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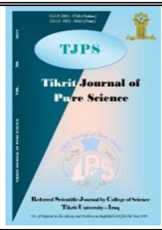

Purification and determination the molecular weight and study the kinetic properties of Glucose-6-phosphate dehydrogenase from Diabetic patients

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Abstract

This study was conducted to purifity Glucose 6-phosphate dehydrogenase from diabetic patients by gel filtration technique on Sephadex G100 and determine the molecular weight of enzyme, concentration and kinetic constant [Km, Vmax], study the effect of optimum temperature, substrate, pH. The study includes [60] patients with diabetes mellitus and [60] healthy individual as control. The activity of G6PD was measured after precipitated by [75%] Ammonium Sulfate concentration, using [Tris-HCl] buffer at pH 8.2. The specific activity was calculated [21.5UI\mg], total activity [706.8UI], number of purification [3.45] enzyme yield [23.188%] and enzyme activity [17.67UI\ml]. and the molecular weight were [57.82] k.D. The effect of substrate concentration was found to be increased with increasing substrate concentration. The optimum pH was found to be [8.4] and the optimum temperature was [38C]. Michaelis- Menten [K_m] value for the enzyme was [3.33mM] and Vmax value was [0.263IU\ml].

Introduction

Diabetes mellitus is a metabolic disease which was clinically and genetically heterogeneous disease characterized by hyperglycemia due to defects in insulin metabolism. If the hyperglycemia of diabetes is not managed properly, it causes long-term damage, dysfunction, and failure of different organism[1]. notably the eyes, kidneys, nerves, heart, and blood[2]. It has been stated that oxidative stress and impaired release of nitric oxide may be the contributory factors in the pathogenesis of diabetes[3].

One of the main causes of diabetes is functional causes such as pancreatic disorders, This occurs when the pancreas is infected with tumors [benign and malignant], internal bleeding or when the pancreas is removed leading to an absolute inability to secrete insulin to lead to diabetes induced by excessive use of thiazide diuretics, anti-inflammatory drugs and antiviral[4]. Genetic causes Individuals with parents who have diabetes or individuals from a family with a family history of the disease are more likely to develop diabetes than others Obesity is also cause of diabetes, Obese people who store high amounts of fat in the abdomen are more likely to develop diabetes[5]. Those who accumulate fat in the limbs and increase the amount of fats affect the blood sugar level because it is one of the main factors that cause insulin resistance Of cells by reducing the sensitivity of insulin receptors on the surface of target cells[4]. One of the other reasons is also lack of physical activity as it affects the increased incidence of diabetes In some cases, emotional emotions have an effect on diabetes, such as anxiety, fear or sudden shocks, and susceptibility to disease[6]. Viral infections have a major role in the development of type 1 diabetes, Self-infection due to viral infection or by the destruction of beta cells in the pancreas.[7]

Diagnosis of diabetes

Clinical Diagnosis: Patients with diabetes of all types have the following symptoms:

Polyuria, polydipsia, lethargy, boils, slow healing wounds, frequent infections persist for a long time. Patients with Type I diabetes suffer from weight loss dehydration, ketonuria, and hyperventilation[6]. Symptoms of type 1 diabetes tend to be short-term, whereas patients with type 2 diabetes tend to have chronic symptoms with longer duration of symptoms[5]. This is a significant difference between the two. Lack of secretion of insulin also causes excessive metabolism of free fatty acids, and this leads to confusion in fat metabolism [7].

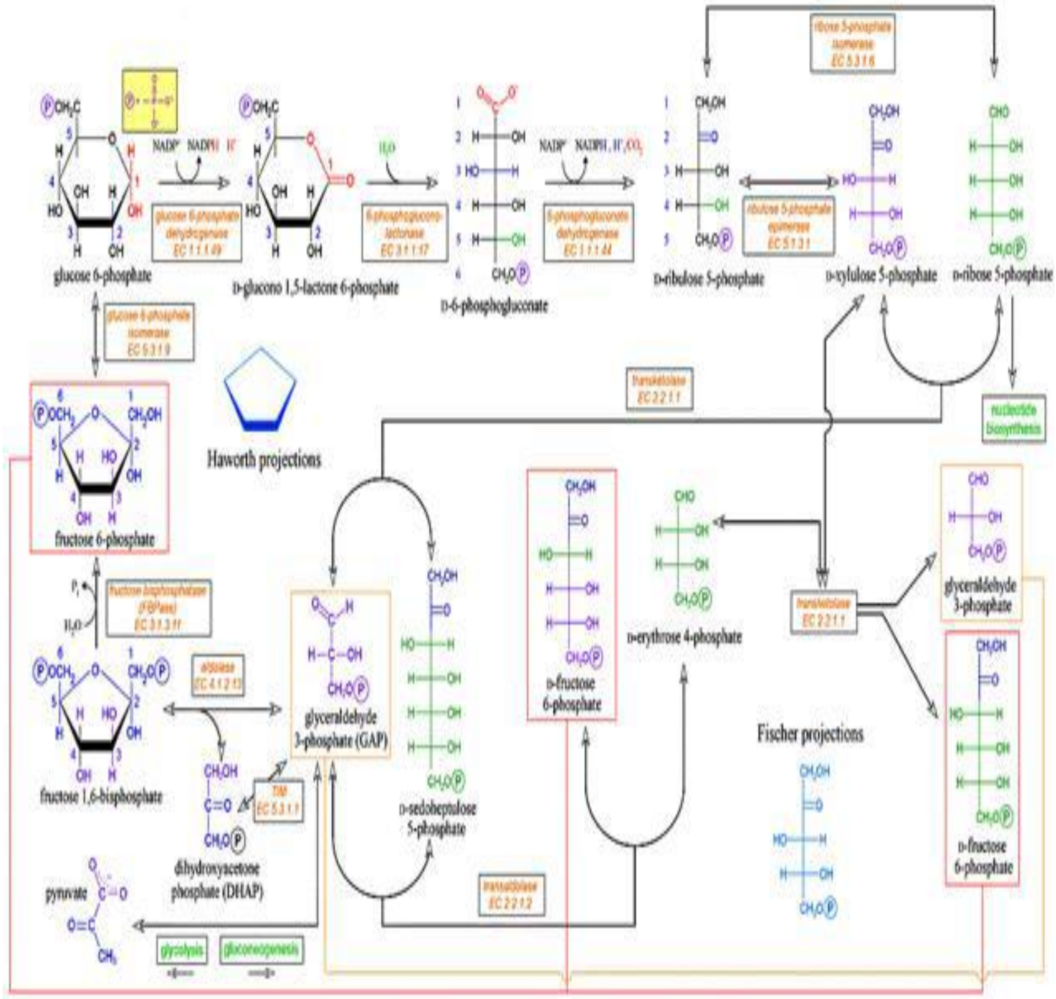
Laboratory diagnosis: High blood sugar hyperglycemia and the emergence of glucose in the blood glucose is a distinctive phenomenon of diabetes, so some tests are used to diagnose diabetes, including: **Fasting Plasma Glucose [FPG],** Random plasma Glucose [RPG], **Oral glucose Tolerance test [OGTT**], **Glycosylated Haemoglobin [HbA1c**], **Glucose in Urine**, Ketone body in Blood or Urine[46,47].

Glucose-6-phosphate dehydrogenase[G6PD]

Enzymes: are vital catalysts that accelerate the rate of chemical reactions. They have a high molecular weight protein structure. Like other proteins, the enzyme is composed of a combination of a large number of amino acids that have one or more polypeptides, It is a three-dimensional form of the protein, Amino acids are found in these sequences according to a particular sequence of each enzyme, leading to a specific vacuum structure that enables the enzyme to accelerate its own reaction [12].

Glucose – 6 - Phosphate Dehydrogenase [G6PD] [Oxidoreductase, EC1.1.1.1-49] is one of the cytoplasmic enzymes is spread throughout the body, especially in the red blood cells, which is one of the most important enzymes of the egg, it is the main enzyme and the key to the Pentose phosphate pathway.[13] It stimulates the oxidation process of the glucose-6-phosphate necotinamide adenine dineuclutide [NADP] and to convert it to an effective reduced form [NADP] to preserve the life-producing pathways of several important substances, particularly in red blood cells because they have no other source of production [NADP] to preserve the life-producing pathways of several important substances, particularly in red blood cells[6]. NADPH product produced by G6PD is complementary to the reduced triglyceride enzyme Glutathione reductase [GR], which converts and converts oxidative glutathione [GSSG]to [GSH] [7], Which protects human red blood cells from partial oxidation, Table [1], showed the pathway of pentose phosphate sugar and the rule of G6PD in reactions of pathway.

Table 1: Pentose phosphate pathway [8]



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Glucose-6-Phosphate Dehydrogenase was first discovered by the scientists Warburg [Christian] in 1931 in the red blood cells of the horse and since then studies and research have been conducted to extract and purify the enzyme from various sources [9].

There are other important reasons to study the change in the effectiveness of this enzyme G6PD enzyme in the body's various tissues is linked to many diseases in humans such as jaundice in children and hemolytic anemia [17]. The number of infected people is 400 million males, females, neonates and other ages, according to the World Health Organization [WHO] report. [1] More than 442 types of enzyme [G6PD variants] have been identified using a large number of biological techniques, including molecular analytical methods, which identify genetic mutations that occur in the genes responsible for the biological processing of different types of enzyme[10]. Gene found that The enzyme gene was found to be carried on the sex chromosome [X][12]. The shortening of enzyme efficacy is associated with genetic and hereditary disorders[11]. This shortage is widespread in the world, especially in the Mediterranean region Patients with this type of hemorrhagic deficiency are generally affected by certain drugs and foods In the case of a paroxysmal or neonatal jaundice found in natural erythrocytes, enzymatic activity decreases with age[11]. Many mutations of this enzymatic deficiency are widespread in the world and by geographic location. Moreover, genetic defects and age can lead to Enzymatic Deficiency An enzyme deficiency leads to the production of some red blood cell anemia due to exposure to certain chemical agents or certain infections and wounds [12].

Classification: Numerous G6PD variants have been described These have been classified by the World Health Organization according to the magnitude of the enzyme deficiency and the severity of hemolysis. This classification gives some approximation of the magnitude of hemolysis an individual might incur in the setting of an oxidative stress. Only class I, II, and III are of clinical significance.

Class I – Class I variants have severe enzyme deficiency [<10 percent of normal] relation with chronic hemolytic anemia.

Class II – Class II variants also have severe enzyme deficiency [<10 percent of normal], but there is usually only intermittent hemolysis, typically on exposure to oxidant stress such as fava bean exposure or ingestion of certain drugs and the classic example is [G6PD Mediterranean]

Class III – Class III variants have moderate enzyme deficiency [10 to 60 percent of normal] with intermittent hemolysis, typically associated with significant oxidant stress the classic example is[G6PD A$^-$].

Class IV – Class IV variants have no enzyme deficiency or hemolysis the wild-type [normal] enzyme is considered a class IV variant, as are numerous other genetic changes that do not alter levels of the enzyme and these variants are of no clinical significance.

Class V – Class V variants have increased enzyme activity [more than twice normal]. These are typically uncovered during testing for G6PD deficiency and they are of no clinical significance [13].

Clinical significance of the enzyme:

Acute hemolytic anemia: Some individuals with G6PD deficiency have acute hemolytic anemia at the site of the wound when some medications, acute diseases, and certain foods are taken [15].

Neonatal jaundice: Anemia and jaundice are most often observed in newborns in individuals with severe enzyme deficiency. [21]

Neutrophil dysfunction: - The enzyme is used in addition to red blood cells in white cells to reduce the oxidizing factors. Some people with severe enzyme deficiency suffer from a defect in the function of white blood cells, which causes weak respiratory resistance to diseases and also weakens The presence of beneficial bacteria in the body [14].

Diabetic mellitus-induced hemolysis: In people with an enzyme deficiency, hemolyticysis starts with an increase in ketone content in diabetics and has the lowest levels when blood glucose levels are abnormal in diabetics[22]. Studies have indicated that high sugar leads to the deposition of decomposed blood in patients with deficiency [15].

The relationship between [G6PD] enzyme and diabetes

An epidemiological study from[16] suggested a positive correlation between diabetes and deficiency. [G6PD] has conducted a study on Indian society being the most potential to give this relationship Serum samples were collected for healthy people and of both sexes.. A higher incidence among Indians provided an excellent opportunity to study the possible association of G6PD deficiency in diabetes mellitus [5]. G6 PD deficiency is one of the common enzymopathy in human being affecting about 400 million people worldwide. It is suggested that there may be a positive association of G6PD deficiency with diabetes mellitus. Although G6PD deficiency is not uncommon in our country but there is scarcity of data on this regards especially on diabetes. Therefore, this study was undertaken to observe the G6PD status in patients with type 2 diabetes mellitus in order to explore the role of this enzyme deficiency as one of the risk factor for diabetes mellitus[7].a positive association of G6PD deficiency with diabetes mellitus Although G6PD deficiency is not uncommon in our country but there is scarcity of data on this regards especially on diabetes. Therefore, this study was undertaken to observe the G6PD status in patients with type 2 diabetes mellitus in order to explore the role of this enzyme deficiency as one of the risk factor for diabetes mellitus[6].

Material and method

Collection of samples:

The total number was [60] samples, blood samples were collected from people with diabetes of both sexes. samples of both types of diabetes were diagnosed of the disease using a blood glucose test.

Blood was drawn from the vein using a 5 ml plastic syringe. The blood was placed in clean, sterile plastic tubes free of anticoagulant. And left to coagulate at room temperature. The blood serum was then separated from the centrifuged at 5000 G for 15 minutes to ensure adequate serum red blood cell extraction. The activity of the enzyme was measured directly in vitro.

Diagnosis kits:

The kits in table[2] were used in this study .

Table 2: The kits and its source

Biolabo- France	Glucose 6-Phosphate dehydrogenase [G6PDH] kit
Aflu-Italia	Total protein kit
Aflu-Italia	Glucose kit

Estimation of Biochemical Parameters in Blood Serum

Estimation of Glucose Concentration in serum

Principle: glucose level in serum was measured using [kit Aflu Italia]depending on enzyme method that stated on Trinder reaction[46]

Determination of Total Protein in serum

Total protein level in serum was measured using [kit Aflu Italia]depending on enzyme method. [47]

Determination of G6PD activity in serum

G6PD activity in serum was measured using Biolabo kit according to equation:[48]

$$G6PD\ (U/ml) = \frac{A\ per\ min * 10^3}{V_{enzyme} * 6.22}$$

A = Absorbance V= enzyme volume in ml. 6.22= absorbance confection of [NADPH] wave length 340 nm.

Separation and purification of the enzyme G6PDH from serum of diabetic patients:

G6PDH was purified from the serum of diabetic patients using the following steps:

Addition ammonium sulphate

Serum proteins were precipitated using gradual concentrations of ammonium sulphate until 75%. 3.75gm of ammonium sulphate was added to 5 ml of serum during 60-45 minutes by incubating sample in ice bath with constant stirring, and then dissolved in 4ml of 1M Tris - HCl regulated solution[pH= 7.8].

Membrane separation (dialysis) :

The purpose of this process is to remove the remainder of the ammonium sulfate added to precipitate the proteins by placing the protein dissolved in step above the bag membrane separation dialysis bag after measuring the activity of the enzyme G6PD and protein concentration , and dunks the bag in buffer solution organizer 1M Tris - HCl pH 7.8 has been changing liquor regulator from time to time for the whole night, this step is conducted at a temperature of [4°C±1°C] to maintain the activity of the G6PD and after the end of the membrane separation process was measured by the activity of the G6PD and protein concentration.

Gel Filtration Chromatography

Gel Filtration technology is one of the most important techniques in the field of biochemistry, one of the methods used in the separation of compounds depending on the size of their molecules and their molecular weights, and were used for the purification of the enzyme G6PD nomination gel column Sephadex G100.

The gel filtration technology is also used as a method for estimating the approximate molecular weight of protein by drawing a graph showing the relationship between the size of the elution and the known molecular weight. The protein with the molecular weight is then passed through the separation column and calculated The size of the Rogan accurately and in comparison with the known molecular weights can estimate the approximate molecular weight of the unknown protein. [18,19]

Used solutions:

1-buffer solution 0. 1M Tris-HCl pH 7.8 Prepare by dissolve 15.76 gm of Tris-HCl per one liter of distilled water and adjust pH at 7.8.

2- Sephadex G100 prepare by dissolve 2.5 gm of the Sephadex G100 column filler in 200 ml of the 0.1M Tris-HCl pH solution 7.2 and leave the solution for 28-24 hours at 4°C. During this time the solution was changed several times to remove the soft minutes from the solution Because its presence reduces the velocity of the flow of the liquid solution through the column.

3- Sodium chloride solution at 500 mM concentration Prepare by dissolve 29.25 g of NaCl in one liter of 0.1M Tris-HCl pH 7.8 solution.

Procedure:

glass column with a diameter of 1.5 cm and a length of 30 cm had been used. A small amount of glass wool is placed at the end of the column to prevent the gel particles from leaking out of the column. The gel solution is slowly and homogeneously poured into the column to prevent air bubbles from forming. [11cm], wash the column with sufficient amounts of 0.1M Tris-HCl pH solution until a flow velocity of 2.5 ml / min was obtained [20]. Add 5 ml of enzyme after membrane separation slowly over the surface of the G100 Sephadex gel and leave for 5 minutes to soak in the gel column. The process of separation was started using 150ml of the structured solution containing 500 ml of NaCl, collecting 5 ml per part. After collecting the extracting parts of the separation column, the efficacy of the G6PD enzyme was evaluated by paragraph and protein concentration by the method [kit Aflu].

Kinetical study of G6PD

The kinetics of G6PD were studied after its separation and partially purified from the serum of diabetic patients by gel filtration. These included:

Effect of Glucose 6-phosphate concentration [G6P] as substrate: The effect of different concentrations of substrate on the activity of G6PD was studied using different concentrations of substrate:
[0.6,0.0512,0.0256,0.0128,0.064.0.048,0.024,0.012] M of substrate using to determine that the enzyme is subject to the Michaelis-Menten equation. The Km values were obtained using the Linover-Burke plot.

Estimated of optimum pH: pH has a significant effect on the enzyme activity for controlling ionization Ionic aggregates at the active site of the enzyme The optimal pH of enzyme stability is an important characteristic of enzymes [38]. The pH effect in buffer solution [0.1M Tris-HCl pH 7.8] was studied at the velocity of the G6PD reaction. Different pH solutions [11, 10, 9, 8, 7.6] were used with G6P at 0.6 mM and 37 ° C [G6PD kit Biolabo], and by plotting the relationship between reaction velocity and pH, the optimal pH was identified.

Effect of temperature: G6PD kit Biolabo was used to measure the activity of G6PD in different temperatures [57, 47, 37, 27, 17 and 7] with the buffer solution [0.1M Tris - HCl pH 7.8] at constant substrate concentration (0.6mM) and then painted the relationship between the reaction velocity and the temperature to find out the optimum temperature of the enzyme reaction.

Results and Discussion

Purification of enzyme:

Precipitation by Ammonium Sulphate: The basic principle of the method is to equalize the charges on the surface of the protein and the degradation of the water layer surrounding the protein and reduce the degree of watering and reduce the solubility of the protein and sedimentation[23]. Add the stages to get rid of some of the protein content with the enzymatic extract[24]. The result in table [4] showing the efficiency of quality [10.77 units / mg] with the number of times of purification [1.73] and the yield [35.02] during the satisfaction rate of ammonium sulfate sulfate estimated [75%]. The results differed with the studies of enzyme extraction. In a study involving red blood cell extraction, the specific activity was 1.251[units / mg] with 121.5 purification number and 53.8% enzymatic yield when using ammonium sulphate salt at 35-65% [25]. Another study showed that the specific efficacy was 0.37 mg / ml and the number of purification times was 39.79 times and the yield of 79.18 units / mg after adding the salt with saturation concentration [40-60%] of the red blood cells of the geese[26]. In the Penicillium duPonti fungi, the salt was used by saturation [45-60%], giving the result a quality efficacy of [1.04 units/mg] and the number of times the purification of 8.67 times and proceeds 63.4% [33].

Gel filtration: The different methods used to purify the enzyme from bacterial, fungal, plant, or animal sources were obtained in obtaining high purity of the enzyme, During this research the use of gel filtration technique with the Sephadex G-100 was the result of the specific efficacy [21.5 units / mg], total activity [706.8 units], purification number 3.45, enzymatic yield [23.188%] and enzymatic efficacy [17.67 units / ml]. Results were obtained with other studies of enzyme purification. In one study, using the Sephacryl-S200 column to purify the enzyme from rat liver it was found that the specific efficacy was 24.75 units / mg and the total efficiency was 198 units with 6.17 times purification and an enzyme yield of 60.57% [28]. In a study that included several steps, first transfer the concentrated enzymatic extract with ammonium sulphate salt on the calcium phosphate column and then transfer the extract to the ion exchanger DEAE-Cellulose and then the gel filtration column Bio-Gel A-150 these steps gave a quality effect of 470 units / mg with a frequency of 2.42 times and an enzyme yield of 10%[29]. A study[45] of the purified enzyme from calf tissue using the gel filter column Sephadex G-25 an enzyme yield of 91% with 450 times purification. Another study included enzyme purification from Coriander Leaves The first two-step purification was performed with ammonium sulphate deposition and the use of the Sephadex G-200 gel. The enzymatic activity was 1.82 units / mg and the enzymatic yield was 26.4% and the number of purification times was 74 [30]. Also using gelatin filtration of the Sephadex G-200 column and concentration of sulphate salts prior to filtration, the results included [326 unit\ mg] the enzymatic yield was 19.9% and the frequency of purification was 2.5 when purifying the enzyme from the pituitary gland of caw.[31] In another study to Saccharomyces cerevisiae include using saturated concentration of ammonium sulphate salt[40-80]and gel filtration column on Sephacryl S-200 gave specific activity 65.68 unit/ mg and the enzymatic yield was 20.62% and the number of purification times was 2.94.[29]

Table 3: steps of enzyme purification

Step of purification	Elute [ml]	Activity [IU⁄ ml]	Protein conc. mg/ml	Specific Activity [IU⁄mg]	Total Activity [IU]	Purification [fold]	Yield [%]	Total Protein conc. [mg]
Crud serum	5	30.48	24.5	6.22	3048	1	100	4.9
Ammonium sulphate [75%]	4	21.54	8	10.77	1077	1.73	35	2
Dialysis	3.5	19.1	6.37	10.49	1049	1.68	34.4	1.82
Gel filtration [Sephadex G-100]	3	17.67	2.46	21.5	706.8	3.45	23.188	0.82

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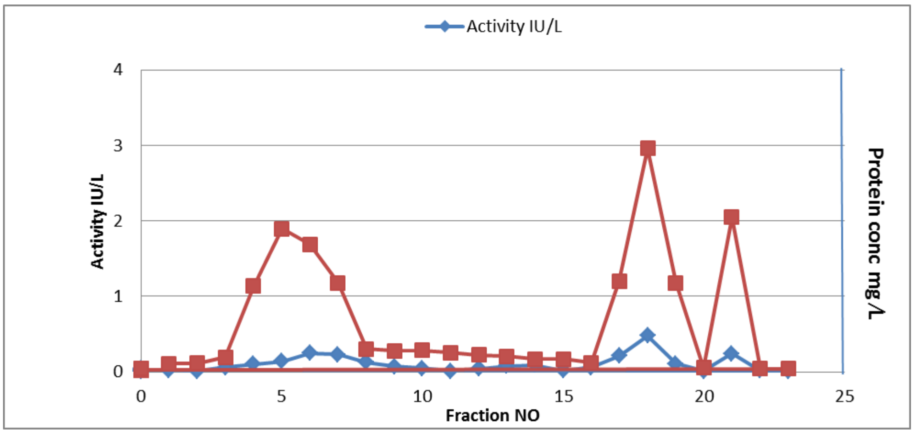


Figure (1) purification of G6PD enzyme with Gill filtration technology on Sephadex G-100 [Elution curve]

Determination of Molecular weight using Gel filtration technology

The researcher's method (Andrews. P)[18] was based on the gel filtration method in estimating the approximate molecular weight of the G6PD enzyme from the protein package [18] which showed the highest concentration of the protein. and passed a number of known molecular weight indicated in Table [5] and the molecular weight ranges between [20000000-13700] Dalton for the purpose of specifying the characteristics of the column In terms of internal volume [Elution Volume Ve] for each material as well as the free or empty size of the granules [Void Volume Vo], which was estimated from the standard curve of blue dextran at 3 ml per part. The Vo value was equal to 33 ml and the recovery volume of the standard Ve proteins to the volume of the recovery of dextran blue Vo, represented by the Ve\Vo relationship[28]. As shown in Table [5] [Sephadex G-100], molecular weight and recovery volume The Elution Volume of each material versus its molecular weight logarithm shows the appearance of a straight line in which the approximate molecular weight of the protein packet separated by the gel filtration technique is shown in [Fig.1]. The recovery volume of the package [18ml] To approximate molecular weight [Fig.2], the approximate molecular weight of the G6PD is [57.82] k.Da for the enzymatic extract using gel filtration technique.

Table 4: relation between molecular weight and elution volume to standard protein

Ve\Vo	Elution volume [Ve]in[ml]	Number of fraction [ml]	Log Molecular weight	Molecular weight [Dalton]	Standard protein
1	33	11	6.3	2000000	Blue Dextran
1.36	45	15	4.82	67000	BSA
1.3	60	20	4.7	58000	alpha-Amylase Enzyme
2.18	72	24	4.63	43000	ovalbumin
2.45	81	27	4.39	23000	Chymotrypsinogen
2.81	93	31	4.13	13700	Ribonuclease

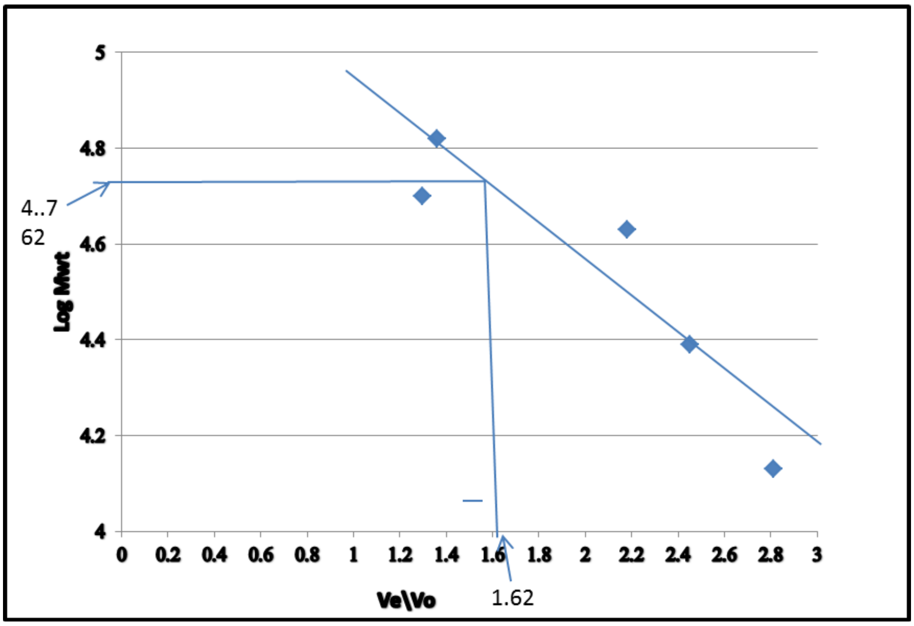


Figure [2] stander curve to determine proximal molecular weight of G6PD enzyme with Gel filtration chromatography

We see these results contrasted with a range of studies and studies conducted to estimate the molecular weight of the enzyme from its various sources[34]. The molecular weight was 133 kDa for the purified enzyme from pig liver using ammonium sulphate and Sephadex G-200 for the bilateral body the single molecule had a molecular weight of 67.50 and other studies showed that the molecular weight of the enzyme was purified from the liver of the mice, and the pituitary gland for cows had a molecular weight of 64 kDa[32]. The molecular weight of the purified enzyme from human red blood cells was estimated to be 43 kDa [34]. and in another study using gelatin filtration with the Sephadex G150 the molecular weight was [40 KDa] from Bean plant.[35] The molecular weight of the purified enzyme from diabetics was within the range mentioned [41], ranging from 22-58 KDa. Due to the difference in the scientific basis in the different methods used in the Dalton of the enzyme purified from yeast Saccharomyces cerevisiae Purification of enzymes The molecular weights vary from one study to another. [40] The difference is also due to the length of the purification steps of some of the different studies which may lead to the enzyme breaking through these long stages and therefore the resulting molecular weights are less than the real G6PD Study of Enzyme kinetics.[44]

G6PD Study of Enzyme kinetics

effect of the pH: - The results of the kinetic study of the enzyme showed that the optimal pH of the enzymatic extract was in the range [8-8.4]as shown in figure [3]. The optimal basis for the stability of the enzyme was [8-9] Extreme pH values affect substrate and the ionic state of the enzyme and lead to enzyme protein mutagenesis by altering the enzyme body [23]. In a study of [43] people with enzymatic deficiency and a group of healthy patients, pH was [8.5-7].as in figure[3].

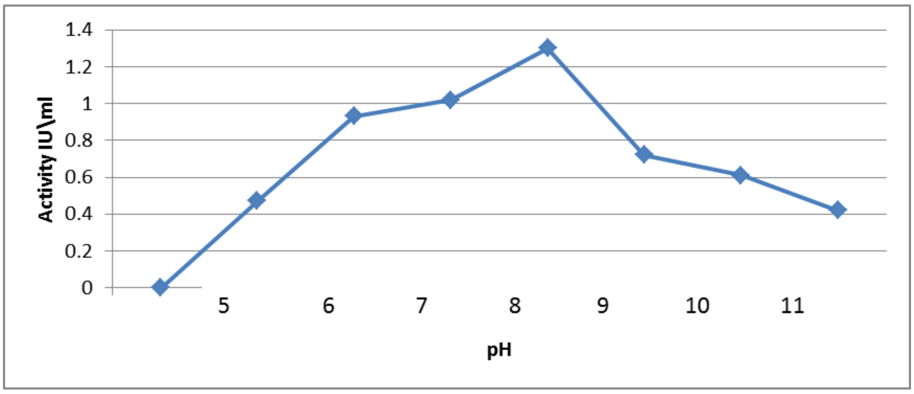


Figure 3: effect of pH on G6PD activity

Effect of temperature on enzyme activity: The optimum temperature of the enzyme activity when the pH was confirmed and the concentration of Substrate was [37-38 C]. The results varied with the studies carried out, including a study on the enzymatic extract extracted from coriander leaves

[30C][31]. and another study indicated that the optimum degree of the enzyme purified from bacteria *Azotobacter* and human placenta is [50C] [36]. In a study of the purified extract from the lamb marrow, the optimum grade was [38C] [37].

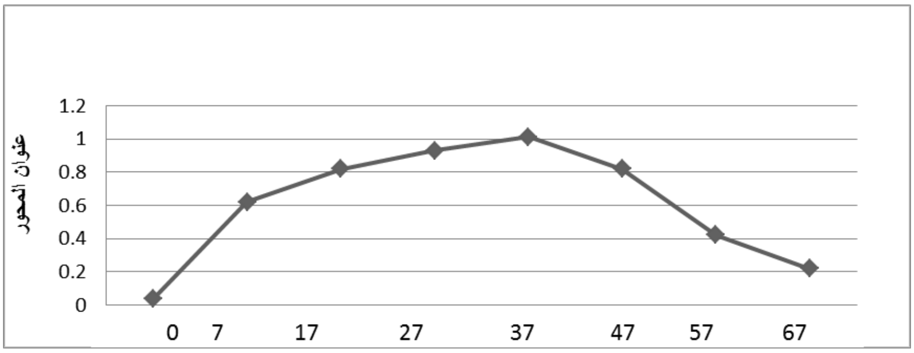


Figure: 4: effect of temperature on G6PD activity

Effect of substrate concentration: The Michaelis-Menten constant [Km] was defined as the affinity between the enzyme and substrate. The higher its value, the less the value of the substance is reduced. In order to stimulate the biological reactions and to determine the stability of enzymes and the effect of inhibitory and activating substances on enzymatic efficacy[40]. The results of the kinetic constants estimated for the enzymatic extract were shown in Figure and table [5]. The constant value of the Michaelis-Menten of substrate[G6P] was[0.263mM] and the maximum velocity value Vmax [3.33 UI\ml]. The differences between all these studies were clear and almost natural as a result of the different sources of the enzyme which were cleared and the different methods In the Iraqi study to purify the enzyme from human blood, the value of Michaelis-Menten was found in the substrate after the use of two purification methods [103 and 114 micro mole] and the maximum velocity[362 and 403 micromole / min /mg] respectively. [41] In another study on coriander leaves, the value of the Michaelis-Menten constant was 0.11 mmol and the maximum velocity was 0.038 units / ml. In a study to purify the enzyme from the pituitary gland for cows, the values of the Michaelis-Menten constant were 0.042 mmol and the maximum velocity was 9 units / ml[32]. In a WHO report for 2015, Michaelis Menten - purified from malaria patients is an average [30-50 micro mole][1]. In a study on fungus *Penicillium duPonti* the Michaelis constant and the maximum velocity were respectively [0.43 mmol, 9 unit / mg]for the purified enzyme of the fungus. [33] The value of the Michaelis constant[Km] from the human placenta was [0.4 mmol] and the maximum velocity was [8 unit\mg][36]. study of the enzyme purification of the local isolation of yeast *Saccharomyces cerevisiae* showed that the value of the Michaelis-Menten[Km] constant was [0.343 mmol] and the maximum velocity of substrate G6P [4.08 mmol\ min][42].

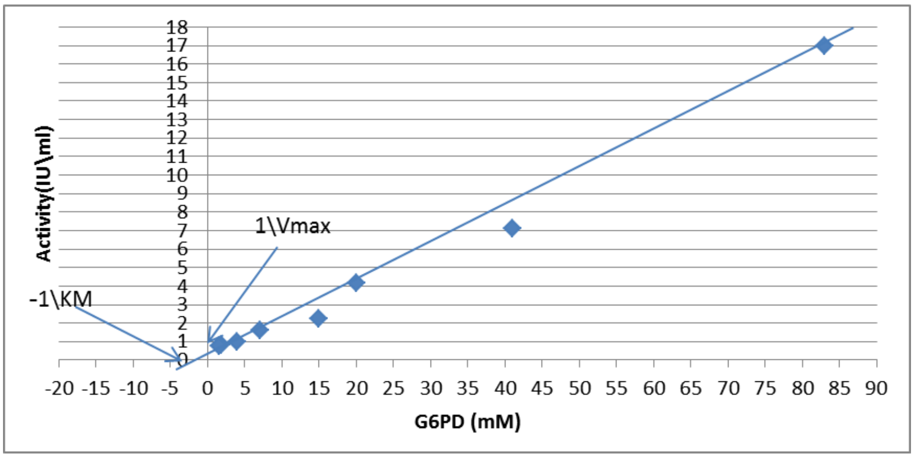
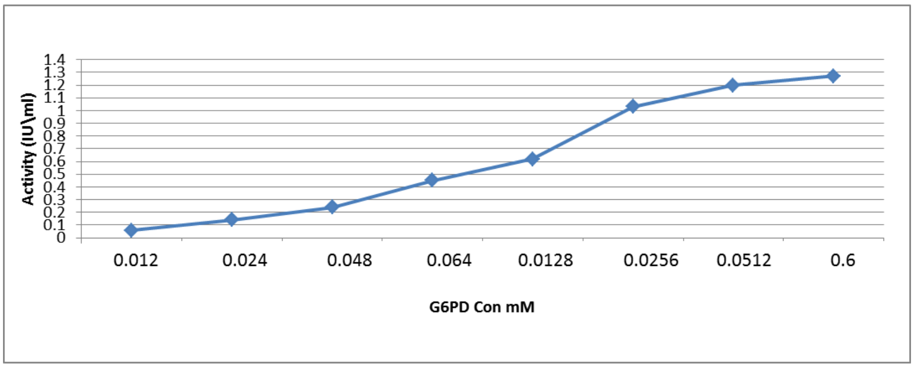


Figure 5: Lineweaver-Burk plot

Table(5) calculation value of Km and Vmax

-1\Km	Km	1\Vmax	Vmax
3.8	0.263 mM	0.3	3.33IU\ml



figure[6] effect of substrate concentration on enzyme activity

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دراسة الخصائص الحركية لإنزيم [G6PD] المستخلص والمنقى من مرضى السكري وتقدير الوزن الجزيئي له

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الملخص

تنقية إنزيم [G6PD] من مرضى السكري باستخدام تقنية بسيطة هي الترشيح الهلامي بواسطة هلام السيفادكس G-100 وتقدير الوزن الجزيئي للأنزيم في المرضى ومقارنته مع الوزن الجزيئي ومقارنته مع النسبة الطبيعية للوزن الجزيئي وكذلك تقدير الثوابت الحركية للإنزيم وهي ثابت ميكاليس– منتن Km وقيمة السرعة القصوى Vmax وتقدير قيم تركيز المادة الأساس ودرجة الحرارة والأس الهيدروجيني الأمثل للإنزيم. تتضمن الدراسة 60 عينة من مرضى السكري و60 عينة من الأصحاء حيث تم قياس الكلوكوز وفعالية الإنزيم في المصل وأظهرت النتائج ارتباط معنوي بين نقصان فعالية الإنزيم ومرض السكري وكذلك تم ترسيب الإنزيم باستخدام كبريتات الأمونيوم بنسبة إشباع [75%] ثم بعد ذلك تم تنقية الإنزيم بتقنية الترشيح الهلامي على هلام السيفادكس G-100 واعتمادا على الطريقة ذاتها تم تقدير الوزن الجزيئي للإنزيم. تم حساب الفعالية النوعية [21.5وحدة/ملغم والفعالية الكلية [706.8 وحدة] وعدد مرات تنقية بلغت 3.45مرة والحصيلة الإنزيمية [23.188%] والفعالية الإنزيمية [17.67وحدة/مل]. وتم قياس الوزن الجزيئي باستخدام نفس الطريقة وكان مقدار الوزن الجزيئي للإنزيم [57.82] كيلو دالتون وتم قياس تأثير زيادة تركيز المادة الأساس على فعالية الإنزيم فوجد إن فعالية الإنزيم تزداد بزيادة تركيز المادة الأساس إلى أن تبلغ مستوى ثابت تتوقف فيه الفعالية عن الزيادة مهما زاد تركيز المادة الأساس وأظهر الرسم البياني بينهما بشكل قطع زائد وتم دراسة تأثير الأس الهيدروجيني على الإنزيم فوجد إن الأس الهيدروجيني الأمثل هو [8.4] وتم دراسة تأثير درجة الحرارة على فعالية الإنزيم فوجد إن درجة الحرارة المثلى لعمل الإنزيم هي [38] وتم تقدير الثوابت الحركية فوجد إن قيمة ثابت ميكاليس – منتن هو [Km=3.33mM] وكانت قيمة السرعة القصوى [Vmax=0.263IU\ml] .

الكلمات المفتاحية: أنزيم الكلوكوز 6–فوسفات, داء السكري, تقنية الترشيح الهلامي, الوزن الجزيئي.