

## **Kinetics of $\alpha$ -amylase enzyme in human serum**

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### **الخلاصة:**

أجريت هذه الدراسة لدراسة الخواص العامة لأنزيم  $\alpha$ -أميليز  $\alpha$ -amylase من خلال دراسة العوامل المؤثرة على فعالية الإنزيمات بصورة عامة والتي هي الدالة الحامضية pH, درجة الحرارة, وتركيز المادة الأساس, و من خلال قياس فعاليته بطريقة كاراوي Carraway وتم اعتماد 25 شخص سليم ومعرفة المدى الطبيعي لهذا الإنزيم, في مصل الانسان ومعرفة الظروف المثلى لفعاليته , حيث وجد أن لهذه العوامل تأثير واضح على فعالية أنزيم  $\alpha$ -أميليز, كما درست فعاليات هذا الانزيم في مصل 20 مريض بالتهاب البنكرياس حيث وجد ان هناك فرق معنوي عند مقارنتهم بمجموعة الأصحاء (السيطرة) مقدار  $<0.005$  كما وجد كل من قيمتي  $V_{max}$  و  $K_m$  للأنزيم والتي كانت 100 و 25mg/dl في مجموعة الأصحاء بينما كانت قيمة كل من  $K_m$  و  $V_{max}$  في مجموعة المرضى المصابين بالتهاب البنكرياس هما 30 و 71.42mg/dl وهذا يدل على ان ميل الانزيم للارتباط بالمادة الأساس لمجموعة الأصحاء اكبر من ميله في مجموعة السيطرة.

### **Abstract:**

**Objective:** The present study was conducted to study the general characterization of  $\alpha$ -amylase (AMS) enzyme by studying of the effect of various factors which affected on the activity of the enzyme ( $\alpha$ -amylase ) by caraway method, in control group and patients group who was studied .

**Method:** Twenty five healthy subject were depended in this study, and indicated the normal level of the enzyme, the factors effect of the activity  $\alpha$ -amylase enzyme are: pH, temperature, substrate concentration, incubation duration. The evolution of the factors exhibit significant effect on the activity of the enzyme, and the  $V_{max}$  and  $K_m$  were found 100,25 mg/dl respectively, in 20 patients with pancreatitis., the  $V_{max}$  and  $K_m$  were found in this group 71.42, 30mg/dl.

**Results:**  $\alpha$ -amylase activity was observed to be very stable enzyme and effective at pH (5- 7.8), temperature (37,40), 0.06 mg/dl substrate concentration.  $V_{max}$  and  $K_m$  were found 25 mg/dl,100 in healthy group but in patients group were found 30 mg/dl,71.42. AMS activity in serum patients of pancreatitis was very stable in pH 7-7.6, temperature 37C° substrate concentration. The results of serum enzyme activity measurement revealed significant ( $p<0.005$ ) elevation of AMS activity in patients of pancreatitis when compared with those of the healthy subject.

**Keywords:**  $\alpha$ -amylase enzyme (AMS),  $\alpha$ -amylase sources.

### **Introduction:**

$\alpha$ - Amylase is an enzyme belonging to the class of hydrolases enzymes that catalyze the breakdown of starch and glycogen [1]. Starch consists of both amylose and amylopectin, amylose is along un branched chain of glucose molecules, linked by  $\alpha$ -1-4 glycoside bonds, amylopectin is branched chain polysaccharides with  $\alpha$ -1-6 linkage at the branch points [2]. The structure of glycogen is similar to that of amylopectin but is more highly branched [3]. Amylase is present at a high concentration in pancreatic juice and saliva and may be extracted from such other tissue as the gonads fallopian tubes, skeletal muscle and adipose tissue [4]. In normal subjects most plasma amylase is derived from the pancreas and salivary glands. Being of relatively low molecular weight, it is excreted in the urine [5]. Estimation of plasma amylase activity is mainly requested to help in the diagnosis of acute pancreatitis, the diagnosis significance of serum and urine  $\alpha$ -amylase

measurements is in the diagnosis of acute pancreatitis, disorder of tissue other than the pancreas can also produce elevation in levels [6]. In which the plasma activity may very high. However it may also be raised in association with other intra and extra abdominal conditions that cause similar acute abdominal pain [7], enzymes activities were affected by factors, such as pH, temperature and substrate concentration:

Increasing the temperature usually increases the rate of a chemical reaction by increasing the movement of molecules, the rate at which intermolecular collisions occur, and energy available for the reaction. Such is the case with enzymatic reactions, until the temperature is high enough to denature the protein composition of the enzyme. The reaction rate increase for every 10 C° increase in temperature, is approximately 2 for enzymes, indicating that this increase in temperature usually doubles the reaction rate [8]. The rate at which an enzymatic reaction proceeds and whether the forward or reverse reaction occurs depend also on substrate concentration. Both Michaelis and Menten hypothesized the role of substrate concentration in formation of the enzyme-substrate complex (ES), according to the hypothesis, with the amount of exceeding the amount of substrate, the reaction rate steadily increases as more substrate is added [9]. Because enzymes catalyze physiologic reaction, the enzyme concentration affects the rate of the catalyzed reaction. As long as the substrate concentration exceed the enzyme concentration, the velocity of the reaction is proportional to the enzyme concentration [10].

The aim of this study is to characterized the factors were effected amylase activity in serum human.

### **Material and method :**

Twenty patients complained from pancreatitis, the patients were admitted to the AL-Hussain General Hospital in Kerbala for management. In addition, 25 healthy individuals were included as control group.

Determination of  $\alpha$ -amylase enzyme activity:

The activity level of  $\alpha$ -amylase was determined by carraway method [11].

The principle of caraway method is: this method was depend of the measurment on the starch unhydrolysis level by addition of starch with particular concentration to blood serum under reaction conditions optimum to convert (change) starch to maltose, this reaction stopped after 15 min from the starting addition of enzyme inhibitor and measured the amount of starch which unhydrolyze, this amount inverse relatively with the amylase activity.

**Determination of pH optima:** To determine the effect of pH on  $\alpha$ -amylase enzyme activity, the enzyme as incubated in a set of different concentrations of phosphate buffers of pH ranging from 2.6 to 7.6 and the reaction was performed at 37 C°, the buffers phosphate by use citric acid (from Na<sub>2</sub>HPO<sub>4</sub>+citric acid) solution are (2.6, 3, 3.4, 3.8, 4.2, 4.6, 5.0, 5.4, 5.8, 6.2, 6.6, 7.0, 7.6).

**Determination of the effect of temperature:** The effect of temperature on enzyme activity was determined by varying incubation temperature the reaction mixture from 10 C° to 60 C°, this temperatures are (10, 20, 30, 37, 40, 50, 55, 60) C° .

**Determination of the effect of substrate concentration :**

To determine the effect of the substrate by various concentration of starch ranging from 0.01 mg/dl to 0.08 mg/dl, this concentrations are (0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08) mg/dl.

Maximum velocity of reaction ( $V_{max}$ ) and Michaela's cnstant ( $K_m$ ) were determined by using Lineweaver-Burk plot and the values were found .

**Results:**

The rang of  $\alpha$ -amylase enzyme activity in normal human serum was (45-160)U/L when use 25 healthy subject (control group), while in 20 serum patients with pacreatitis the rang was (88-370)U/L. This were expressed as mean  $\pm$  SD and analyzed statistically by student t test, the results of serum enzyme activity measurement reveled significant ( $p < 0.005$ ) elevation of AMS activity in patients of pancreatitis table (1).

Table (1): serum activity in patients of pancreatitis with healthy subject.

Parameter	Subject	Mean $\pm$ SD	P value
$\alpha$ -amylase U/L	Control	68.54 $\pm$ 18.22	<0.005
	Patients	170.44 $\pm$ 19.30	

From the doing experiments showed that:  $\alpha$ -amylase activity hasalso been observed that the enzyme is very stable and effective at pH (6.6, 7.6) (fig (1)):

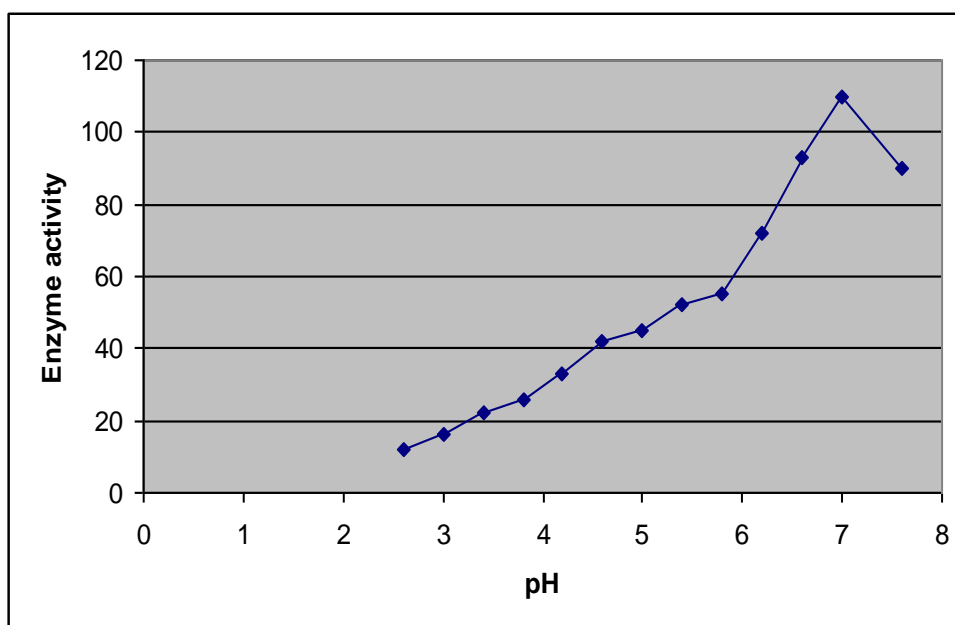
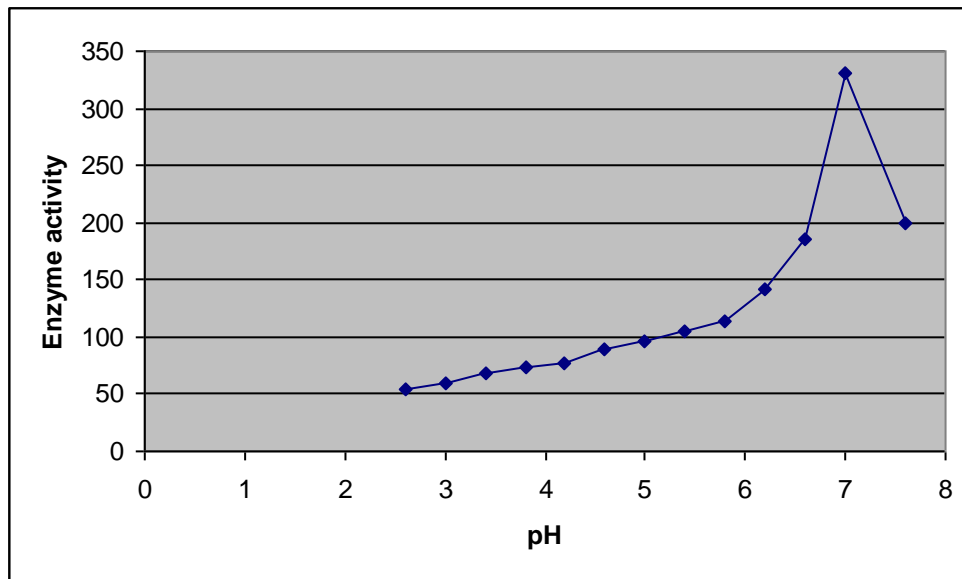


Figure (1): Relationship between pH and activity of  $\alpha$ -amylase in serum healthy subject.

But with serum patients pancreatitis enzyme activity was stable in pH (7,7.6) Fig (2).



Figure(2): Relationship between pH and activity of  $\alpha$ -amylase in serum patients with pancreatitis.

The study of the effect of different temperatures on the activity of  $\alpha$ -amylase revealed that with the rise in temperatures, the activity of the enzyme increases up to (37, 40) C° (fig (3)).

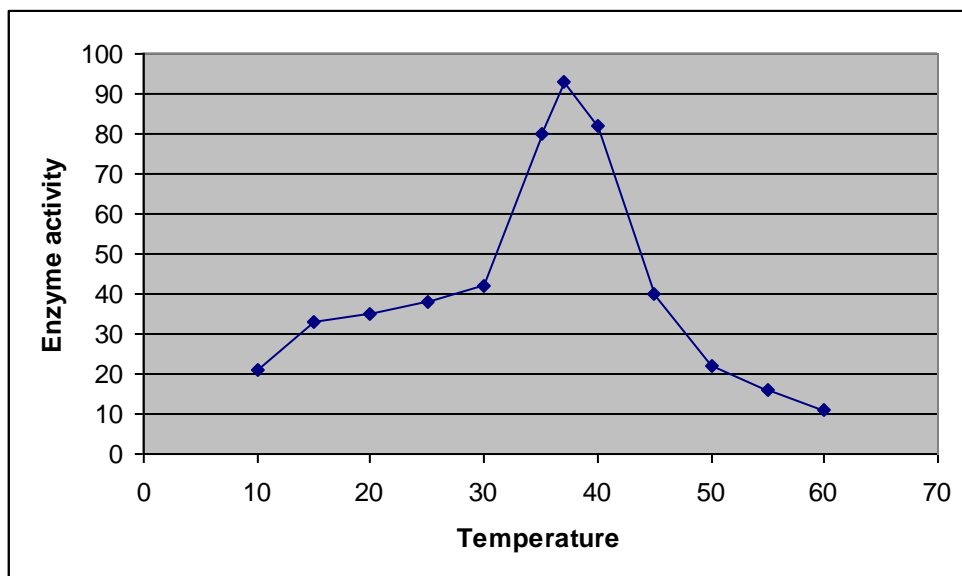


Figure (3): Relationship between temperature and activity of enzyme in serum healthy subject.

But with serum patients pancreatitis enzyme activity was stable in temperatures, the activity of the enzyme increases up to (37) C°fig (4).

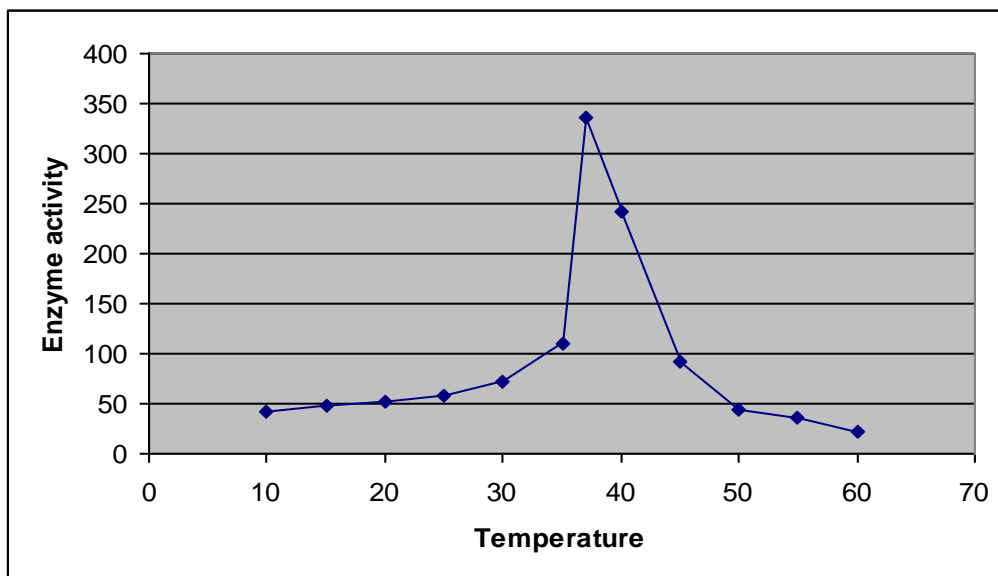


Figure (4): Relationship between temperature and activity of enzyme serum in patients with pancreatitis.

It was also observed that with the increase in substrate concentration, the enzyme activity also increased in 0.07 mg/dl (fig (5)).

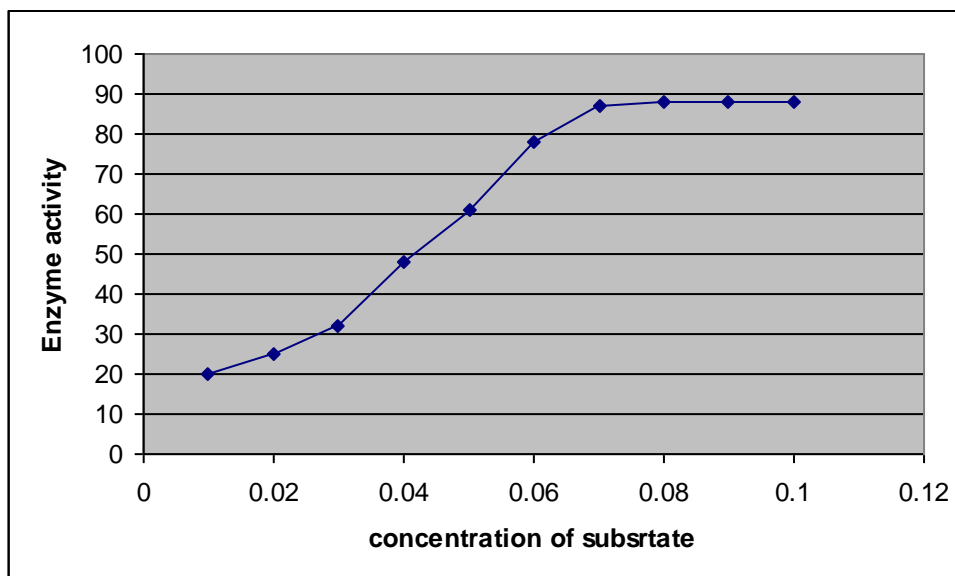


Figure (5): Relationship between substrate concentration and activity of enzyme in serum healthy subject.

While with serum patients of pancreatitis enzyme activity was increased in 0.08 mg/dl (fig (6)).

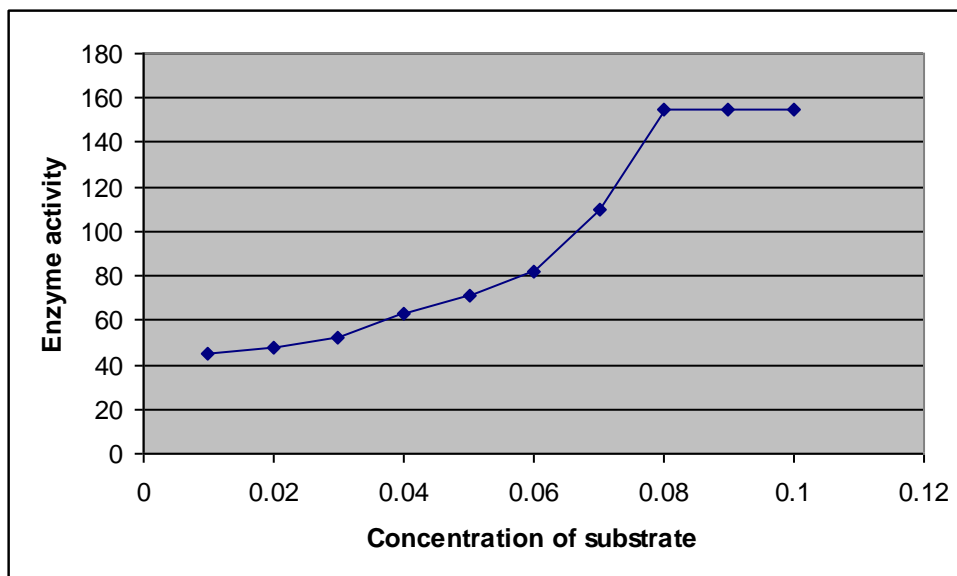


Figure (6): Relationship between substrate concentration and activity of enzyme in serum patients with pancreatitis.

The  $K_m$  and  $V_{max}$  with serum enzyme activity in healthy were 25 mg/dl and 100 by Lineweaver-Burk plot (fig (7)):

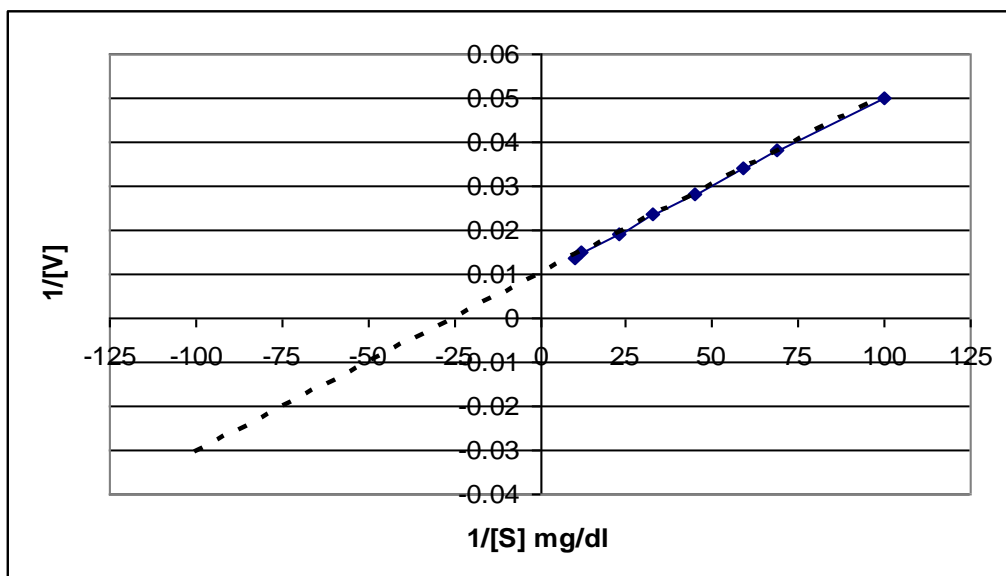


Figure (7): The Lineweaver-Burk plot for activity of enzyme in serum healthy subject. While the  $K_m$  and  $V_{max}$  with serum patients of pancreatitis enzyme activity were 30 mg/dl and 71.42 by Lineweaver-Burk plot (fig (8)):

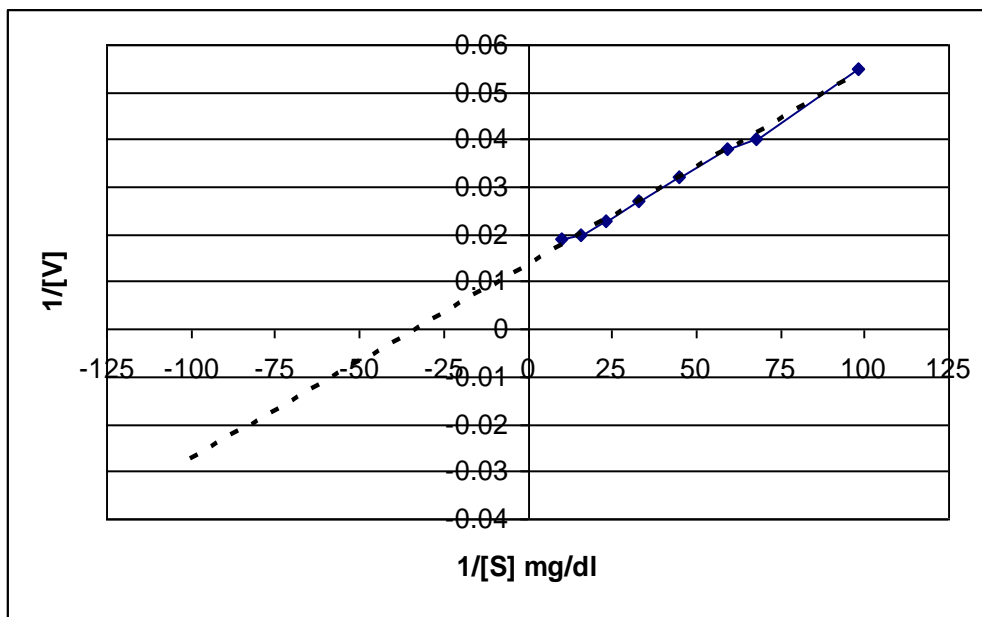


Figure (8): The Lineweaver-Burk plot for activity patients of pancreatitis enzyme activity.

### Discussion:

An elevated  $\alpha$ -amylase level is a nonspecific finding, however, the degree of elevation of AMS enzyme is helpful, to some extent, in the differential diagnosis of acute pancreatitis, serum AMS levels begin to rise 2 to 12 hours after the onset of an attack, peak at 24h, and return to normal levels within 3 to 5 days. Values can reach much higher levels[12].

The broad peak of the curve in fig (1) and fig (2) significance that pH has very more influence on the activity and stability of enzyme [13].

The study of the effect of different temperatures on the activity of  $\alpha$ -amylase revealed that with the rise in temperatures in both groups, the activity of the enzyme increases with (37, 40) C° fig (3) and fig (4), and thereafter decrease slightly in activity probably because of enzyme denaturation [14], fig (3) and (4), The studies of enzymes reported to have optimum activity at (37, 40) [14].

It was also observed that with the increase in substrate concentration, the enzyme activity also increased up to 0.07 mg/dl, following with the increase in substrate concentration, and thereafter the enzyme activity was reduced [15, 16]. This might be due to maximum saturation fig (5) and (6).  $V_{max}$  (maximum velocity of reaction) and  $K_m$  (Michaelis's constant) values of enzymes activity were determined by using Lineweaver – Burk plot and the values were found to be 100 and 25 mg/dl, respectively in serum healthy subject, while in patients of pancreatitis  $V_{max}$  and  $K_m$  were 71.42 and 30 mg/dl. This results suggestive of AMS enzyme in healthy group tend to more than patients group substrate (starch). [6, 16].

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