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Purification of the Acetyl CoACarboxylase-1 from Serum of Breast Cancer Women after Mastectomy

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Abstract

Acetyle CoA Carboxylase-1was purified from serum of premenop- ausal women with breast cancer (after Mastectomy or treatment) by Gel Filtration using Sephadex G-100 and by Ion Exchange using DEAE-Cellulose A-50, also the molecular weight was estimated by the Electrophoresis on Acrylamide in the absence of denaturing elements . The result showed that a single band was obtained at 210 KD by Gel Filtering while Ion Exchange showed one band at 210 KD. The optimum temperature of purified Acetyle CoA Carboxylase-1 was 40 ℃ , optimal pH at 7.5 and the optimum substrate concentration at 1.8mM. Michaelis-Menten constant (km) was 0.3mM and Vmax was 23mM.min^{-1}.

Introduction:

Breast cancer (BC) continues to abruptly disrupt the lives of millions of women. Just this year (2018), 2.1 million new cases of BC are expected to be diagnosed worldwide [1,2].Breast cancer classification and patient stratification is crucial in terms of determining treatment strategy in clinic.

Breast tumors are classified into two groups: in situ (20% of all cases) and invasive breast tumors (80% of all cases). Based on their location, in situ breast cancers are further classified into two groups: ductal carcinoma in situ (DCIS 80% of in situ breast cancers) and lobular carcinoma in situ (LCIS20% of in situ breast cancers). About 20–50% of DCIS tumors can eventually progress to an invasive carcinoma [3]. Younger age at menarche, parity, and older age at first full-term pregnancy are well established risk factors for breast cancer. These risk factors may influence breast cancer risk through long-term effects on sex hormone levels in premenopausal women, through long-lasting changes in breast tissue, or by other biological mechanisms [4].

Acetyl-CoA carboxylase (ACC) (EC 6.4.1.2) is the mediates the first step of fatty acid synthese by carboxylation of acetyl-CoA to form malonyl-CoA and functions as a rate-limiting enzyme in fatty acid synthese. Two isoforms of ACC with distinct subcellular distribution and

physiological roles have been identified, of which the cytosolic isoform ACC1 is predominant in control of the fatty acid synthesis, while the mitochondrial isoform ACC2 mainly regulates the fatty acid oxidation through inhibition of carnitine palmitoyltransferase I by localized malonyl-CoA production [5,6] Increased ACC1 expression is associated with several cancers including breast , liver lung , and prostate [7] . Elevated ACC expression is observed in the early stage of breast cancer,ACC activity is regulated in cancer cells through phosphorylation by AMPK [8,9].

Early studies of purified rat liver enzyme indicated subunit MR of 215 - 230 kd which could be further converted by proteolysis into two nonidentical subunits of MR 118 kd and 125kd . In more recent reports, two major protein bands (MR=240 and 260 kd) were found after SDS-PAGE when enzyme was purified from rat liver using polyethylene glycol precipitation . but only one major band (MR=260 kd) when an avidin affinity column was used to purify the enzyme .Regardless of the method of purification, determination of the subunit molecular weights of the purified enzyme may not reflect those of the native enzyme because the process may either alter its structure or favor the purification of one form preferentially [10].

Aim of study: Purification of the Acetyl CoA Carboxylase-1 after chemotherapy and radiotherapy for breast cancer patients and Calculate values km ,V max of enzymatic reaction.

Material and Methods

Material: All Chemical and Reagents were purchased from Sigma Aldrich / Germany, Fluka/ Switzerland, BDH / England, Pharmacia fine Chemical /Sweeden.

Methods:

Blood Sampling: The serum was obtained by intravenous extraction of blood in an amount of 3 ml, then the samples were placed in sterile tubes and left for about half an hour at room temperature to coagulate. After that, the serum was separated by a centrifuge at(4500g) for 10 minutes and then kept at (-20 ° C) until the required tests were performed on it.

Estimation of ACC-1 activity: ACC-1 Activity was estimated spectrometry according to Kroeger method Include: the assay mixture (450 µl) contained 100 mM buffer phosphate (pH 7.8), 5 mM $MgCl_2$, 4 mM DTT, 10 mM $NaHCO_3$, 4 mM ATP, 0.4 mM NADPH, 0.4 mM acetyl–CoA. The reaction (at 37° C) was started by the addition of serum , Absorbance is measured at 365nm , photometric measurements were performed using a 500 µl cuvette with a 1-cm path length [11].

Estimation of Protein: protein was determined by Kite Biomagrap Tunis.
Reference range: Serum total protein is 6 to 8 g/dl [12,13].

P**urification of ACC-1**: The proteins were precipitated using different concentrations of ammonium sulfate from 30-60%, so the optimum degree of saturation was 40% by adding (1.4gm) of ammonium sulfate gradually to the serum with stirring for 15 minutes, then separating the precipitate from the filtrate by a centrifuge cool at a temperature of 0 ° C with a speed of 10000g for 30 minutes, then the precipitate was dissolved with a little buffer phosphate at (100 mM, pH 7.8) and the activity and total protein were measured as shown in

table 1 and proceeded into a further purification by gel filtration Chromatography applying into a Sephadex G-100 Column (2.5 x 40 cm) using phosphate buffer (pH 7.8, 100 mM) as elution buffer, The flow rate (1 mL/min) from the column and 5 mL of each fraction were collected . Where 125 ml of phosphate buffer was passed through the gel column to ensure the descent of the enzyme by adding gradually and slowly so as not to crack the surface of the gel. The resultant was extra purified by Ion Exchange Chromatography using DEAE-Cellulose Column(2.5 x 25 cm) and a washing solution consisting of phosphate buffer Solution (20mM, pH7.8) which contains (10 mM of sodium citrate) and the dilution solution that consists of phosphate buffer (0.13M, 75ml; 0.75M, 75ml; pH7.8) which contains (10mM) of Sodium Citrate[14-16]. The flow rate (1 mL / min) from the column and 5 mL of each fraction were collected, After each purification step, concentrate the perfusion solution with glucose for 4 minutes before measuring activity and total protein. Therefore, the volumes used by the gel filtration and ion exchange method became few and concentrated.

Electrophoresis: Used Garfine method for electrophoresis Polyacrylamide under non denatured condition [17]. The molecular weight of the purified enzyme ACC-1 was measured compared to standard proteins standard proteins solution**:** Prepare to dissolve standard proteins Bovine albumin, albumin Eggs, Chymotrypsinogen, Lysozyme, Glucose Oxidas, Alcohol dehydrogenase and Immunoglobulins In the sample solution at a concentration of 5 mg/ml.

Temperature Effect: The effect of temperature on the purified enzyme (ACC) from sera at different temperatures (20,25,30,37,40,45,50◦ C) and the temperature was adjusted by thermostat and the activity was measured used Kroeger method [11].

pH Effect: The studied effect of the acid function on the purified serum solution the reaction mixture solutions were prepared in different acid function (4.5 ,5,5.5,6,6.5,7,7.5,8,8.5,9) and the pH of the prepared solutions was adjusted by Hydrochloric acid (1M) and sodium hydroxide(1M) and the activity was measured used Kroeger method at optimum temperature.

Substrate Concentration Effect: The studied effect of the substrate (Acetyl Co-A) on the purified serum solution prepared concentrations different of substrate (0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.2, 1.4, 1.8,2 mM) and the activity was measured Used Kroeger method at optimum temperature and optimum pH [11]. The value(km)of the Substrate (Acetyl Co-A) of the ACC-1 purified enzyme from the blood Serum of women with breast cancer was calculated using the Lineweaver- Burk equation.

Results and Discussion

The result of ACC-1purification protocol could be illustrated in table1, Fig.1, Fig. 2and Fig. 3

Table 1: purification of Enzyme ACC(ACC-1) from breast cancer serum for premenopausal after treatment or Mastectomy.

Step	Elute (ml)	Activity (IU/ml)	Total activity (IU)	Total protein (g/dl)	Specific Activity (IU/g)	fold	% Yield
Grude Serum	5	24.24	121.2	7.30	16.60	1	100
Ammonium Sulphates	4	19.39	77.56	0.80	96.95	5.84	63.99
Dialysis	3	17.81	53.43	0.33	161.90	9.75	44.08
Gel Filtration	3	10	30	0.15	200	12.04	24.75
Ion exchange	2	6.90	13.8	0.04	345	20.78	11.38

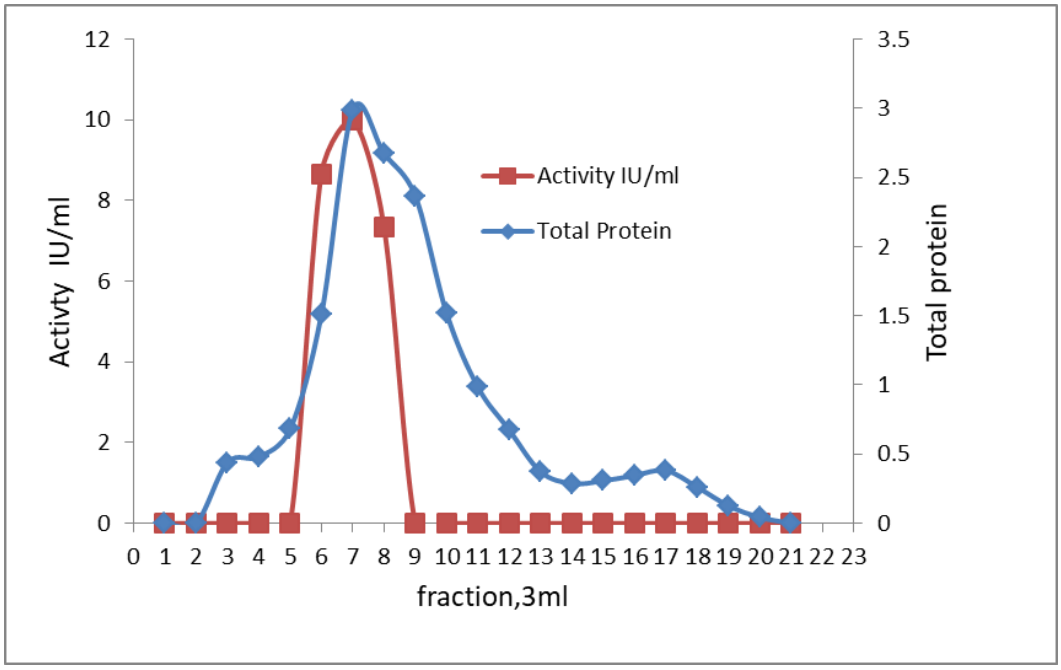

Figure 1: Gel filtration of ACC1 from breast cancer serums blood in premenopausal group after treatment or Mastectomy.

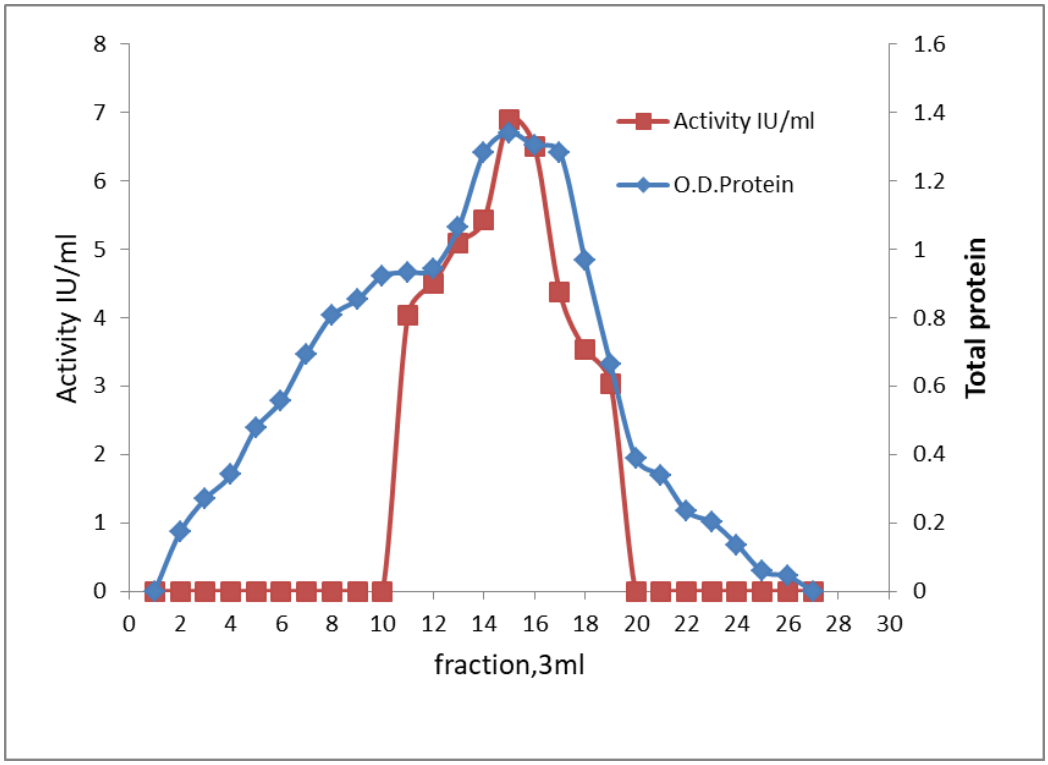

Figure 2: Ion exchange of ACC1 from serum with premenopausal breast cancer after treatment or Mastectomy

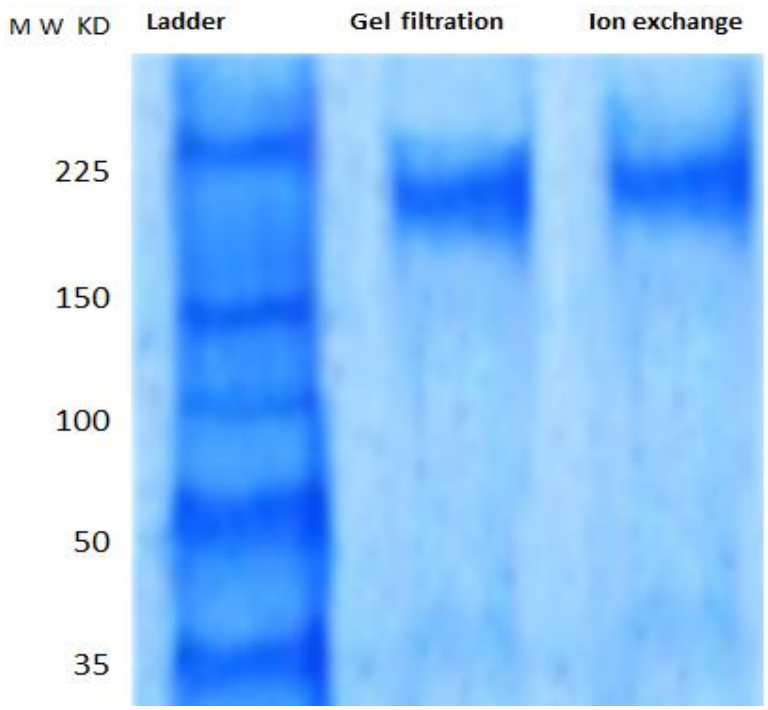

Figure 3: Electrophoresis of the purified ACC-1 enzyme from the serum of women with breast cancer premenopausal group for after treatment or Mastectomy.

Where the results showed a single peak in the gel filtering Chromatography and a single peak in the ion exchange Chromatography, as shown in Fig. 1 and 2. Electrophoresis on acryl amide, in the absence of denatured agents, the above results are proven, as it was observed that a single band appeared in the exudate part of the gel filtration as shown in Fig

.3 molecular weight (210 kd) and ion exchange and molecular weights (210 kd) as shown in Fig .3.

These results are agree with the results of Beaty and his group, Where he is obtained one single peak of the purified ACC enzyme from chicken liver and obtained a band 210KD. [18]. It also agreed with Dehaye and his group where he obtained one single peak of the purified ACC enzyme from the seeds of the gel filtration method and he found the protein band at 210KD [19]. It also agreed with the findings of Roessler, where he obtained a band 200KD molecular weight using gel filter method [20], as well as with the chenge and his group, where the ACC enzyme was purified from humans and Rat, where a band was found at 210KD [21].

Effect of Acidic function

The Effected the acidic on speed of the enzymatic reaction through its effect on the ionic groups associated with the surface of the enzyme, where the ionic residues are taken in the active site of the enzymes and the creation of an enzymatic reaction that maintains the catalytic activity of the enzyme and Substrate , this pH agree with Manning and his group particularly the ACC-1 multiple enzyme that contains ionized residues in the centers reaction, the activity of charting the relationship between different degrees of the acidic function and reaction speed of the ACC-1 enzyme. The reaction velocity is observed with an increase in pH to the limit of reaching the maximum speed at an acidic function of 7.5. [22,23], as shown in Fig. 4.

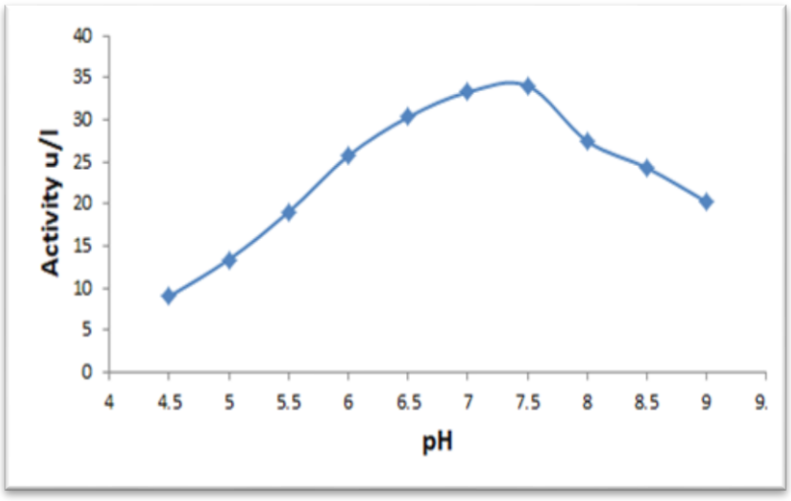

Figure 4: The effect of pH and Activity on ACC-1

Temperature Effect

Fig .5 shown the effect of temperature on the reaction speed of the ACC-1 enzyme where a temperature rise is observed to reach the maximum reaction speed at a temperature of 40 ℃, then the reaction speed begins to decrease when the temperature increases above 40 ℃ and It is agree with Manning and his group Where the temperature affects in the case of ionization of the active groups present on the surface of the enzyme and the Substrate which leads to an increase in enzyme activity with increasing temperature because the enzymes are complex protein molecules, their catalytic activity affects the three-dimensional and even four-dimensional structure as the number of collisions between the enzyme and the substrate increases with increasing temperature At the same time, the enzyme cannot perform its

catalytic role when the temperature rises to the extent that leads to a change in protein synthesis and the occurrence of denaturation processes, and then the enzyme loses its catalytic activity[22,23].

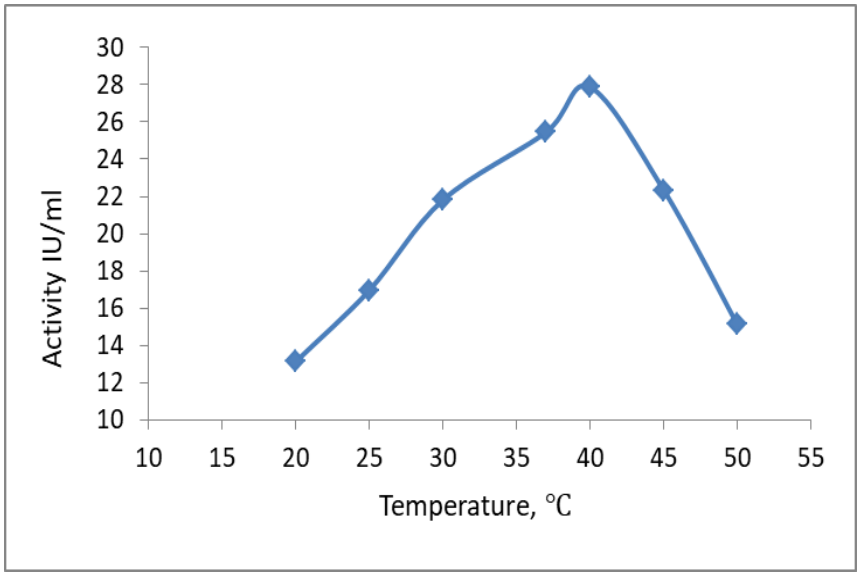

Figure 5: The effect of temperature on purified enzyme ACC-1

Effect of Substrate Concentration (Acetyl CoA)

The effect of the Substrate concentrations (Acetyl Co-A) on the speed of the enzymatic reaction was studied and the optimum concentration of this substance was determined, which gives the maximum reaction speed. Fig. 6 shows the effect of the concentration of (Acetyl Co-A) on the speed of the ACC-1 reaction. An increase in the reaction velocity is observed with an increase in the concentration of the Substrate until the maximum reaction velocity is reached at a concentration of (1.8mM.) Then the reaction speed is established as a result of saturation of sites activity of the enzyme ACC(ACC-1).

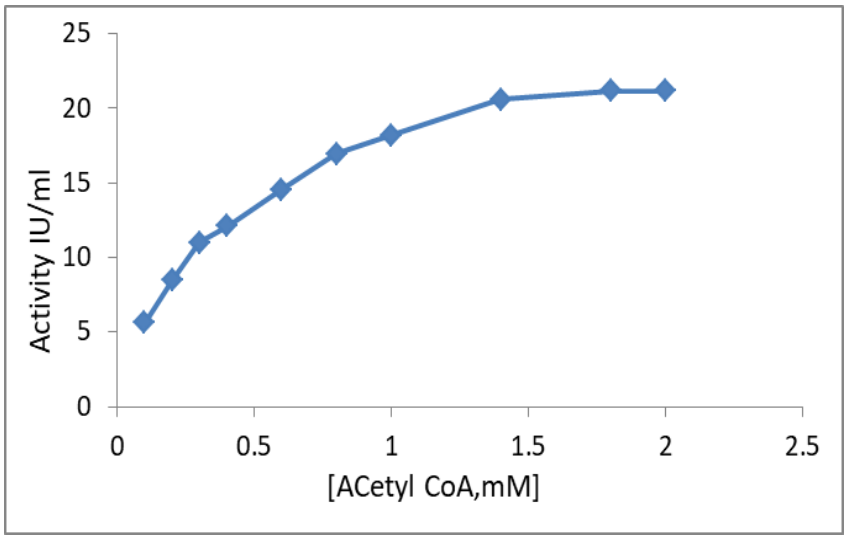

Figure 6: The effect of Substrate concentrations on the Activity of the purified ACC-1

The Michaelis-Menten constant is one of the important physical constants in the kinetic studies of life compounds as it reflects the enzyme affinity of the substrate and it can be known that substrate concentration when the velocity of the enzymatic reaction is half of its maximum velocity (Vmax), Fig. 7.

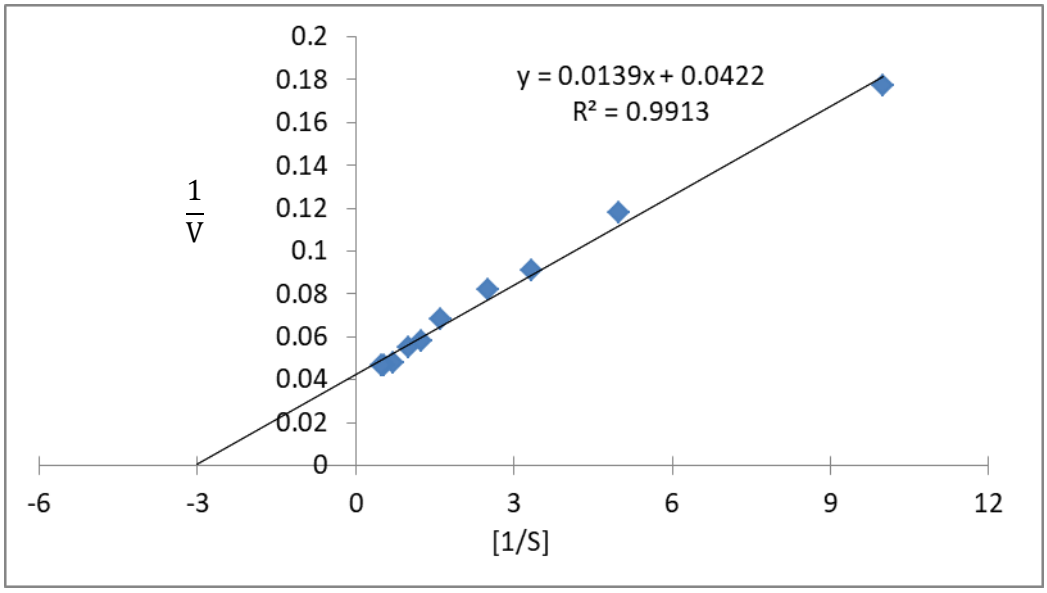

Figure 7: Lineweaver- Burk method for calculating the (Km)

Table 3: Values of Michaelis-Menten constant (km) and Vmax for ACC-1

Equation	Substrate	Con optimum	T optimum	Km(mM)	Vmax
Lineweaver-Burk	Acetyl CoA	1.8mM	40℃	0.3	22mM min^{-1}

Where shows table 3 the value of km and Vmax calculated according to the Lineweaver-Burk for Acetyle CoA the Substrate for the enzyme ACC(ACC-1) purified from serum with breast cancer at optimal conditions for the reaction the Km reflects a high affinity of the enzyme for substrate because a low concentration of substrate is needed to half saturate the enzyme that is reach a velocity of ½ Vmax [24].

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تنقية الاستايل كواي كاربوكسليز-1 من مصل النساء المصابات بسرطان الثدي بعد استئصال الورم

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البحث مستل من أطروحة دكتوراه الباحث الاول

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الكلمات المفتاحية:

سرطان الثدي، الاستايل كواي كاربوكسليز، الفعالية، التنقية، لينوفربيرك

الخلاصة:

تم تنقية انزيم الاستايل كواي كاربوكسليز من مصل النساء المصابات بسرطان الثدي لفئة قبل سن اليأس بعد استئصال الورم بواسطة كروماتوغرافيا الترشيح الهلامي باستخدام السيفادكس G100 و كروماتوغرافيا التبادل الايوني باستخدام ثنائي اثيل امين اثيل –سليلوز A50 وتقدير الوزن الجزيئي بواسطة تقنية الترحيل الكهربائي على الاكريل امايد بغياب العوامل الماسخة .لقد اظهرت النتائج قمة وحزمة بروتينية منفردة بكلا التقنيتان عند وزن جزيئي 210KD تقريبا ,ان درجة الحرارة المثلى 40 ℃ ,عند دالة حامضية 7.5 وان التركيز الامثل للمادة الاساس (الاستايل كواي)1.8 mM, و السرعة القصوى للتفاعل الانزيمي 23mM/min وثابت ميكالس- منتن 0.33mM