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# Separation and purification of amylase enzyme from the sera of breast cancer patients

## فصل وتنقية إنزيم الأميليز من امصال مريضات سرطان الثدي

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## الخلاصة

تمت تنقية انزيم الأميليز من مرضى سرطان الثدي باستخدام كروماتوجرافيا التبادل الأيوني باستخدام ومحلول sephadexG100,باستخدام عمودDEAE,تقنية الترشيح بهلام السليلوز لفصل الانزيم. (7.2) مع الرقم الهيدروجيني (Tris–HCL)منظم أظهرت النتائج زيادة في نشاط إنزيم الأميليز، وهو ما يتناسب طرديا مع الزيادة في تركيز الركيزة. وكان 0.363mm)الشكل الناتج مستقيما، قيمة ثابت ميكايليس– مينتن ( درجة الحرارة المثلى لعمل الإنزيم هي (٣٧) مئوية. أعلى نشاط للإنزيم بعد حضانة خليط التفاعل الإنزيمي لمدة (١٥) دقيقة. أظهرت النتائج أن الوزن الجزيئي للإنزيم المنقى بالرحلان الكهربائي بلغ ٦٠ كيلو دالتون.**الكلمات المفتاحية: إنزيم الأميليز ، سرطان الثدي.**

## Abstract

Amylase was purified from breast cancer patients using ion exchange chromatography using the DEAE cellulose gel filtration technique, using a Sephadex G100 column, and using a buffer solution of (Tris-HCL) with pH (7.2) to separate the enzyme.

The results showed an increase in amylase enzyme activity, which is directly proportional to the increase in substrate concentration. The resulting shape was straight, and the Michaelis-Menten KM value was (0.363) mm). The optimum temperature for enzyme action is (37) C. The highest activity of the enzyme after incubation of the enzyme reaction mixture for (15) minutes. The results showed that the molecular weight of the enzyme purified by electrophoresis reached 60 KD.

**Keywords: Amylase Enzyme, Breast cancer**

## Introduction

There has been increased interest in using saliva as an additional test that improves traditional methods of medical evaluation of serious systemic diseases, including breast cancer screening and detection. Studies dedicated to the analysis of saliva protein and transcript showed the great potential of this biological fluid for breast cancer detection. It has previously been shown that there is a positive relationship between the expression of several of biomarkers in serum and saliva, as patients with cancer have higher expression of specific biomarkers compared to patients without cancer[1] Carbohydrates are the main ingredients For the human diet, sugars are one of the most important The main components of carbohydrates that are mainly It plays an important role in power supply. The carbohydrates must first be broken down to monosaccharides by certain enzymes in the digestive system , since only sugars can do that it is absorbed into the intestinal lumen . α Glucosidase and α-amylase are the main enzymes that Participate in the digestion of carbohydrates[2] αAmylase hydrolyzes complex dietary carbohydrates to oligosaccharides and polysaccharides that are eventually converted to monosaccharides by α- glucosidase. Amylases are broadly classified into α, β, and γ subtypes, of which the first two have been the most widely studied. α-Amylase is a faster-acting enzyme than β-amylase[3] The most prevalent enzyme in the saliva of humans and many other animals is alpha- amylase (EC 3.2.1.1). Salivary amylase is secreted predominantly in the parotid gland in humans. Its activity varies

greatly both between and within a person. The polysaccharides -(1,4)- glycosidic linkages are known to be broken by the enzyme. Moreover, it is crucial for the fundamental biomechanics of humans, aiding the metabolism of carbohydrates[4].

**Material and methods**

DEAE-Cellulose, Sephadex G-100, Ammonium Sulfate(NH4)2SO4, Tris –HCL, Sodium hydroxide (NaOH)

**methods**

**1-ammonium sulfate for partial purification**

Serum proteins were precipitated using graduated concentrations of ammonium sulfate $(NH4)_2SO4$, as (9.3 grams) of ammonium sulfate were added to (15) ml of serum during a period of (45-60) minutes. The precipitate was then dissolved in (10)Mm buffer solution Tris HCl of PH7.2 0.1 M

**2-Membranous separation (dialysis)**

It is one of the most important and well-known methods used to partially purify enzymes, and its purpose is to take out the remaining ammonium sulfate added to precipitate the protein. The dissolved protein was placed in a dialysis bag after measuring the amylase enzyme activity and protein concentration. Its bag was dipped in the buffer solution for (18) hours, and the buffer solution was changed from time to time. After the membrane separation process was completed, amylase enzyme activity and protein concentration were determined.

**3- Ion exchange (DAEA-Cellulose)**

The basis of the work of this technology is the difference in charge, as it was used to purify the analog separated by filtration using a filter column containing the material DAEA-cellulose

**solutions**

1- Buffer solution 10 mM Tris-HCl – PH 7.2
It was prepared by dissolving( 1.58gm) of Tris-Hcl in 1000ml of distilled water and adjusting the pH at 7.2
٢-filtration solution DAEA-cellulose
The solution was prepared by dissolving (8) g of DAEA-cellulose in (200) ml of distilled water and leaving the solution for (22-24) hours at a of 4℃. During this period, the buffer solution was changed several times to remove fine particles from the solution because their presence reduces the flow velocity of the mature solution through the column.

**The method:-**

1- A glass column with a diameter of (2.3 cm * 20 cm) was used. A bit of glass wool was placed at its end to prevent the DAEA-cellulose particles from leaking out of the column. The DAEA-Cellulose suspension was poured into the column slowly to prevent the formation of air bubbles that hinder the separation until it reached a height of (10 cm). The column was washed with sufficient amounts of buffer solution until a flow velocity (1.5 mL/min) was obtained.
2- (5) ml of the enzyme after membrane separation was added slowly to the surface of DAEA-Cellulose and left for (5) minutes.
3- The separation process was started by using (150) ml of the buffer solution, collecting (5) ml for one part
4- After collecting the mature parts from the separating column, the enzyme activity and protein concentration were measured

**Gel filtration**

The basis for the work of this technique is the difference in molecular weight, as it was used to purify the analog separated by gel filtration chromatography using a gel filter column on Sephadex G100.

**Solution**

The solution was prepared by dissolving (3) grams of Sephadix G100 in (200) ml of distilled water, and leaving the solution for (22-24) hours at a temperature of 4 degrees Celsius. During this period, the buffer solution was changed several times to remove fine particles from the solution because their presence reduces the flow velocity of the mature solution through the column.

**method**

1-A fractionation column( 2.3cm*20cm)was used, and a little glass wool was placed at its end to prevent the Sephadix G-100 particles from leaking out, The solution was poured slowly to prevent the formation of bubbles until the gel height reached (10) cm. The column was also washed several times to obtain At a flow rate of (1.5) ml/min
2- (5) ml of the enzyme after membrane separation was added slowly to the surface of Sephadix G-100 and

left for (5) minutes

3- The separation process was started by using (150) ml of the buffer solution, collecting (5) ml for one part.
4- After collecting the mature parts from the separating column, the enzyme activity and protein concentration were measured.

## Results& Discussion

**Molecular purification of amylase in breast cancer patients**

Sera from patients with the highest amylase activity were collected and mixed to obtain a total volume of 15 ml. The mixture was exposed to ammonium sulfate at a saturation rate of 65%. It was found that at this percentage, the specific activity of the resulting enzyme was 0.00088 units/mg, which is a noticeable decrease compared to 0.0011 units/mg of the specific activity of the crude enzyme. Activity is shown in (Table 1.1).
After purification using DEAE cellulose chromatography, the enzyme activity increased further. Figure (1.1) shows a diagram of the amylase enzyme and its specific activity. In the dialysis step, (elution was done at 0.4 M NaCl) the α-AMY activity reached 0.0006 U/mL. After completion of ion exchange chromatography, active fractions representing amylase were collected and pooled onto a Sephadex G-100 column (Dimensions) that had been pre-equilibrated with phosphate buffer. The results shown in (Figure1.2) showed only one absorption peak representing amylase with maximum activity. The gel filtration technique purified the enzyme by approximately (1.68) purification folds with a maximum activity of 0.013 units/ml and yielded 6.01%.

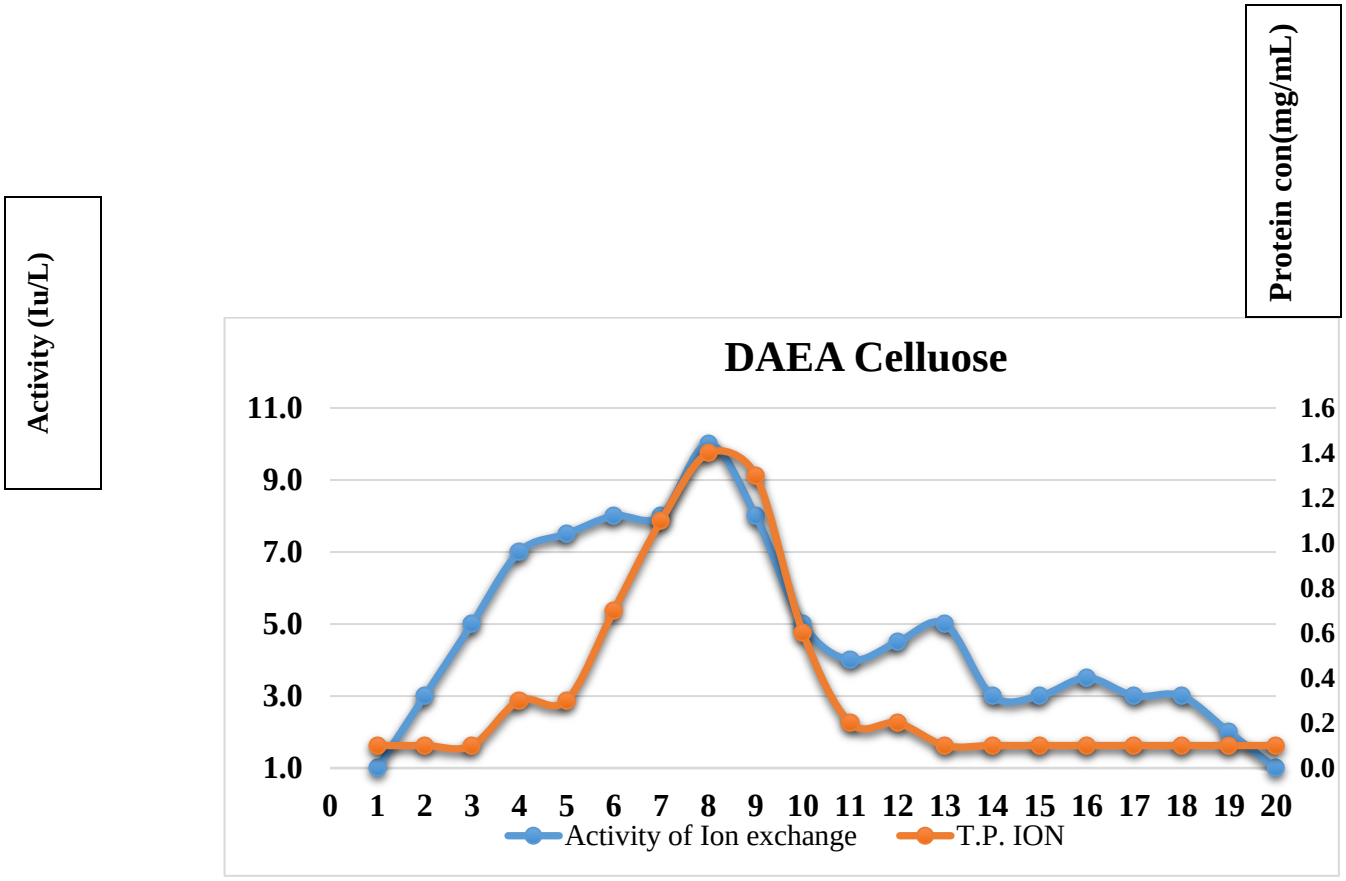


**Figure (1.1): Purification of amylase enzyme by DAEA cellulose**

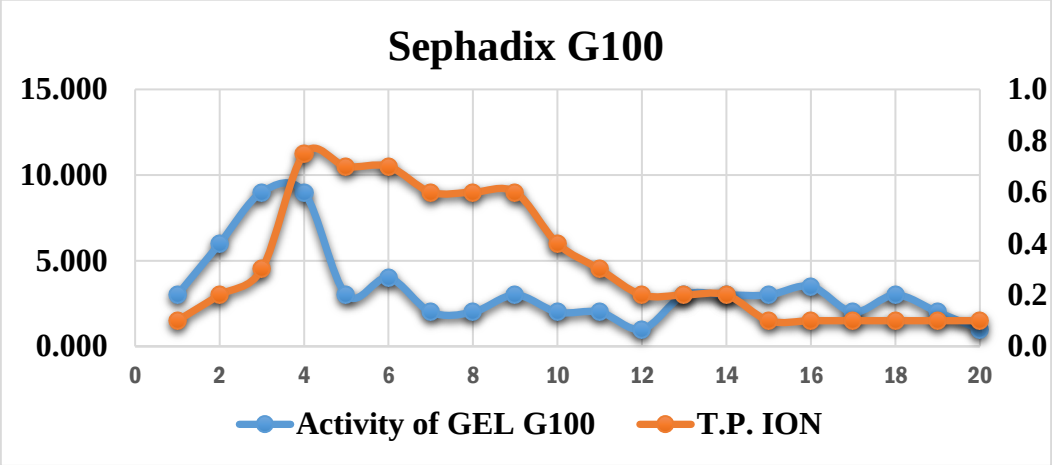


**Figure (1.2): Purification of amylase enzyme by sephadix gel G100**

**Table (1.1) : Separation and purification of amylase enzyme from breast cancer patients**

| Purification Steps | Volume (ml) | Activity (IU/ml) | Total Activity | Protein Concentration (mg/ml) | Specific Activity (U/mg) | Recovery Yield % | Fold of purification | Total protein |
|---|---|---|---|---|---|---|---|---|
| **Crude** | **15** | 0.072 | 1.08 | 63 | 0.0011 | 100 | 1 | 945 |
| **Precipitation** | **13** | 0.044 | 0.572 | 50 | 0.00088 | 52.9 | 0.8 | 650 |
| **Dialysis** | **14** | 0.012 | 0.168 | 20 | 0.0006 | 15.5 | 0.54 | 280 |
| **Ion exchange (DAEA-Cellulose)** | **5** | 0.015 | 0.075 | 10 | 0.0015 | 6.9 | 1.36 | 50 |
| **Gel Filtration** | **5** | 0.013 | 0.065 | 7 | 0.00185 | 6.01 | 1.68 | 35 |

**Effect Of Substrate On The Activity of Amylase Enzyme In Breast Cancer Patients**

The effect of the substrate concentration on the reaction rate of the partially purified amylase enzyme separated from a Sephadex G100 column was studied. It was observed that there was an increase in the enzymatic reaction rate with increasing substrate concentration. Figure (1.3) demonstrates how the rate of the enzymatic reaction rises as the substrate concentration does (ALFA AMYLASE) This is consistent with the study (Kiran et al), which indicated that the amylase activity increases with the increase in the concentration of the substrate, and becomes stable gradually[5]

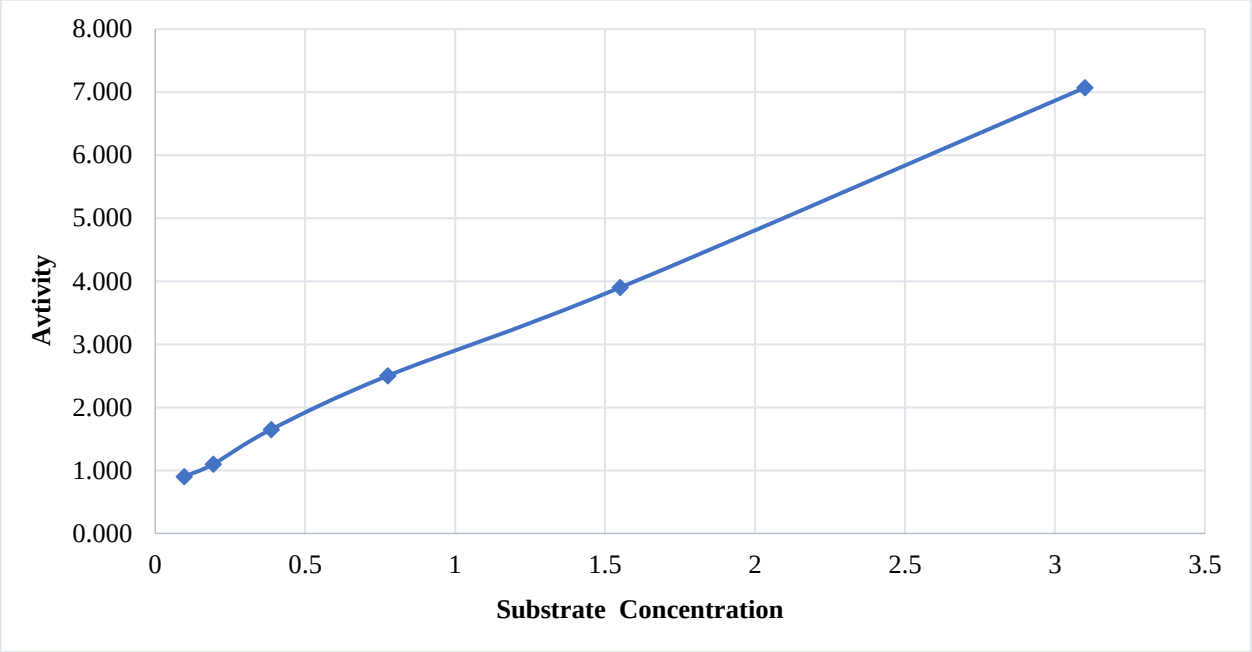


**Figure (1.3) : Effect Of Substrate On The Activity of Amylase Enzyme In Breast Cancer Patients.**

**Find Michaelis-Menten Constant Km**

There are several methods for calculating the value of Km, but the linear method of Lineweaver-Burkin calculating the value of Km is the most accurate and the best from a practical point of view, due to the lack of arithmetic operations in it and its efficiency in indicating the accuracy of the experiment, and the value of Km obtained from it is relatively accurate to calculate the Km constant for the purified enzyme using gel filtration, the procedure was followed as illustrated in Figure (1.4), resulting in a Km value equal to ٠,٣٦٣ Mm , Vmax 4. He( S. A. Fincan and B. Enez )indicated that the value of the Michaelis-Menten constant for the amylase enzyme was 0.015 mM and the value of Vmax 1.424[6]

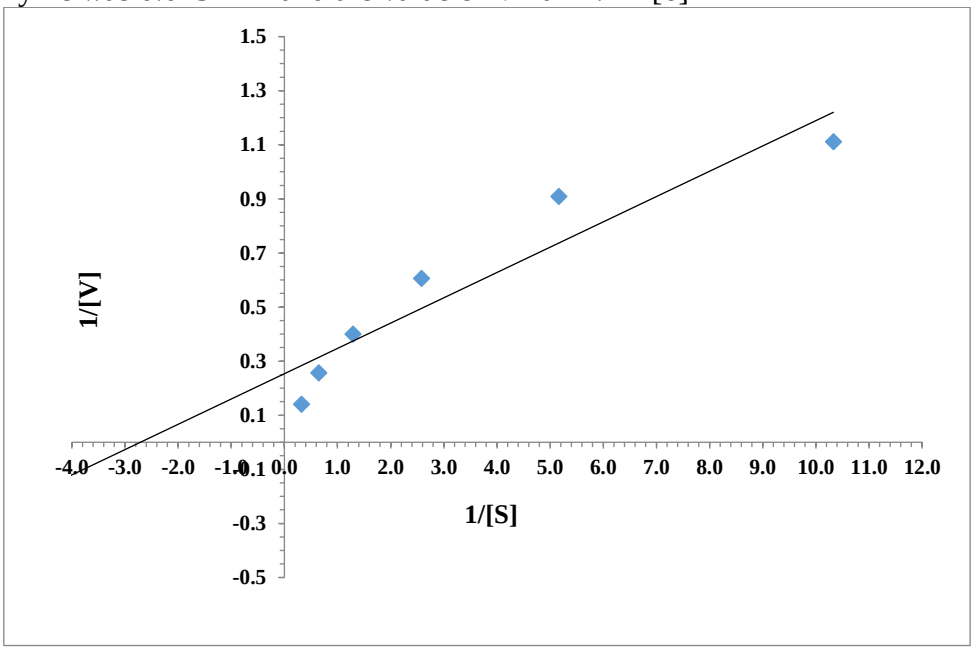


**Figure (1.4) : Lineweaver-Burk plot to calculate the value of Km of amylase enzyme in breast cancer patients**

**Effect Of pH On The Activity of Amylase Enzyme In Breast Cancer Patients**

The pH level significantly affects enzymatic activity due to variations in the enzyme's nature and chemical composition, as well as the presence of multiple ionic groups carried by the enzyme. Enzymes function optimally at specific pH levels as they are highly sensitive to changes in hydrogen ion concentration. When using different pH levels for the buffer solution (carbonate-bicarbonate) used in the amylase enzyme reaction, there is an increase in the reaction rate with higher pH levels until reaching the maximum speed at the optimal pH, which was (pH 7.2). After that point, the enzyme's activity decreases due to internal electrostatic repulsion

or loss of internal electric charge on the side chains of amino acids resulting from the unfolding of the enzyme protein molecule. As a consequence, the enzyme becomes unable to form the enzyme-substrate complex (ES complex). Figure (1.5) you get is consistent with what you get( S. Kiran et al)[5]

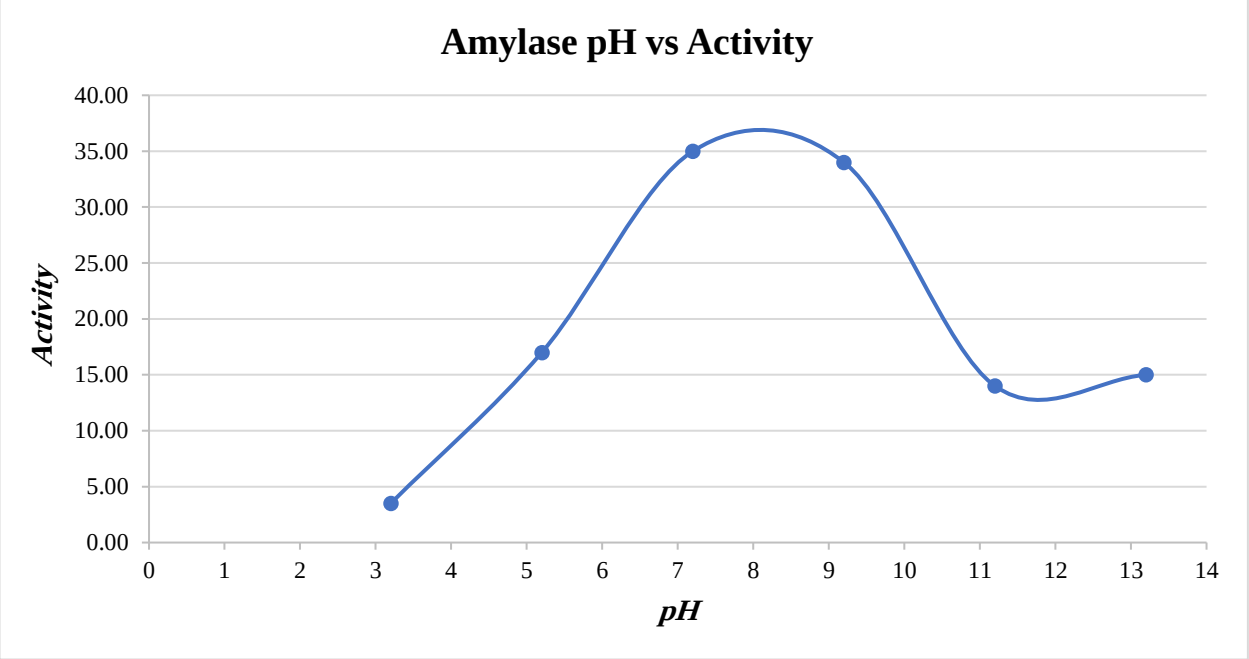


**Figure (1.5) : Effect Of pH On The Activity of Amylase Enzyme In Breast Cancer Patients**

**Effect Of Temperature On The Activity of Amylase Enzyme In Breast Cancer Patients**

The optimum temperature for enzymatic activity is the highest temperature at which the rate of the enzymatic reaction reaches its maximum and the enzyme maintains its maximum activity. This period is not less than the time required to evaluate the enzyme activity.

Several factors influence the determination of the optimal temperature for enzymatic activity, including the solubility of the enzyme, the pH of the reaction solution, the thermal stability of the enzyme, the quality and ionic strength of the buffer solutions used, and the ionization state of the active groups and substrate.

The rate of the enzymatic reaction increases with increasing temperature until it reaches the optimum temperature for the reaction, after which it gradually decreases. This decrease is due to denaturation and destruction of the enzyme molecule, which leads to a decrease or loss of enzyme activity. This decrease is due to effects on the conformational structure of the enzyme and the possibility of denaturation and damage of the enzyme molecule. High temperatures increase the kinetic energy of molecules, breaking bonds between active amino acids, and resulting in loss of enzyme activity. In general, the enzyme cannot perform its effective role at high temperatures that exceed what affects its structural form. This results in the removal of its natural form, resulting in loss of enzymatic activity.

The results showed an increase in the reaction rate of the amylase enzyme with increasing temperature, and the highest rate was observed at a temperature of 37°C. Figure(1.6) These results are consistent with the findings of (Yalçın & Çorbacı ) regarding the optimal degree of action of the enzyme α-amylase for Aspergillus oryzae at 30-37 °C[7]

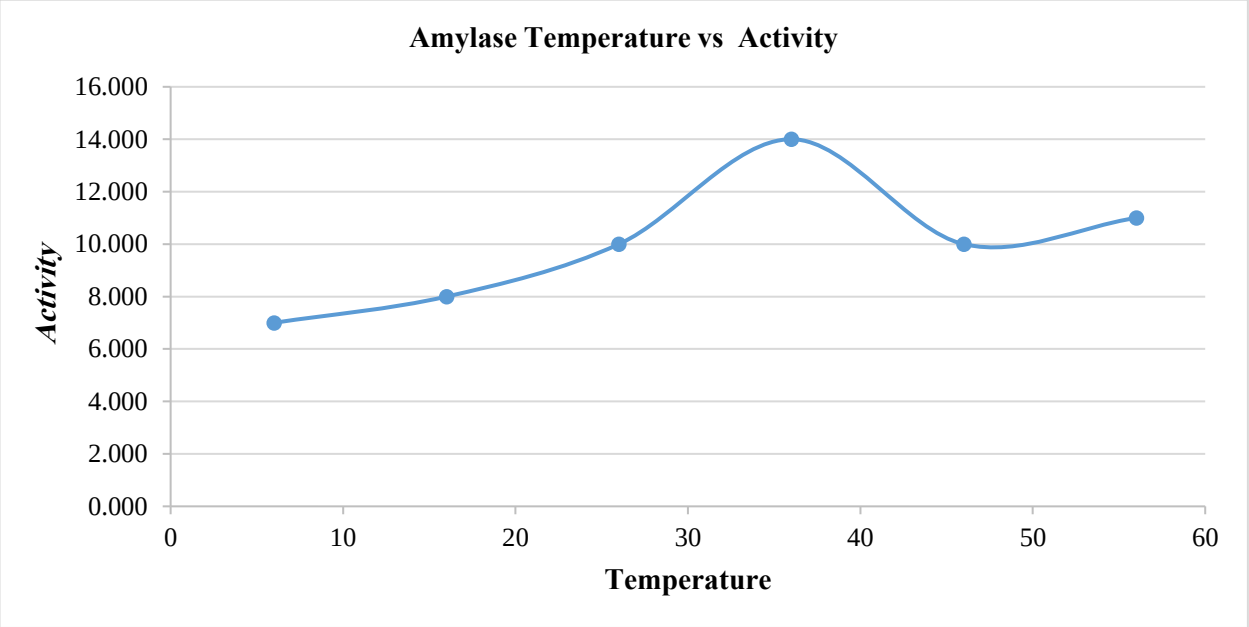


**Figure (1.6) : Effect Of Temperature On The Activity of Amylase Enzyme In Breast Cancer Patients**

**Effect Of Incubation Time On The Activity of Amylase Enzyme In Breast Cancer Patients**

The study investigated the effect of reaction time on the activity of the purified enzyme from breast cancer patients' sera by incubating the enzyme with its substrate at a temperature of 37°C for different time intervals (1 - 25) at pH 7.2. The results showed an increase in enzyme activity with increasing incubation time, and the maximum enzyme activity was observed at 37°C after a 15-minute incubation period with the reaction mixture.

However, after 15 minute period, the enzyme's activity started to decrease, as shown in Figure(1.7) The results of this study do not match the findings of (LIU, Xu Dong) (W. H. Abdulaa). They indicated that the purified enzyme was incubated for 60 minutes[8][9]

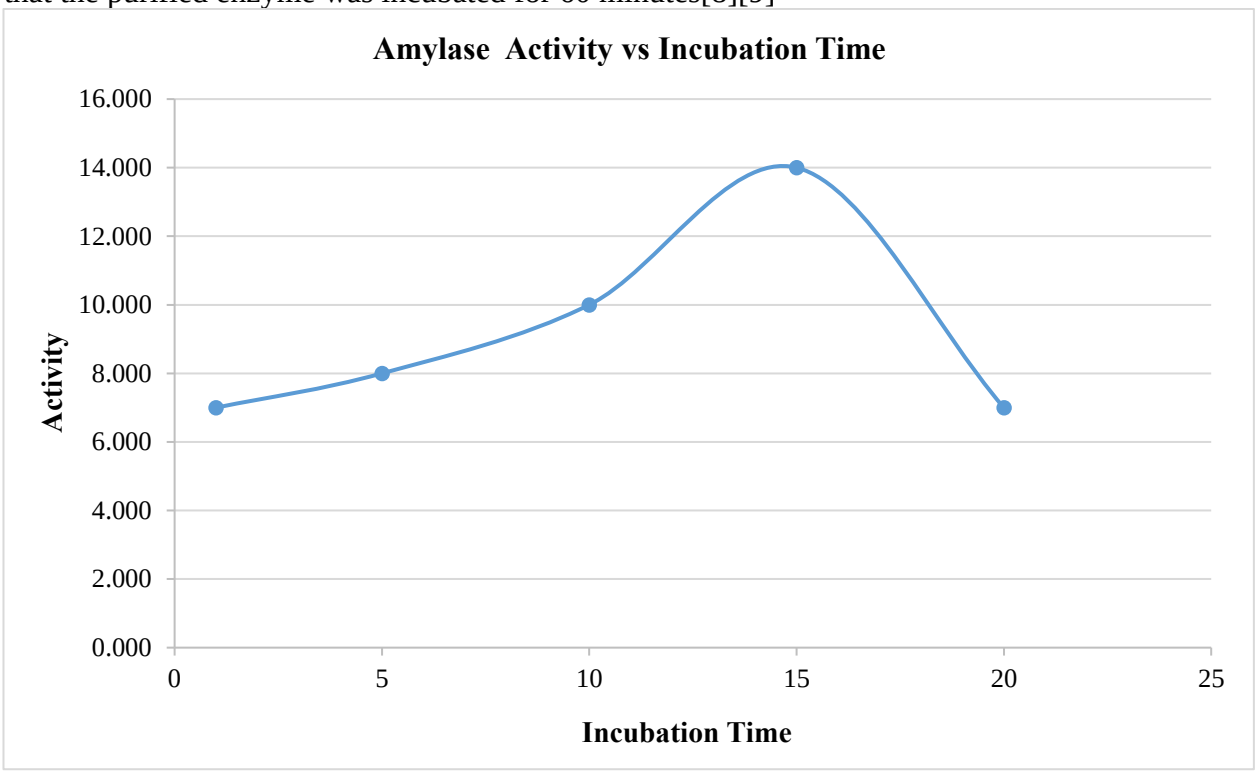


**Figure (1.7) : The Effect Of Incubation Time On The Activity Amylase Enzyme In Breast Cancer Patients**

**Detection of Amylase Purity by Electrophoresis**

The movement of enzymes during electrophoresis depends on several factors, including molecular weight. When other factors such as charge and electricity arise, proteins move over different distances depending on the molecular weight. Figure (1.8) shows the shape of the alpha-amylase enzyme when it migrates in standard solutions[10]. The molecular weight of α-amylase purified from individuals with breast cancer (60KD) is shown in the figure below.

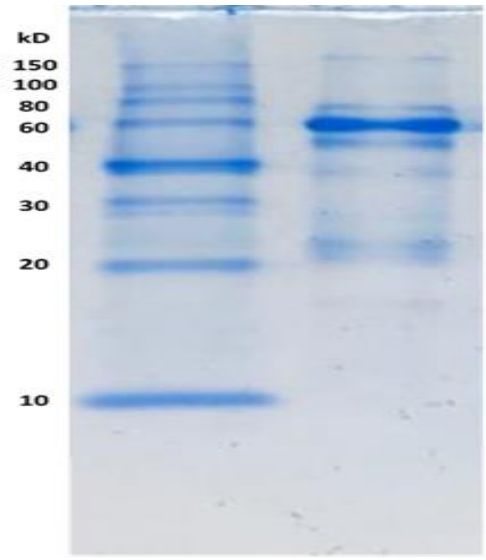


**Figure (1.8): Electrophoresis of partially purified alpha- amylase to determine the molecular weight**

**Conclusions**

Amylase partially purified by DEAE-Cellulose column, yield (6.9%) and specific activity (0.0015).

Amylase partially purified by ion exchange column, yield (6.01%), and specific activity (0.00185). The values of Km and Vmax were (Km = 0.363Mm) and (Vmax 4).

The molecular weight of the enzyme purified from patients' serum was (60 KD).

**Recommendations:**

Study and purification of other enzymes in breast cancer patients.

**References**

**1-** L. V Bel'skaya, E. A. Sarf, D. V Solomatin, and V. K. Kosenok, "Metabolic Features of Saliva in Breast Cancer Patients," *Metabolites*, vol. 12, no. 2, p. 166, 2022

**2-** B. Nikavar and N. Yousefian, "Inhibitory effects of six Allium species on α-amylase enzyme activity," 2009.

**3-** S. C. B. Gopinath *et al.*, "Biotechnological processes in microbial amylase production," *Biomed Res. Int.*, vol. 2017, 2017.

**4-** C. Boehlke, O. Zierau, and C. Hannig, "Salivary amylase–The enzyme of unspecialized euryphagous animals," *Arch. Oral Biol.*, vol. 60, no. 8, pp. 1162–1176, 2015.

**5-** S. Kiran, A. Singh, C. Prabha, S. Kumari, and S. Kumari, "Isolation and characterization of thermostable amylase producing bacteria from hot springs of Bihar, India," *Int. J. Pharma Med. Biol. Sci.*, vol. 7, no. 2, pp. 28–34, 2018.

**6-** S. A. Fincan and B. Enez, "Production, purification, and characterization of thermostable a-amylase from thermophilic Geobacillus stearothermophilus," *Starch/Staerke*, vol. 66, no. 1–2, pp. 182–189, 2014, doi: 10.1002/star.201200279.

**7-** H. T. Yalçın and C. Çorbacı, "Isolation and Characterization of Amylase Producing Yeasts and Improvement of Amylase Production.," *Turkish J. Biochem. Biyokim. Derg.*, vol. 38, no. 1, 2013.

**8-** X. D. Liu and Y. Xu, "A novel raw starch digesting α-amylase from a newly isolated Bacillus sp. YX-1: purification and characterization," *Bioresour. Technol.*, vol. 99, no. 10, pp. 4315–4320, 2008.

**9-** W. H. Abdulaal, "Purification and characterization of α-amylase from Trichoderma pseudokoningii," *BMC Biochem.*, vol. 19, no. 1, pp. 1–6, 2018.

**10-**S. Roy and V. Kumar, "A practical approach on SDS PAGE for separation of protein" *Int. J. Sci. Res.*, vol. 3, no. 8, pp. 955–960, 2014