activity with Determination of Michaelis-Menten's Constant (Km) and Maximum Velocity (Vmax)

The activity of the TPO was measured in the presence of different concentrations of Disodum phenyl phosphate on TPO ( 33, 16.50, 8.25, 4.13, 2.06, 1.03 mMol/L), as substrate. It was found that the maximum activity of the TPO was obtained by using a concentration ( 16.50 mmol/L ) of disodium phenyl phosphate, which is (0.0068 IU/L). figure (3) shows increasing enzyme interaction velocity with increasing concentration of substrates.

**Table (2): The Impact of Substance conc. on TPO activity.**

| Conc.[S] mMol | Activity IU/L | 1/ [S] | 1/ V |
|---|---|---|---|
| 33 | 0.0056 | 0.0303 | 21.00 |
| 16.50 | 0.0068 | 0.0606 | 21.11 |
| 8.25 | 0.0025 | 0.1212 | 26.32 |
| 4.13 | 0.0030 | 0.2424 | 37.04 |
| 2.06 | 0.0040 | 0.4854 | 50.00 |
| 1.03 | 0.0068 | 0.9709 | 100.00 |

The same figure showed that purified TPO enzyme from serum subjected to Michaelis-Menten's equation where the resulting figure was of the hyperbolic curve and showed direct correlation.




**Figure(3). Michaelis-Menten's plot showed the effect of Substrate Concentration on TPO activity.**

There are several methods for calculating the value of the MichaelsMenten constant (Km) when the enzyme is subject to the MichaelsMenten's equation, Km can be calculated for it, as the Line weaver-Berke method was the most accurate and best in practice, due to its ease of use and the lack of calculations in it and its efficiency in showing the accuracy of experimenting, Results in this study showed from Line weaver–Burk equation by plotting the reciprocal of the initial velocity versus the reciprocal of the substrate concentration. A linear relationship was obtained with a Km and Vmax value of the enzyme (4 mM and 0.0435 IU/L) for TPO, Km and Vmax were obtained using Line weaver– Burk plots showed in figure (4). The reciprocal of substrate concentration (1/S) was plotted against the reciprocal of reaction rate (1/V) according to the following equation:

$$v_i = \frac{V_{max}\ [S]}{K_m + [S]}$$

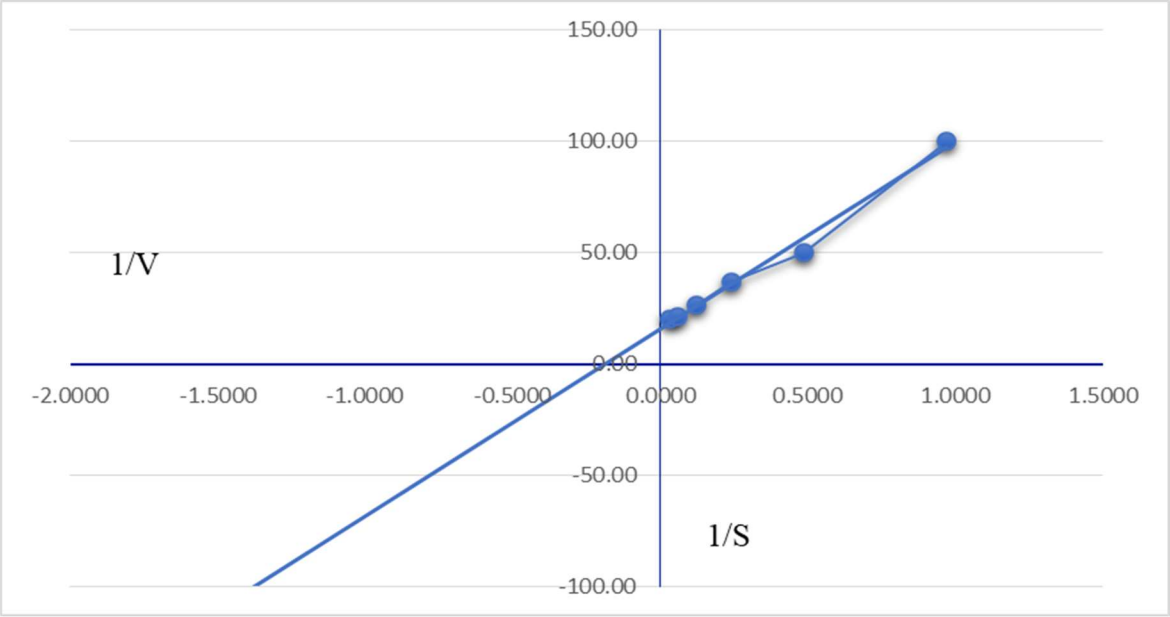


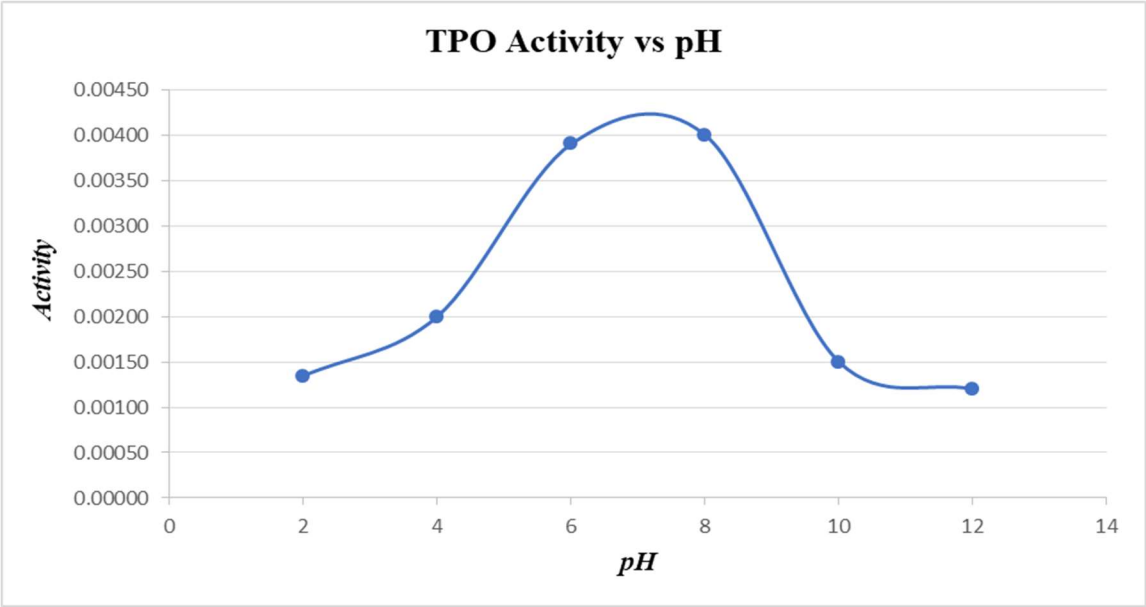

**Figure (4) Line Weaver–Burk plot for partially purified TPO**

### 3. 3.2 Effect of pH on Enzyme Activity

The various effect of pH on the TPO enzyme activity was studied, and different grades of PH were tested to determine optimal pH for the TPO enzyme activity (2, 4, 6, 8, 10, and 12 pH), as shown in figure (5). The maximum enzyme activity was discovered at (pH 7.4), purified TPO. The different nature of the enzyme and its chemical makeup, as well as the presence of multiple ionic groups carried by the enzyme, all contribute to the pH effect on enzyme activity. Enzymes function at the ideal pH because they are extremely sensitive to changes in hydrogen ion concentration H+[7].

The pH affects the rate of enzyme-catalyzed reactions. It also helps enzymes maintain their stability, enzymes have a pH optimum and frequently produce bell-shaped velocity. graphs of - versus-pH [8]. When the pH of an enzyme is too high or too low, it completely loses its activity. Due to changes in the ionic state of the substrate, changes in the pH environment of the reaction in the active site, or complex enzyme substrate interactions at substrate concentrations greater than

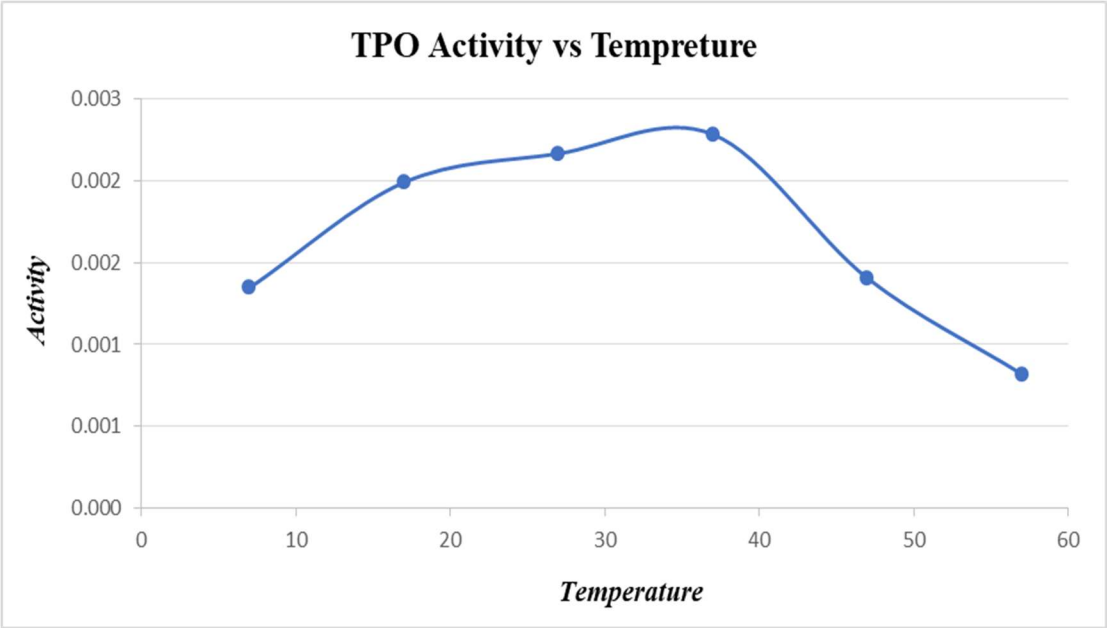
Km, enzyme activity may be reduced at low pH. The enzyme will be decisive if the concentration of the substrate is low. The tertiary structure of the enzyme is also impacted by pH . As a result, at high pH levels, the enzyme's activity may be irreversibly denaturized[9].




**Figure(5) Effect of pH on TPO activity**

### 3. 3. 3 Effect of Temperature on Enzyme Activity

Different temperatures were tested to determine the optimal temperature for TPO enzyme activity (7, 17, 27, 37, 47, and 57°C). The optimal temperature was 37 °C, and the findings show that increasing the temperature led to an increase in TPO activity, followed by a decrease in TPO activity, as shown in figure (6). It is well known that a particular reaction's speed rises with increasing temperature up to the reaction's ideal temperature, after which it gradually falls. because The enzyme molecule has become denaturated or damaged as a result. The binding between active amino acids disintegrates at high temperatures due to the increase in the kinetic energy of molecules, which impairs enzyme function. a modification to the enzyme's engineering configuration explains the decrease. The ionisation of groups on the surface of the enzyme and its substrate is influenced by high temperatures. Complex protein molecules called enzymes have a structure that affects their ability to catalyse reactions. An enzyme's tertiary structure is mostly maintained by non-covalent connections; however, when a molecule absorbs too much energy, the tertiary structure is destroyed, and the enzyme is denatured, losing its activity [10]. Below 37 °C. TPO activity decreases due to insufficient energy required to perform enzyme-substrate complex [11].




**Figure(6) Effect of Temperature on TPO activity**

### 3.3.4. Effect of Reaction Time on Enzyme Activity

The figure (7) has shown the enzyme activity dependence on incubation time ( 0, 5, 10, 15, 20 and 25) minutes, first minutes (1 min) at 37 C° was adopted as the standard incubation time throughout the work because it relates to a linear region of the curve and presents reliable absorbance values. This may be related to the temperature ascribed to the enzyme where the more time the heat breaks the bonds between two amino acids.

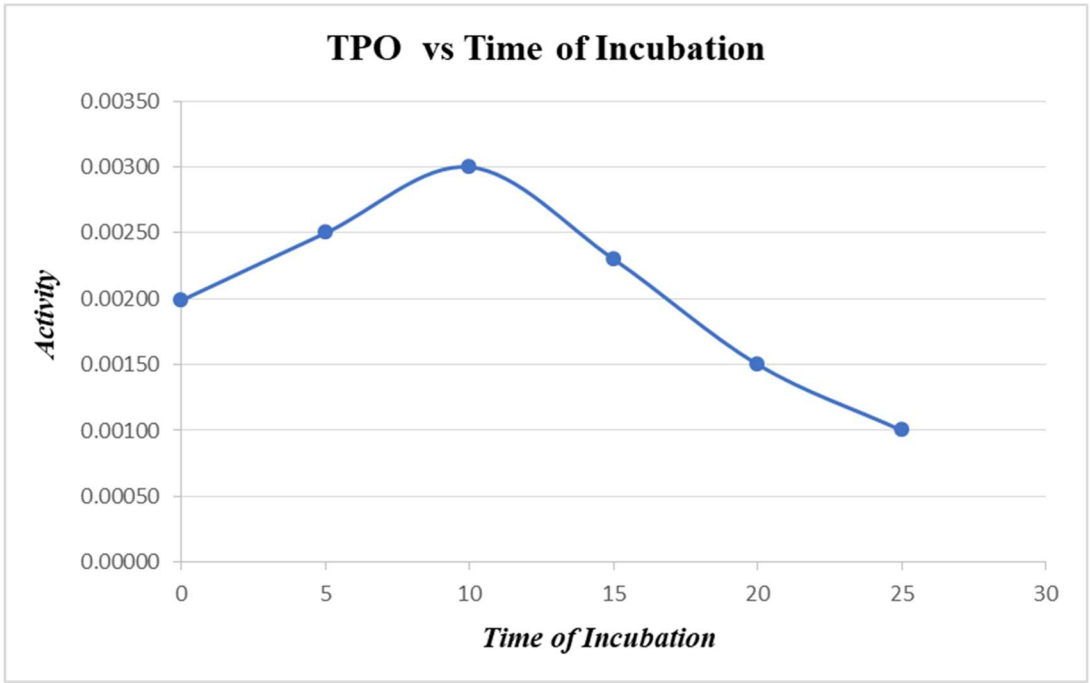



**Figure (7) Optimization incubation time of TPO**

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