ISSN: 1343-4292
Volume 140, Issue 05, August, 2022

Partial purification of cardiac creatine kinase (CK-MB) from patients with myocardial infarction and study of kinetic properties.

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ABSTRACT— This study was done to partial purification Creatine kinase(CK-MB) from serum of myocardial infection patients and study enzyme kinetics under different conditions, a specimens were taken from the intensive care unit at Salah El-Din General Hospital for patients with myocardial infarction between 1/11/2021 and 28/2/2022 and the purification process by Sephadex G100 .the results of Kinetic studies showed that the optimal concentration of substrate was (100mmol), the Michael's-Menten constant was (Km=47.1mmol), and the maximum velocity was (Vmax=24.1 U/L), whereas the optimum temperature was (37°C) and the ideal pH was (7.3). Where the above practical results showed the activity of the enzyme at different pH, temperature and concentration of substrate concentration.

KEYWORDS: Creatine Kinas(CK-MB), Myocardial infection,

1. INTRODUCTION

Creatine kinase (CK) is an enzyme present in nearly all muscle cells that catalyzes the reaction between ATP and creatine, thus producing cellular energy. The most important vital indicators that rise in the blood of an organism and is known by another name is creatine phosphokinase or phosphocreatine kinase and its classification number is (Ec 3.4.21.21). It is produced in the liver by three amino acids: ArLginine, Glycine, and Methionine [1].

Proteins in general are large molecules consisting of amino acids and linked by peptide bonds. Protein purification is a series of processes through which a specific protein can be isolated in order to conduct chemical tests on it. The purification process depends largely on the source of the sample, size, shipment and degree Acidity and solubility in general depend on the physical properties, so the methods and techniques depend on the type of protein to be purified. Many different mechanical, physical and chemical techniques and methods for protein purification [2].

The multiplicity of types of proteins and their life activities and the presence of chemical differences between them made purification and extraction processes one of the basics of biochemistry. One of the important requirements in the purification process of proteins is to release proteins from the cell without causing damage to their activity by means of mechanical mixing. There are also several methods, including sonication, fractionation by pressing and grinding. By means of sand. One part of the cell, if protein is found in it, is considered a raw material for purification. The detection of protein purity is carried out in the following way (Protein solubility, as each of the different types of proteins has a different solubility in a specific solvent from which the protein can be extracted and purified, Electrophoresis of the protein, Chromatographic separation method, By detecting the components in proteins such as minerals, Immunological methods, Ultra-high-speed centrifugation) [3].

2. Materials and Methods

1- The process of adding ammonium sulphate, where the proteins in the blood were precipitated by using gradual concentrations of ammonium sulfate solution until a saturation rate of 80% was obtained, where 9.5 gm of ammonium sulfate was added to 15.5 ml of serum. In a time period of (60 - 45) minutes, then we put the serum in an ice bath with continuous stirring of the resulting solution, then the filtrate is separated from the formed precipitate, and then the resulting precipitate is dissolved with the least amount of the buffer solution (Tris - HCl).), whose concentration is 10mM) while the acidic function is (pH = 7.3), then the resulting filtrate is taken and then we add an amount of ammonium sulfate salt in order to precipitate the largest amount of proteins.

2- Dialysis, which is one of the methods used in the process of separation and purification of enzymes and is considered one of the oldest, as the purpose of this process is to remove the remaining ammonium sulfate, which is added to precipitate proteins, where the dissolved protein (in the previous step) is placed in Dialysis bag After this step, the membrane separation bag is immersed in buffer solution (10mM) (Tris - HCl pH 7.3) and the buffer solution is changed continuously from time to time for a period of 20 hours, as this step was performed at a temperature of (C) (° 4 ± 1) in order to maintain the enzyme structure and consequently to maintain the activity of the CK-MB enzyme, and after the end of the membrane separation process (dialysis), the concentration level of MB-CK enzyme and the level of protein concentration were measured.

3- Gel filtration The gel filtration technique used to separate chemical compounds is one of the most important techniques in the field of biochemistry and the basis of this technique is the difference of chemical compounds in terms of their molecular weight and the size of their molecules, as large proteins that have large molecular weights cannot The molecules can penetrate through the gel, where these molecules move out of the gel layer and are accompanied by the solvent, which is displaced sequentially. Often the solvent used is mostly distilled water or can use a diluted buffer solution. Thus, the large molecules that have Large molecular weights are initially filtered during the separation process, while small molecules, which have small molecular weights, can penetrate and enter between the granules of the separation material (the gel), so the large molecules are filtered before the small molecules are filtered, and after this step the components formed from the gel filtration are collected through the parts collector (Fraction Collector) and then calculate the volume of distilled water that was used (or dilute buffer solution) to displace each type on One of the types of separated protein from the purification column, and then the protein materials are isolated by reading the absorbance of the separated protein at the wavelength 546 nm, or by using the ready kit to measure the total protein concentration (60). The gel used in this method is Sephadex G-100.

2.1 Used solutions

1- Buffer 10mM Tris - HCl pH 7.4 The buffer solution was prepared by dissolving 1.576 gm of (Tris - HCl) in one liter distilled water and then the pH was set at (7.3) 2- The suspension solution of Sephadex G100 was prepared. By dissolving (2.5 gm of column packing material (Sephadex G100) in a volume of (200 ml) of 10mM Tris-HCl pH 7.3 buffer solution, then leaving the prepared solution for a period of time of (24-20) hours at a temperature of (4°C) °) During this period of time, the prepared buffer solution was changed several times in order to remove the fine particles that are present in the solution because the presence of these minutes works to reduce the speed of the solution flowing into the column.

2.2 Procedure

1- A transparent, graduated glass column with a diameter (1.5 cm) and a length of (30 cm) was used, and a

ISSN: 1343-4292
Volume 140, Issue 05, August, 2022

little glass wool was placed at the end of the column in order to prevent the gel granules inside the column from leaking out of the glass column, and after fixing the column, the solution was poured The suspension of the gel into the purification column in a relatively slow and homogeneous manner in order to prevent the formation of air bubbles in the separation column, which hinder the separation process until the height of the gel in the separation column reached (12 cm), and then the column was washed from the buffer solution (10mMTris). – HCl) (pH 7.3) in sufficient quantities until a flow velocity of the solution into the column was obtained by (1 ml / min). 2. Then (10.5 ml) of the prepared form (enzyme) after the membrane separation process and slowly over The surface of the gel is Sephadex G100 and then it is left for a period of 5 minutes in order to absorb into the gel column 3. Then the separation process was carried out by using 100 ml of buffer solution that contains (500 mmol of NaCl) where (5) ml per portion.

4- After collecting all exudative fractions from the column, the concentration of creatine kinase enzyme (CK-MB) was estimated according to the method and the total protein concentration. 2-6-2 Kinetic studies of creatine kinase enzyme CK-MB after the process of separation and partial purification of the enzyme (CK-MB) from the serum of patients with myocardial infarction and through gel filtration method, the kinetic characteristics of the enzyme were studied, and this study included the following: 2-6- 1-2 Effect of the concentration of the substrate on the activity of the enzyme (CK-MB).

Where the activity of CK-MB enzyme was studied at different concentrations of the substrate and the extent of the effect of these concentrations on the enzyme, which was partially purified, by using different concentrations of the substrate, which are 100, 50, 25, 12.5, 6,25, 3,125. In order to know the effect of the concentration of the substrate on the work of the CK-MB enzyme, where the rate of the enzymatic reaction was measured according to the method mentioned previously, and by drawing the resulting relationship between the speed of the enzymatic reaction and the concentration of the base substance, in order to know that the enzyme is subject to the Michal-Menten equation, and through that it was obtained On the values of Michaels-Menten's constant Km, by using the Lenofer-Burke graphical method, which links between the inverse values of each of the velocity and the base material concentration (1/[S] vs. 1/v).

The effect of changing the pH value of the buffer solution ((10mM Tris-HCl)) pH 7.3 on the rate of the reaction of CK enzyme was studied, where solutions with different pH value were used (3,7,5,4,10) with The presence of the substrate with a concentration of 100 mmol/L and a temperature of 37 °C, where the CK-mb enzyme activity was measured by drawing the relationship between reaction rate and pH, and then the optimum pH was identified.

The effect of temperature, where the enzymatic reaction was carried out at different temperatures, which included (57, 47, 37, 27, 17, 7) °C and in the presence of the buffer solution (10mM Tris-HCl) with a pH value of 7.3 and with a concentration of The base material is 100 mmol, and then the resulting relationship between reaction rate and temperature was drawn, in order to find out the optimum temperature for the enzymatic reaction.

3. Results

The proteins are recognized in the first phase of enzyme by getting rid of very much water Thus obtaining a high degree of purity and often the salts for this purpose, including ammonium sulphate (NH4. 2SO4), and is due to it’s good soluble in water as well as lack of effect in the installation of enzymes Add to its own licenses and provide them with a great deal, with salt-salt process as a result of its owners on the surface of the protein, which leads to the protein deposition by reducing the melting of protein and this is called external armament Out, this step is important to significantly adopted most of the previous studies. Of

proteins and water so as to interfere with the effectiveness of enzyme where they are added in stages to get rid of some protein substances associated with enzyme extract [4], [5].

Therefore, the process of separating and purifying the cardiac creatine kinase enzyme (CK-MB) was carried out from the sera of patients with myocardial infarction in multiple stages. Then the excess salt used in the precipitation was removed during the Dialysis process by (Tris-HCl) as a buffer solution with pH 7.3, where the degree of enzyme purification for this stage reached (1.9) times, with an enzyme yield (57%) and a specific activity (0.57 U/mg) and as shown in Table (1-1), and then the process of purifying the enzyme was completed by using the method of gel filtration chromatography and using Sephadex G100 gel (1-1).

Results were obtained from other studies on enzyme and purification. This study agreed with other studies [6], [7].

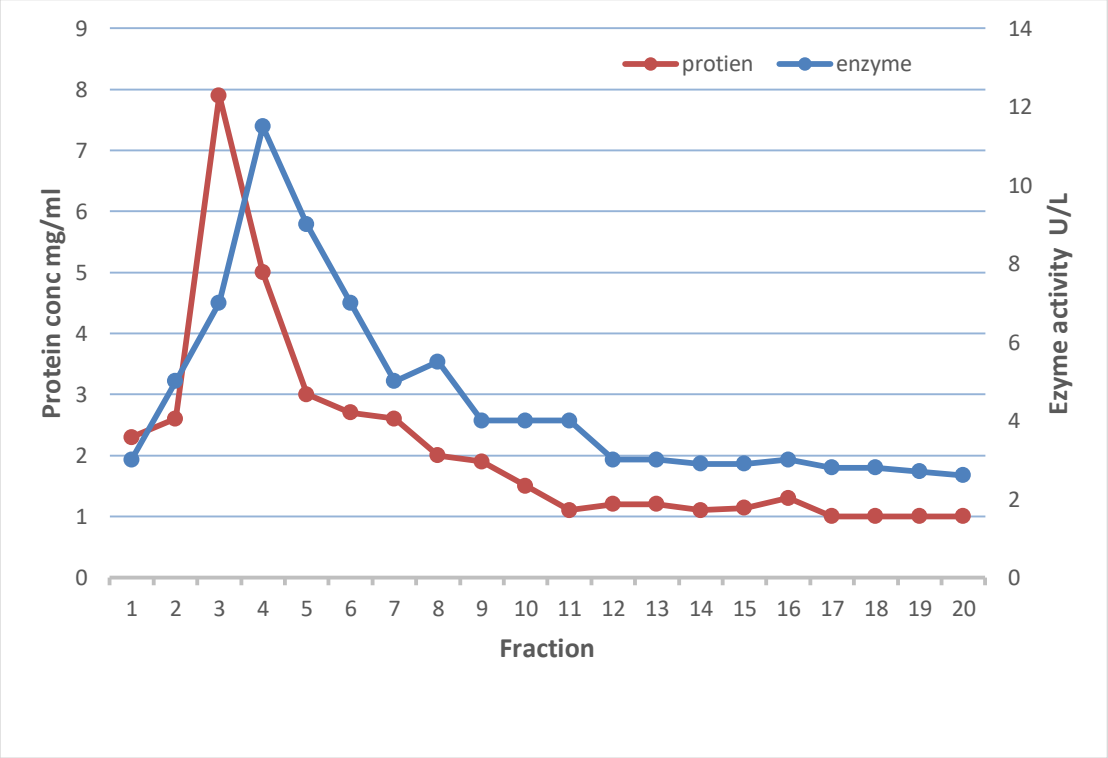

Figure (1-1) Purification of cardiac creatine kinase (CK-MB) by gel filtration chromatography

Table (1-1) of the purification stages of cardiac creatine kinase enzyme CK-MB from patients with myocardial infarction

Purification stages	Volume ml	Efficiency UI/L	Total activity	Protein concentration mg/ml	Total protein concentration mg	Specific activity IU/mg	Number of purification times	Output 100%

Crude serum of ck-mb	14	15.5	217	60	250	0.25	1	100
ammonium sulphate	9.7	13.3	130	42	210	0.31	1.5	60
Dialysis	10.2	12.25	124.95	21.2	106	0.57	1.9	57.5
Gel filtration Sephadex G100	6	11.7	70	8	48	1.46	5.6	33

Study of the kinetic properties of cardiac creatine kinase enzyme (CK-MB) partially purified from patients with myocardial infarction (MI).

1-Studying the effect of the concentration of the main substance on the activity of the enzyme and finding the value of Km and v max.

The effect of the concentration of the substrate on the reaction speed of the cardiac creatine kinase enzyme (CK-MB), which was partially purified from patients with myocardial infarction and separated from the Sephadex G100 column, was studied. The maximum reaction at the concentration (l/ mmol100), and Figure (1-2) shows the increase in the rate of the enzymatic reaction with the increase in the concentration of the base substance. The same figure also shows that the cardiac creatine kinase enzyme CK-MB purified from the serum of patients is subject to the micelles-menten equation, as the resulting figure is an ellipse, and there are several ways to calculate the value of the Michaelis-Menten constant Km, which is known as the affinity between the enzyme and the base material. The higher the value of this constant , the less the affinity between Between the enzyme and the substrate, and when the value of this constant decreases, the affinity between them increases, through which it is possible to determine the efficiency of the enzymatic analogues to stimulate vital reactions and to know the extent of the stability of the enzymes and the effect of the inhibitory and activating substances on the enzymatic activity [8], or the concentration of the base substance when the rate of speed is half the value of the speed Maximum (Vmax) [9], when the purified enzyme is subject to the micales-mentin equation, its constant value (Km) can be calculated, as the Lineweaver-Berke method is the most accurate and best method from a practical point of view, due to the ease of use of the equation and the lack ofcalculations.in it and for its efficiency in indicating the accuracy of the experiment procedures, To calculate the values of the constant (Km) for the purified CK-MB enzyme by chromatography gel, the above method was followed as in Figure (1-3). The same is equal to (24.1 U / ml), and if these results obtained contrasted with some previous studies where they were conducted to determine the kinetic constants of the purified enzyme and the differences in the results between all these studies were clear and these differences are almost normal due to the different sources from which the enzyme was purified As well as the different methods used in purification, and these studies purify the enzyme from E.Coli bacteria [10].

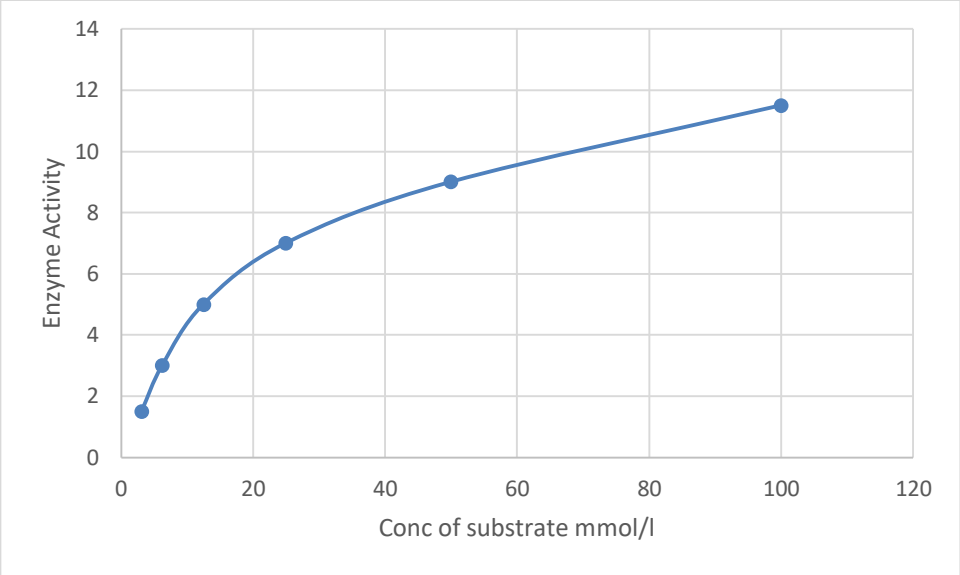

Figure (1-2) Effect of the concentration of the main substance on the activity of purified cardiac creatine kinase enzyme(CK-mb) from patients with myocardial infarction

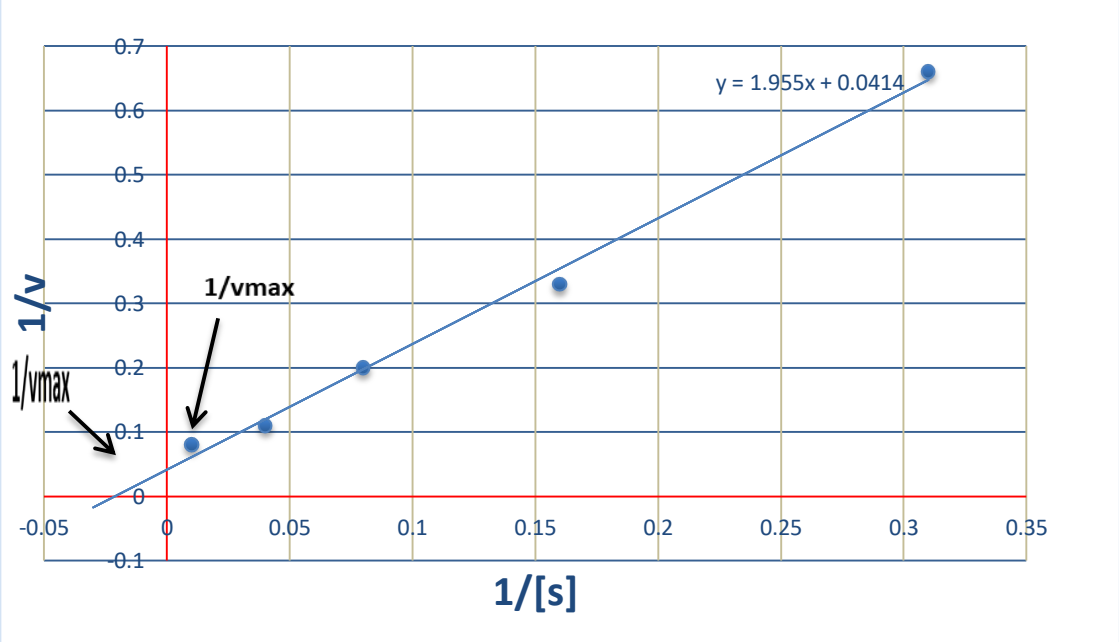

Figure (1-3) Lineweaver-Park diagram for calculating Km and vmax of creatine kinase-MB (CK-mb) purified from MI patients cardiac

2- Studying the effect of temperature on the enzyme cardiac creatine kinase CK-mb

The rate of the enzymatic reaction is known to increase with the increase in temperature until it reaches the optimum temperature for the enzymatic reaction, then the rate of the reaction begins to decrease gradually. Which causes the bond between the active amino acids to break, and this causes a loss of enzyme activity, which explains this decrease due to a change in the geometric arrangement of the enzyme. High temperatures affect the ionization state of the groups on the surface of the enzyme as well as its base material. The state of ionization affects the catalytic activity in the triple structure of the organization, so the enzyme in this case is not able to perform its natural active role when the temperature rises to the extent that the temperature affects the structure of the enzyme, this rise causes a change in its natural shape, which

ISSN: 1343-4292
Volume 140, Issue 05, August, 2022

causes the loss of Enzyme activity [11]. The results obtained differed with other studies conducted Previously, including a study involving the enzyme extracted from coli-E [7].

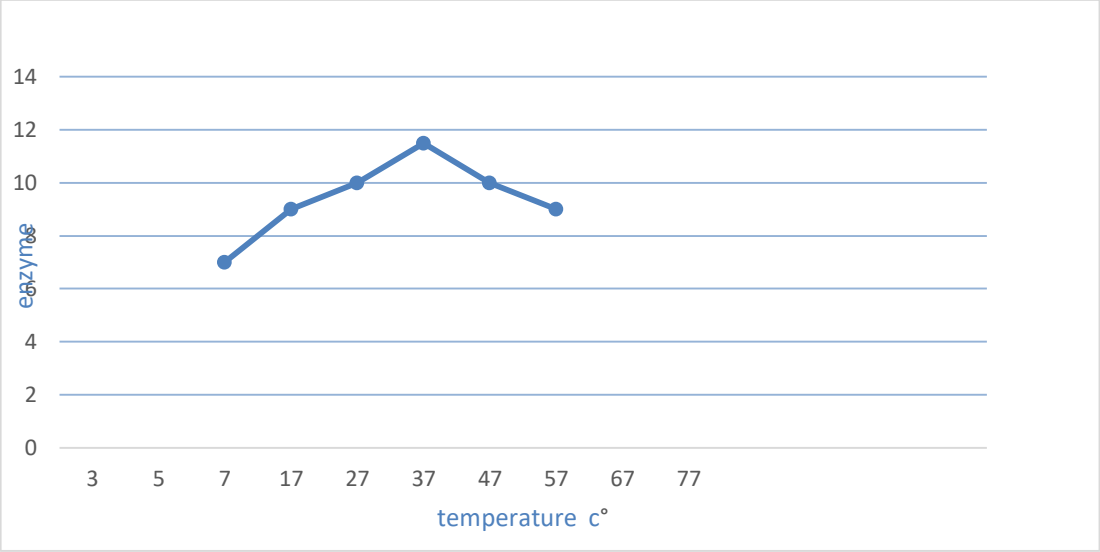

Figure (1-4) Effect of temperature on the activity of purified enzyme from myocardial infarction patients

3-Studying the effect of pH on the enzyme cardiac creatine kinase CK-mb

The degree of pH greatly affects the activity of the enzyme as a result of the different nature of the enzyme as well as the difference in its chemical composition, in addition to the presence of multiple ionic groups that the enzyme carries, where enzymes operate at an optimum pH degree (7.3) due to being very sensitive to any change in ion concentration Hydrogen H+. The rate of the enzymatic reaction increases gradually with an increase in pH until the reaction reaches the maximum speed at the optimum pH of the reaction, then after that the rate of the enzymatic reaction begins to decrease. pH = 7.3 Figure (1-5) shows a gradual increase in the rate of the enzymatic reaction with an increase in pH until the maximum speed of the enzymatic reaction is reached at pH (pH = 7.3), then after this value the reaction speed begins to decrease and on this basis the pH is considered (pH = 7.3) is the optimum pH for the work of this enzyme. An increase in pH leads to internal electrical repulsion or leads to a loss of internal electrical charge on the side of the amino acid chains, which is the result of opening the protein molecule constituting the enzyme (leading to protein denaturation). As a result, the enzyme is unable to form a complex - base material (ES) (12) As the results of the kinetic study of the enzyme showed that the optimum pH of creatine kinase (CK-MB) was (7.3) as shown in Figure (1 - 5). Where the results of the pH differed from the results of previous research, including those of the purified enzyme.

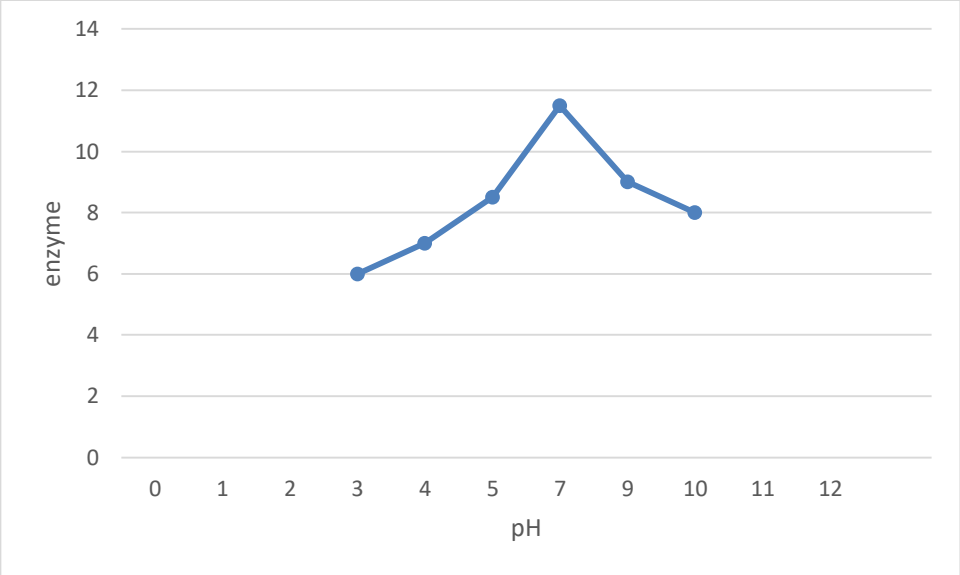

Figure (1-5) Effect of pH on the activity of cardiac creatine kinase enzyme CK-MB purified from sera of patients with myocardial infarction

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