Kinetic and Thermodynamic Studies on the Binding of ^{125}I –Anti CA 15-3 Antibody to the Isolated CA 15-3 from Human Breast Tumor Homogenate [+]

دراسات حركية وثرموديناميكية لارتباط الضاد المتخصص ^{125}I –Anti CA 15-3 Antibody مع CA 15-3 المعزول من مجانسات اورام الثدي في الانسان

Salwa H.N.Al-Rubae'i [*] **Sami A. AL-Mudhaffar**[**] **Raad K. Muslih** *

Abstract:

This research include, the studies of kinetic and thermodynamic parameter associated with the binding of ^{125}I-anti CA15-3 antibody to isolated CA15-3 from benign (Fibroadenoma) and malignant breast tumors tissues (IDC) were investigated. It was shown that the reaction in all studied cases follow pseudo-first order reaction kinetics. The maximum binding (B_{max}) of isolated CA15-3 in benigen tissue homogenates was $10.48x10^{-3}$ mg.mL^{-1} after 90 minutes incubation at 37^{o}C, while the (B_{max}) of isolated CA15-3 in malignant tissue homogenates was $13.38x10^{-3}$ mg.mL^{-1}. The (B_{max}) was decreased with increasing temperature. The values of affinity constant (K_a) were dependent on the temperature, K_a increased from 14.18 mg^{-1}.mL at 5^{o}C to 31.65 mg^{-1}.mL at 45^{o}C in benigen tissue homogenates , while K_a was increased from 13.87 mg^{-1}.mL at 5^{o}C to 23.81 mg^{-1}.mL at 45^{o}C in malignant tissue homogenates. The association constant K_{+1} increased with temperature in (Fibroadenoma). On the other hand, K_{+1} was independent of temperatures in (IDC). Time course, scatchard, Van't Hoff and Arrhenius plots led to theoretical determination of thermodynamic parameters of both the standared state (i.e., ΔH^o, ΔG^o, ΔS^o) and transition state (i.e., Ea, ΔH^*, ΔG^*, ΔS^*) for the formation of (^{125}I-antiCA15-3 antibody /CA15-3) complex. The thermodynamic data shown that the binding reaction is an entropically driven reaction and suggest an involvement of hydrophobic forces in the stabilization of complexes.

المستخلص:

يتضمن البحث دراسة الخصائص الحركية والثرموديناميكية لارتباط الضاد المتخصص ^{125}I-anti CA15-3 antibody مع المستضد الكاربوهيدراتي CA15-3 المعزول من انسجة اورام الثدي الحميدة والخبيثة. اشارت النتائج الى ان التفاعل يتبع حركيات المرتبة الاولى الكاذبة في كلا الحالتين. و كان اعلى ارتباط (B_{max}) للـــ CA15-3 المعزول من مجانسات الانسجة الحميدة ١٠,٤٨×١٠$^{-٣}$ ملغم. مل$^{-١}$ بعد ٩٠ دقيقة من الحضن بدرجة حرارة ٣٧ م^o ، بينما كان اعلى ارتباط (B_{max}) للـــ CA15-3 المعزول من مجانسات الانسجة الخبيثة 13.38×١٠$^{-٣}$ ملغم. مل$^{-١}$. اشارت النتائج الى ان (B_{max}) تقل بزيادة درجة الحرارة.

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[*]Assistant Professor /College of Science, Al-Mustansiriya University

[**] Professor/ College of Science, Baghdad University

اظهرت النتائج اعتماد قيم ثابت الفة الارتباط (K_a) على درجة الحرارة اذ ازدادت قيمته من ١٤,١٨ ملغم $^{-1}$.مل بدرجة ٥ م^{o} الى ٣١,٦٥ ملغم $^{-1}$.مل بدرجة ٤٥ م^{o} في انسجة مجانسات الثدي الحميدة ، بينما لوحظ زيادة قيم (K_a) من ١٣,٨٧ ملغم $^{-1}$.مل بدرجة ٥ م^{o} الى ٢٣,٨١ ملغم $^{-1}$.مل بدرجة ٤٥ م^{o} في انسجة مجانسات الثدي الخبيثة . استعملت نتائج المتابعات الزمنية لدرجات الحرارة المختلفة ورسومات سكاجرد وفانتهوف وارينيوس في حساب المعاملات الثرموديناميكية القياسية(ΔG^o وΔH^o و ΔS^o) والخالة الانتقالية (Ea وΔH^* و ΔG^* و ΔS^*) لتكوين المعقد . واشارت النتائج بان القوة الدافعة للتفاعلات الارتباطية تعتمد على دالة التغير الانتروبي مما تدل على اهمية التاثرات الكارهة للماء في تكوين المعقدات الناتجة .

Introduction:

Tumor markers may be used to indicate the risk, presence, status, or future behavior of cancer [1]. Carbohydrate antigen (CA15-3) assay used in monitoring of breast cancer [2]. The specific reaction between an antibody (Ab) and an antigen (Ag) is usually driven by electrostatic forces between oppositely charged amino acids, hydrogen bonding, and hydrophobic interactions. The equilibrium reaction, termed "biospecific interaction", is characterized by the affinity of reactants to form Ag-Ab complex [3].

Kinetic studies supplement the information for differences between the initial, final states of each reactant and an intermediate activated complex, (i.e, the pathway taken by the reactants reach the final product) [4]. On the other hand, thermodynamics of the binding describes the system in its initial, final states. Using kinetic and equilibrium data also determined thermodynamic formation constant.

Al-Mudhuffar et.al, have many studies on the kinetic and thermodynamic of protein-protein interaction in human breast tissue, like kinetic and thermodynamic of purified steroid receptor of malignant breast tumors with hormone [5], also kinetic and thermodynamic studies on the binding of lectin in human malignant breast to glycoprotein [6].

In this research, the basic mathematical analysis was described and used to explain the mechanism through kinetics of binding of CA15-3 from both breast tumor homogenates (fibroadenoma and Infiltrating ductalcarcinoma) to its antibody to form (^{125}I-anti CA15-3 antibody / CA15-3) complex in partially purified fraction.

Materials and Methods

Chemicals

All chemicals and reagents used in this study were of analytical grade, Immunoradiometric assay kit for CA 15-3 level from Diasorin Inc. Na_2HPO_4, NaH_2PO_4 , NaCl, and NaN_3 from BDH,limited,Poole (UK).

Instruments

The instruments used during this study were Gamma counter type 1270-rack gamma II from LKB. pH meter, cooling centrifuge; with a maximum speed 5000 r.p.m. from Hettich. Memmert water bath, memmert incubator from West Germany. SM-shaker from England.

Patients

Two groups of breast tumors patients were included in this study.

Group I : Consisted of 40 patients with benign breast tumors

Group II : Consisted of 32 premenopausal patients with breast cancer (IDC)..

All patients were admitted for treatment to (Baghdad Teaching Hospital), (University Hospital, Al-Nahrain College of Medicine), (Nursing Home Private Hospital) and (Al-Arabi Private Hospital). Patients suffered from any disease that may interfere with this study were excluded. All surgical operation of breast tumors were carried out under the supervision of the following surgeons:Dr. Saab Sedeq, Dr.Munthir Al-Aubaidi, Dr.Azam Qanbar Agha, Dr. Abd Al-Salam Al-Tai, Dr. Zuhair Abid Al-Hadi. The host information of all patients and normal healthy subjects is summarized in table (1).

Table (1): The host information of breast tumors patients and healthy subjects studied.

Group	**Patients**	**No.**	**Age**	**Type of tumor**	**Metastases**
I	Benign breast tumor	40	18-42	fibroepithelial tumor (fibroadenoma)	–
II	Premenopausal malignant breast tumor	32	34-52	Infiltrative Ductal carcinoma	2 lymph nodes

Collections of Specimens

The tumors tissues were surgically removed from breast tumor patients by either mastectomy (cancer patients) or lumpectomy (benign tumor patients). The specimens were cut off and immediately rinsed with ice-cold isotonic saline solution. They were collected individually in plastic receptacle and stored at –20 ^{o}C until homogenization.

PBS–Buffer

Phosphate –buffered saline (PBS) 0.05 M, PBS buffer pH 7 containing 0.02% sodium azide was prepared as following:

A: Disodium basic phosphate (0.5M); 7.0980g Na_2HPO_4 and 9.0g of NaCl
were dissolved in a final volume 1L deionized distilled water.

B:.Monobasic sodium phosphate (0.15M) 5.9990g of NaH_2PO_4 and 9.0g NaCl
were dissolved in a final volume 1L deionized distilled water.

Phosphate buffer saline pH 7 was prepared by mixing a volume of solution A with appropriate amounts of solution B to obtain the required pH.

Preparation of Breast Tumors Tissue Homogenates

The frozen tissue were weighed, sliced finely and scalped in Petri dish standing on ice bath, and then homogenized with fivefold volumes of PBS buffer pH7.2, using manual homogenizer [7]. The homogenate was filtered through four layers of nylon gauze in order to eliminate fibers connective tissues, and then centrifuged at 4000 r.p.m for 45 min. at 4 ^{o}C in order to precipitate the remaining intact cells and the intact nucleus. The supernatant fraction at this speed was separated, divided in aliquots and freeze -20 ^{o}C until use.

Methods

Isolation of CA15-3 by Sepharose CL-4B Column

This part was carried out according to Al-Rubae'i thesis [8].

Protein Determination

Total homogenate protein content was determend by the method of Lowry [9] using bovine serum albumin as the standard.

Kinetic Studies

A-The Time-Course of the Binding of ^{125}I-anti CA15-3 Antibody with CA15-3 in Breast Tumor Homogenate

1. One hundred microliters of partially purified CA15-3 from benign breast tumor (fibroadenoma) and premenopausal malignant breast tumor (Infitrating ductal carcinoma, IDC) containing (150 and 100 mg.mL^{-1} protein) respectively, were added to (20 and 25 µL) of ^{125}I-anti CA15-3 antibody containing (0.140 and 0.175 mg.mL^{-1}) respectively.
2. The volume of reaction were completed to 500 µL with PBS buffer pH 7.0.
3. All tubes were incubated at 37^{o}C at different time intervals (30,60,90,120,150,180) min.
4. Two additional tubes, containing 50µL (0.35 mg.mL^{-1})of ^{125}I –anti CA15-3 antibody only, for total counts were set-aside until counting.
5. At the end of incubation, the assay tubes were centrifuged at 4000 r.p.m for 45 min at 4^{o}C.
6. The supernatant were decanted, the rims at the tube were swabbed with cotton piece.
7. The radioactivity of the complex were counted using gamma counter.
8. To determine the time-course of partially purified CA15-3 binding to ^{125}I-anti CA15-3 antibody at different temperatures, step 1,2,3 and 4 in the same experiment were repeated at different temperatures 5, 15, 25 and 45C^{o}.

Calculation

Calculations

1. The counted radioactivity in each tube (expressed in c.p.m.) represents the bound fraction (B), (i.e., ^{125}I antiCA15-3 antibody/CA 15-3 complex).
2. The counted radioactivity in the tubes containing ^{125}I-anti CA15-3 antibody only represents the total count (T).
3. The (B/T) ratio for each tube counted as follows:

$$(B/T)\% = \frac{\text{Sample Counts (B)}}{\text{Total Counts (T)}} \times 100$$

4. The (B/T) ratio plotted against incubation time at each temperature for both types of homogenates.

B-Determination of Kinetic Parameters of ^{125}I-Anti CA 15-3 Antibody Binding with Partially Purified CA 15-3 in Benign and Malignant Breast Tumors

Determination of the affinity constant (K_a) and the maximal binding capacity (B_{max}) of:

Partially Purified CA15-3 in Benign Breast Tumor Homogenate Binding with ^{125}I-Anti CA15-3 Antibody

1. One hundred microliters of partially purified CA15-3 from benign breast tumor (Fibroadenoma) containing (150 □g.mL^{-1} protein) were added to increasing volumes (4, 8, 12, 16, 20 and 24 µL) of ^{125}I-anti CA15-3 antibody containing (0.0280, 0.0560, 0.0841, 0.1121, 0.1402 and 0.1684 mg.mL^{-1}) to each assay tube. The final volume of each assay tube was completed to 500 µL with PBS buffer pH 7.0.
2. All tubes were incubated for 90 min at 37^{o}C.
3. Steps 4, 5, 6 and 7 in experiment (A) were repeated at different temperatures (5, 15, 25 and 45^{o}C).
4. The time of incubation required to reach the equilibrium state are reported in table (2) and figure (1):

Table (2): The time of incubation for benign and malignant breast tumor homogenate at different temperatures .

Temp. ^{o}C	Time (min.)

	Benign breast tumor homogenate (Fibroadenoma)	Malignant breast tumor homogenate (IDC)
5	180	180
15	60	90
25	90	150
37	90	90
45	180	90

Calculations

1- The B/F ratio was computed for each tube, where:
   B: is the bound radioactivity (mean counts in c.p.m), which represent the formation of (^{125}I-anti CA15-3 /CA15-3) complex.
   F: is the free radioactivity (mean counts in c.p.m.), which represents the (unbound or unreacted), ^{125}I-anti CA15-3 antibody.
   T: is the total activity (mean counts in c.p.m.)

   F = T (total counts) - B (bound radioactivity)

2- The concentration of (^{125}I-anti CA15-3/CA15-3) complex in mg.mL^{-1} which found after time (t) was calculated from the following equation:

$$B(mg.mL^{-1}) = \frac{B(c.p.m)}{T(c.p.m)} x Concentra\ tion\ of\ ^{125}I - anti\ CA15-3\ antibody\ in\ the\ incubation\ medium\ in\ mg.mL^{-1}$$

3- The affinity constant and maximal binding capacity were determined according to Scatchard equation [10].

$$\frac{B}{F} = \frac{1}{K_d} x\ (B_{max} - B)$$

$$K_a = \frac{1}{K_d} = \frac{K_{+1}}{K_{-1}}$$

Where: K_a = affinity constant
K_d = dissociation constant
B_{max} = maximal binding capacity

The value of affinity constant of the binding Ka at each temperature can be calculated from the slop of the straight line in figure (2), while the value of the total concentration of CA15-3 (B_{max}) in breast tumor homogenate for each group was calculated from the intercept of the x-axis.

Partially Purified CA15-3 in Human Malignant Breast Tumor Homogenate Binding with ^{125}I-Anti CA15-3 Antibody

1. One hundred microliters of partially purified CA15-3 from premenopausal malignant breast tumor (IDC) containing (100 µg.mL^{-1} protein) were added to increasing volumes (5, 10, 15, 20, 25 and 30 µL) of ^{125}I-anti CA15-3 antibody containing (0.035, 0.070, 0.105, 0.140, 0.175 and 0.210 mg.mL^{-1}) to each assay tube. The final volume of each assay tube was completed to 500 µL with PBS buffer pH 7.0.

2. All tubes were incubated for 90 min at 15^{o}C
3. Steps 4, 5, 6 and 7 in experiment (A) were repeated at different temperatures (5, 25, 37 and 45 ^{o}C).
4. The times of incubation required to reach the equilibrium state are reported in table (2).

Calculations

The method outlined in experiment (.A) was followed exactly to obtain the values of K_a and B_{max} at each temperature as shown in figure (3).

The Thermodynamic Studies of ^{125}I-Anti CA15-3 Antibody Binding to CA15-3 in Benign and Malignant Breast Tumors

The same steps mentioned in kinetic studies were performed using the dialyzable protein fraction of benign and malignant breast tumor homogenate from fibroadenoma and (IDC) as CA15-3 source.

Calculation

1. The thermodynamic parameters of standard state were obtained from Van't Hoff plot, the values of the natural logarithm of equilibrium constant (affinity constant K_a) obtained at different temperatures were plotted against the reciprocal values of the absolute temperature in Kelvin (1/T), according to the following equation:

$$\ln K_a = \frac{\Delta S^o}{R} - \frac{\Delta H^o}{RT}$$

Where:

ΔH^o = the enthalpy change of the standard state.

ΔS^o = the entropy change of the standard state.

R = the gas constant (8.314 $J.K^{-1}.mol^{-1}$).

ΔH^o value obtained from the slop of a linear relationship of the plot.

The change in Gibbs free energy of the standard state ΔG^o was obtained from the following equation:

$$\Delta G^o = -RT \ln K_a$$

Where Ka is the affinity constant, while the standard state entropy change was obtained from:

$$\Delta S^o = \frac{\Delta H^o - \Delta G^o}{T}$$

2. The thermodynamic parameters of the transition state were obtained from Arrhenius plot of Ln K_{+1} values against (1/T) values, that given a linear relationship according to the following equation:

$$\ln K_{+1} = \ln A - \left(\frac{E_a}{RT}\right)$$

Where:

A: Arrhenius constant .

The values of activation energy (E_a) of the binding reaction can be determined from the slop of the straight line.

The enthalpy of transition state ΔH* was obtained from:

$$\Delta H^* = E_a - RT$$

Transition state of free energy change ΔG* is calculated from the following equation:

$$\Delta G^* = -RT \ \ LnK_{+1} + RT \ \ Ln \frac{KT}{h}$$

where K and h were Boltzmann and Plank's constant which equal (1.38×10^{-23} $J.K^{-1}$), (6.62×10^{-34} $J.sec^{-1}$) respectively.

The change in entropy of the transition state AS* is calculated from the following equation:

$$\Delta S^* = \frac{\Delta H^* - \Delta G^*}{T}$$

Results and Discussion

Kinetic Studies

The Time - Course of the Binding of ^{125}I-anti CA15-3 Antibody with CA15-3 in Breast Tumor Homogenate

Figure (1.A & B) shows the time – course of the formation of (^{125}I-anti CA15-3 /CA15-3) complex at five different temperatures (5, 15, 25, 37 and 45^{o}C) of partially purified CA15-3 from benign and malignant breast tumors homogenates samples.

The concentration of (^{125}I-anti CA15-3/CA15-3) complex formed after time (t) was calculated from the following equation:

[^{125}I-antiCA15-3/CA15-3] in mg.mL^{-1} after time (t) = **Count (c.p.m.) of ^{125}I-antiCA15-3 specifically bound after time (t)** / **Total counts (c.p.m.) of ^{125}I-anti CA15-3 used in the incubation** x **Concentration of ^{125}I-antiCA15-3 in the incubation (mg.mL^{-1})**

The results of time-course pattern at different temperatures indicated that the equilibrium binding studies is temperature and time dependent process. In case premenopausal malignant breast tumor (IDC) the maximum binding occurs at 15^{o}C (after incubation for 90 minutes), while in benign breast tumors (fibroadenoma) the maximum binding occurs at 37^{o}C at the same incubation time. This is may be due to the different source of CA15-3. Several authors studied the time – course of purified steroid receptors of malignant breast tumors [5], others studied the time – course on the binding of lectin in human malignant breast to glycoprotein [6], these studies revealed that the time-course must be done to find the maximum binding at different incubation time as a step to prepare the kinetic and thermodynamic studies.

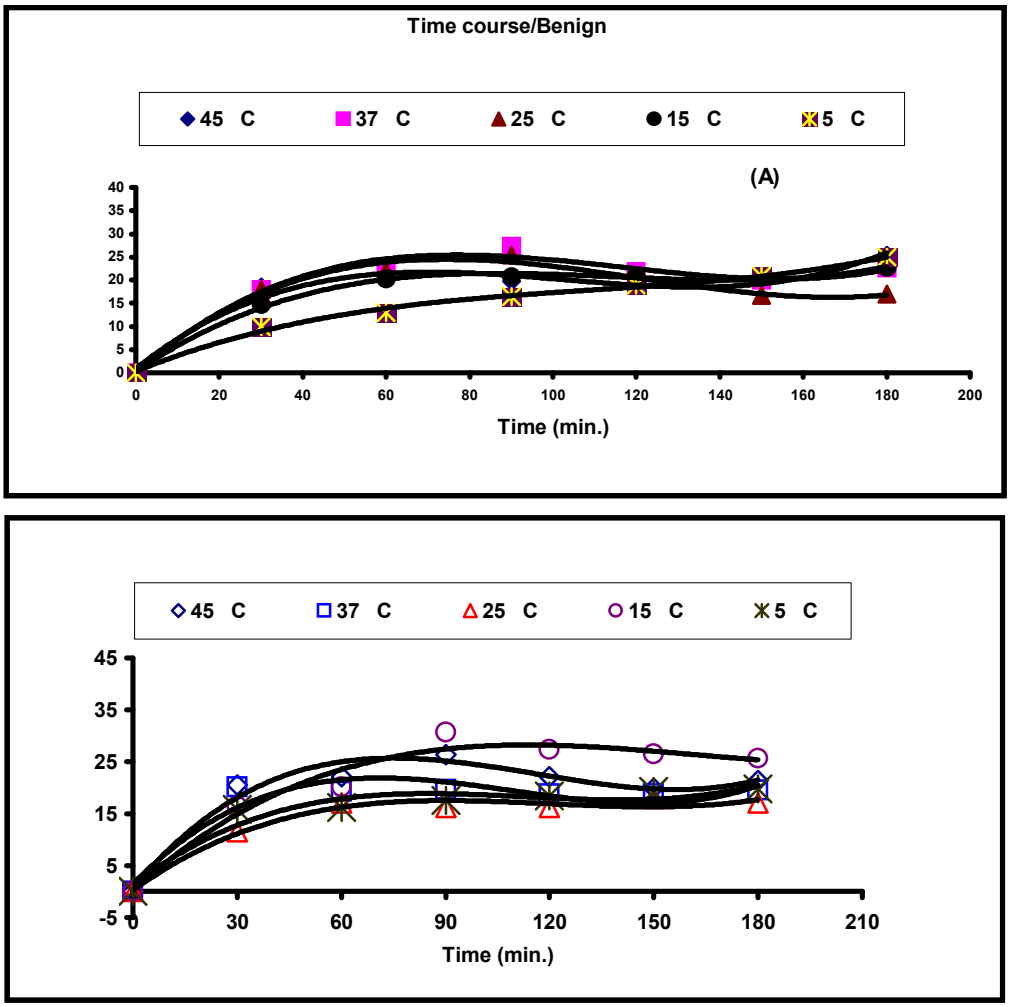

Figure (1): Time-Course of ^{125}I-anti CA15-3 binding to partially purified CA15-3 in:
(A) Benign tumor (Fibroadenoma) tissue homogenate.
(B) Malignant tumor (IDC) tissue homogenate.

Determination of Kinetic Parameters of ^{125}I-Anti CA15-3 Antibody Binding with Partially Purified CA15-3 from Benign and Malignant Breast Tumors

The time course of (^{125}I-anti CA15-3/CA15-3) complex formation was carried out to describe the kinetic parameters of the binding. The simplest proposed model representing this interaction is:

$$^{125}\text{I-antiCA15-3} + \text{CA15-3} \underset{K_{-1}}{\overset{K_{+1}}{\rightleftharpoons}} [^{125}\text{I-antiCA15-3/CA15-3}]$$

Where:
K_{+1}: is the association rate of ^{125}I-anti CA15-3 to /or CA15-3.

K_{-1}: is the dissociation rate of (^{125}I-anti CA15-3/CA15-3) complex formed.

At equilibrium:

$$K_a = \frac{[^{125}\text{I} - \text{antiCA 15} - 3/\text{CA 15} - 3]}{[^{125}\text{I} - \text{antiCA 15} - 3][\text{CA 15} - 3]} \quad \ldots\ldots\ldots\ldots(2)$$

$$K_d = \frac{[^{125}I - antiCA\ 15-3][CA\ 15-3]}{[^{125}I - antiCA\ 15-3 / CA\ 15-3]} \ldots\ldots\ldots\ldots(3)$$

Thus:

$$K_a = \frac{1}{K_d} = \frac{K_{+1}}{K_{-1}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(4)$$

Where:

The value K_a and maximal binding capacity (B_{max}). Were calculated from Scatchard plot at five different temperatures at incubation time of 90 minutes, figure (2) and (3).

It is clear from table (2), that the affinity constant (K_a) is depended on the type of the tumor (i.e., benign or malignant) and on the temperature. K_a increased with increased temperature for the same tumor (Fibroadenoma), K_a increased from 14.18 mg^{-1}.mL at 5^oC to 31.65 mg^{-1}.mL at 45^oC. Whereas the values of dissociation constant (K_d) was calculated by using equation (4), which show that the lowest K_d value of (^{125}I-anti CA15-3/CA15-3) complex occurs at 45^oC at time of incubation 180 minutes.

The concentration of CA15-3 in partially purified fractions of (Fibroadenoma) was determined to be 10.48x10^{-3} mg.mL^{-1} and the maximum binding (B_{max}) occurred after 90 minutes incubation at 37 ^oC. While in the same table the maximum K_a value for the binding ^{125}I-anti CA15-3 antibody with CA15-3 present in partially purified fraction of (IDC) occurred at 15^oC and it was increased with temperature in the following order: 5 >15 >25 >37 > 45 ^oC.

The lowest K_d value of (^{125}I-anti-CA15-3 /CA15-3) complex occurs at 45 ^oC at the time of incubation.

Scatchard plot analysis gave straight line as shown in figure (2) and (3) indicating that the (^{125}I-anti CA15-3/CA15-3) complex is directed against the same epitopes on CA15-3 molecules. On the other hand, the maximum binding occurred at 15^oC and was 13.38x10^{-3} mg.mL^{-1} also shows that the (B_{max}) decreased with increasing temperatures of incubation.

Table (3): The Kinetic parameter of ^{125}I-anti CA15-3 antibody binding to partially purified CA15-3 in breast tumor homogenate.

Temp ^oC	Benign breast tumors (Fibroadenoma)			Malignant breast tumors (IDC)		
	Binding Capacity B_{max}x10^{-3} (mg.mL^{-1})	K_a (mg^{-1}.mL)	K_dx10^{-2} (mg.mL^{-1})	Binding Capacity B_{max} x10^{-3} (mg.mL^{-1})	K_a (mg^{-1}.mL)	K_dx10^{-2} (mg.mL^{-1})
5	9.22	14.18	7.05	10.82	13.87	7.21
15	8.05	16.73	5.98	13.38	20.78	4.81
25	9.02	16.38	6.10	12.57	20.84	4.79
37	10.48	18.66	5.36	9.63	22.22	4.50
45	6.67	31.65	3.16	11.67	23.81	4.20

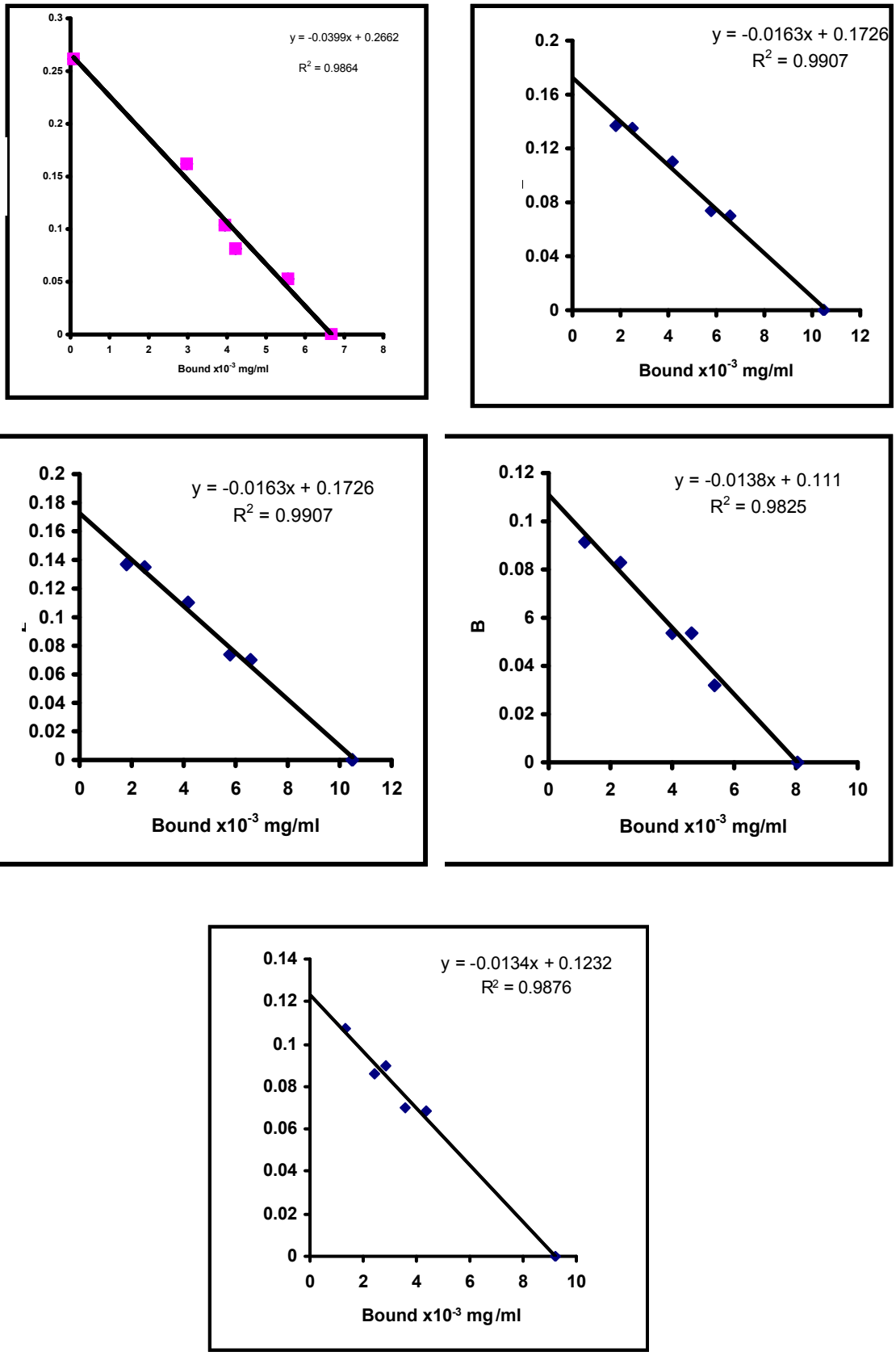

Figure (2): Scatchard plot of ^{125}I-anti CA15-3 antibody binding to the partially purified CA15-3 in benign breast tumors (Fibroadenoma) at five different temperatures.

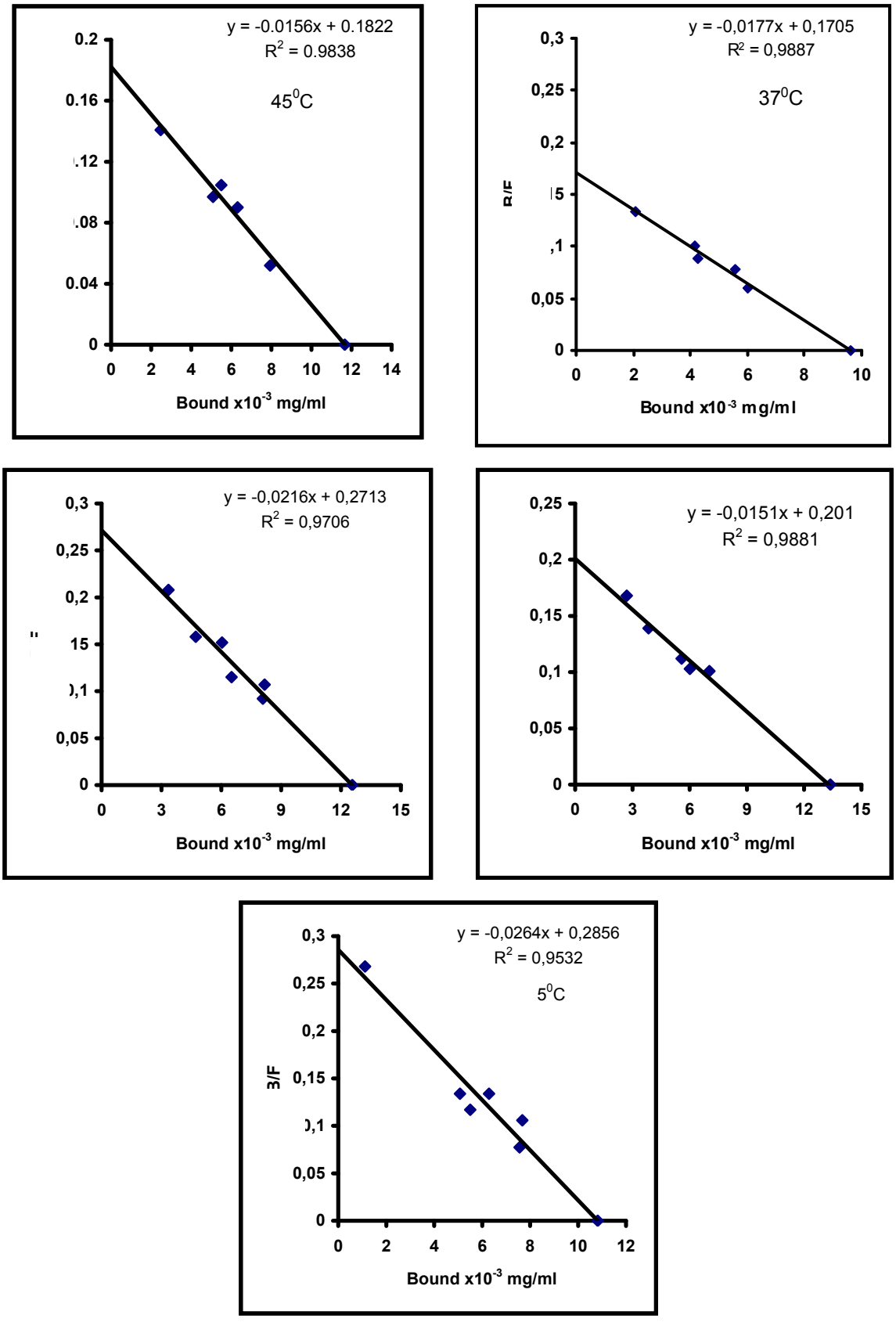

Figure (3): Scatchard plot of ^{125}I-anti CA15-3 antibody binding to the partially purified CA15-3 in Malignant breast tumors (IDC) at five different temperatures.

However, the time-course data shown in figure (1) could be used to determine the reaction order of CA15-3 binding to its specifically ^{125}I-anti CA15-3 using the following equation [12]:

$$Ln[AbAg]_e\left[\frac{[Ab]_t-[AbAg]_t[AbAg]_e/[Ag]_t]}{[Ab]_t[AbAg]_e-[AbAg]_e}\right]=K_{+1}t\left[\frac{[Ab]+[Ag]_t-[AbAg]_e}{[AbAg]_e}\right]\text{.....(5)}$$

Where:

k_{+1} : is the kinetic association constant in mg^{-1}. min^{-1}. mL.

$[AbAg]_e$: is the concentration of (^{125}I-antiCA15-3/CA15-3) complex formed at equilibrium.

$[AbAg]_t$: is the concentration of (^{125}I-antiCA15-3/CA15-3) complex after time (t).

$[Ab]_t$: is the total concentration of ^{125}I-anti CA15-3 antibody in mg. mL^{-1}.

$[Ag]_t$: is the total concentration of CA15-3 in mg. mL^{-1}.

Equation (5) represents the second order kinetics, but the percent of binding was in some cases, small and most labeled antibody remains free and only small fraction binds even at equilibrium, i.e , $[Ab]_t >> [AbAg]_e$

Thus :

$$[Ab]_t >> \frac{[AbAg]_t[AbAg]_e}{[Ag]_t}$$

So that the following equation [3] could be used in order to fit the pseudo-first order kinetics:

$$Ln\frac{[AbAg]_e}{[AbAg]_e-[AbAg]_t}=K_{+1}t\frac{[Ab]_t[Ag]_t}{[AbAg]_e}\text{.....(6)}$$

Against time (t) $\ln\frac{[AbAg]_e}{[AbAg]_e-[AbAg]_t}$ On the other hand, figure (4) and (5) show the plot of in both benign and malignant breast tumors, which give a straight line with a slope equal to the observed value of first rate constant K_{bos} in min^{-1}. The rate constant (k_{+1}) in mg^{-1}. mL. min was calculated at five different temperatures by using the following equation [13]:

$$K_{obs}=K_{+1}\frac{[^{125}I-antiCA\ 15-3]_t[CA\ 15-3]_t}{[^{125}I-antiCA\ 15-3/CA\ 15-3]_e}\text{.....}\ \text{...(7)}$$

The value of k_{-1} at five temperatures was calculated by using equation (4). Whereas, the half-life time of association $(t\ ½)_{ass.}$, Which represented the time needed for the formation of half amount of the complex at equilibrium was determined from the concentration of the complex at equilibrium and the time-course curve. The half-life time of dissociation $(t\ ½)_{diss.}$, was calculated from the following relation:

$$(t_{1/2})_{diss.}=\frac{\ln 2}{k_{-1}}=\frac{0.693}{k_{-1}}$$

$$(t_{1/2})_{ass.}=\frac{\ln 2}{k_{obs}}=\frac{0.693}{k_{+1}}$$

The value of $k_{obs.}$, k_{+1}, k_{-1}, $(t\ ½)_{ass.}$,$(t\ ½)_{diss.}$ at five different temperatures are summarized in table (4). Data analysis in this table shows that highest rate for the association reaction k_{+1} , in benign breast tumors (Fibroadenoma) and malignant breast tumors (IDC) occurs at 37°C and 15°C respectively , while the lowest rate occurs at 45°C. This means the dependence of reaction rate on temperature (Table 4) that also shows the values of the rate constant for the reverse reaction k_{-1} calculated from equation (4). Results show that the rate of dissociation of ^{125}I-anti CA15-3 antibody, from its CA15-3 is temperature independent.

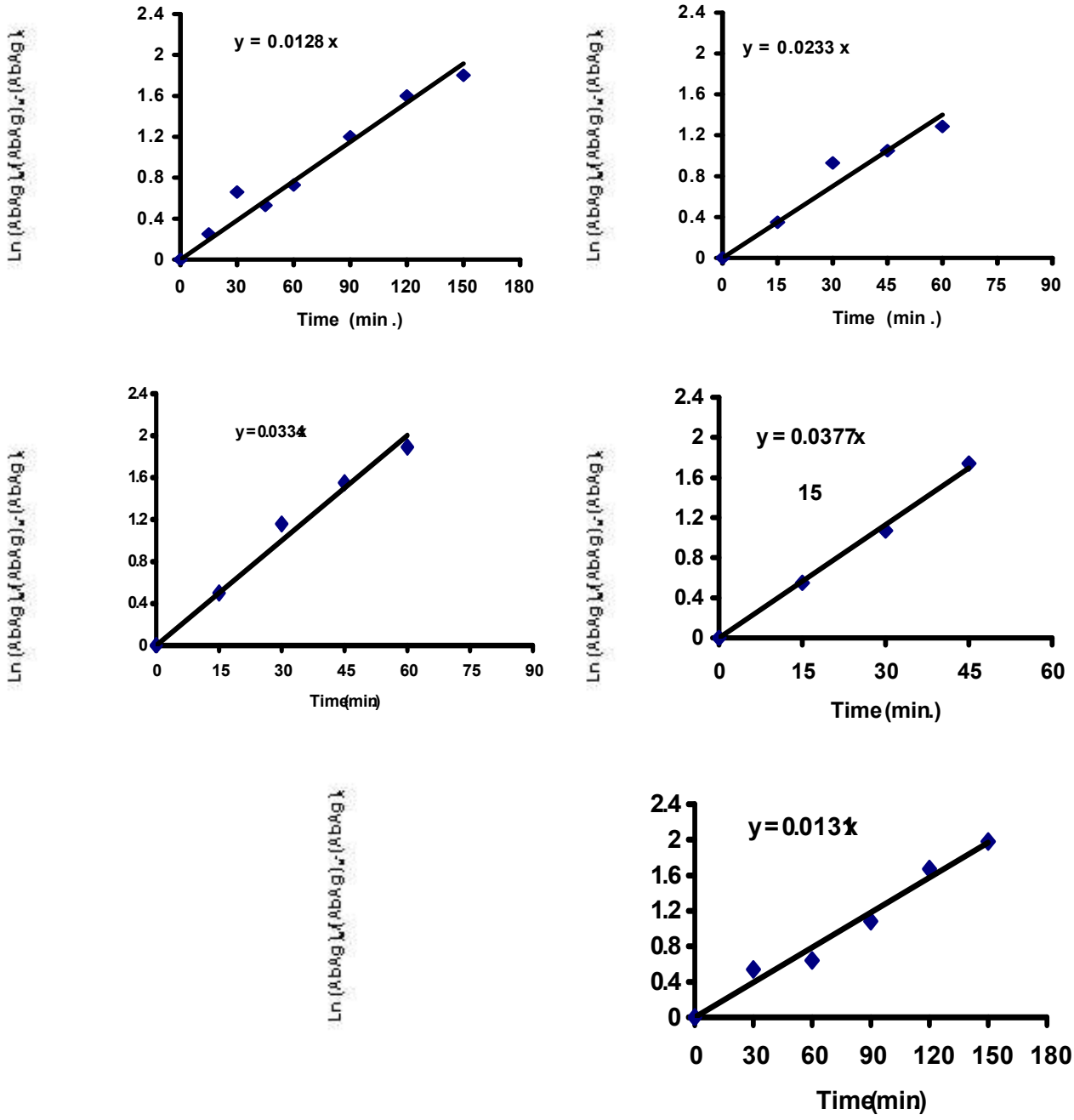

Figure (4): Kinetics of ^{125}I-anti CA15-3 antibody binding to partially purified CA15-3 in benign breast tumors (Fibroadenoma).

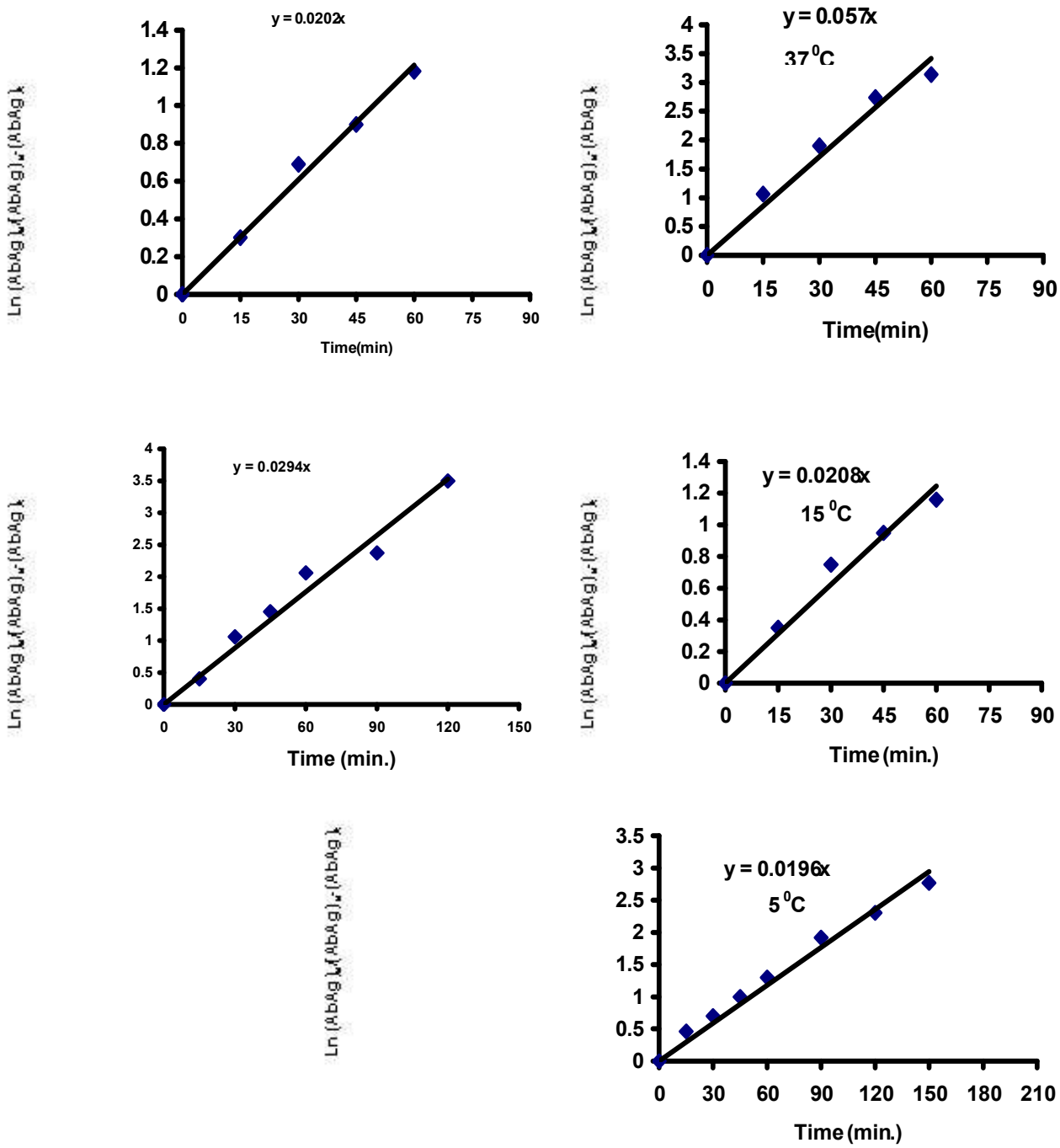

Figure (5): Kinetics of ^{125}I-anti CA15-3 binding to partially purified CA15-3 in malignant breast tumors (IDC).

Table (4): The effect of temperature on the kinetic parameters of ^{125}I-anti CA15-3 binding to partially purified CA15-3 in benign and malignant breast tumors at five different temperature.

Temp. °C	$k_{obs} \times 10^{-3}$ (min^{-1})		K_{+1} $mg^{-1}.ml.min^{-1}$		$k_{-1} \times 10^{-1}$ (min^{-1})		$(t_{1/2})_{ass}$ (min)		$(t_{1/2})_{diss}$ (min)	
	Benign (Fibroadenom)	Malignant (IDC)	Benign (Fibroadenoma)	Malignant (IDC)	Benign (Fibroadenoma)	Malignant (IDC)	Benign (Fibroadenoma)	Malignant (IDC)	Benign (Fibroadenoma)	Malignant (IDC)
5	12.8	20.20	48.69	45.81	15.38	34.68	54	34	45	20
15	23.3	57.00	60.65	116.16	32.50	73.75	30	12	21	9
25	33.4	24.9	93.98	35.48	57.37	18.07	21	28	12	38
37	37.7	20.30	103.54	46.61	61.89	22.43	18	34	11	31
45	13.1	19.60	35.40	21.34	24.96	10.58	53	35	28	66

The Thermodynamic Studies of ^{125}I-Anti CA15-3 Antibody to the Partially Purified CA15-3 in Benign and Malignant Tumors

Thermodynamic Parameters of Standard State

Figure (6) and (7) show Van't Hoff plot of the binding of ^{125}I-anti CA15-3 antibody to the partially purified CA15-3 in benign breast tumors (Fibroadenoma) and malignant breast tumors (IDC) respectively, at different temperatures (5 , 15 , 25 , 37 and 45 °C).

These figures revealed that the equilibrium binding constant (affinity constant) for CA15-3 to its antibody is a temperature dependent. The results indicated that ΔH°, in general, had small values and their positive sign ascertains that the reaction was nearly endothermic. The ΔH° value in the case of the binding of ^{125}I-anti CA15-3 antibody to partially purified CA15-3 in benign breast tumors 12.71 KJ.mol^{-1} was higher than that in case of binding in malignant breast tumors (IDC) 6.7 KJ.mol^{-1}, so more energy is needed in case of benign breast tumor for the reaction (binding) to occur. The small positive value of ΔH° may indicate a favorable interaction between ^{125}I-anti CA15-3 antibody with partially purified CA15-3 in both cases.

These include the non-covalent interaction, which are fundamentally electrostatic in nature such as charge-charge, charge-dipole, dipole-dipole, charge-induced dipole, dipole-induced dipole interactions, and hydrogen bonds. The sum of these types of interactions can yield some stabilization to the folded structure of the complex [11].

The other values of thermodynamic parameters of standard state at five temperatures, such as ΔG° values and ΔS° values are summarized in table (5) and (6).

Table (5): Thermodynamic parameters at standard state of ^{125}I-anti CA15-3 to the partially CA15-3 in benign breast tumors (Fibroadenoma).

Temp. °C	**ΔH° KJ .moL^{-1}**	**ΔG° KJ .moL^{-1}**	**ΔS° J .mol^{-1}.K^{-1}**
5	12.71	-36.87	137.20
15	12.71	-38.59	138.42
25	12.71	-39.88	138.10
37	12.71	-41.82	139.01
45	12.71	-44.30	143.30

Table (6): Thermodynamic parameters at standard state of ^{125}I-anti CA15-3 to the partially purified CA15-3 in malignant breast tumors (IDC).

Temp. °C	**ΔH° KJ .moL^{-1}**	**ΔG° KJ .moL^{-1}**	**ΔS° J .mol^{-1}.K^{-1}**
5	6.70	-36.82	156.55
15	6.70	-39.11	159.06
25	6.70	-40.48	158.32
37	6.70	-42.27	157.97
45	6.70	-43.54	157.99

The negative values of ΔG° reflects the stability of the complex hence. The high affinity of the reactants. The high negative values of ΔG° for the binding reaction are controlled by high

positive ΔS° values of the complex formed. So, our system is characterized by the sole contribution of ΔS° to the stability of the complex formed, which ΔH° has little or no effect [12]: . Whereas, the negative values of ΔG° indicates that the reaction is spontaneous at the standard condition. On the other hand, the high positive of ΔS° suggest that the binding was entropically driven. Entropy has a driven force for the occurrence of the binding reaction, this indicates that the hydrophobic interactions played an important role in the stability of complex formation [13]:.

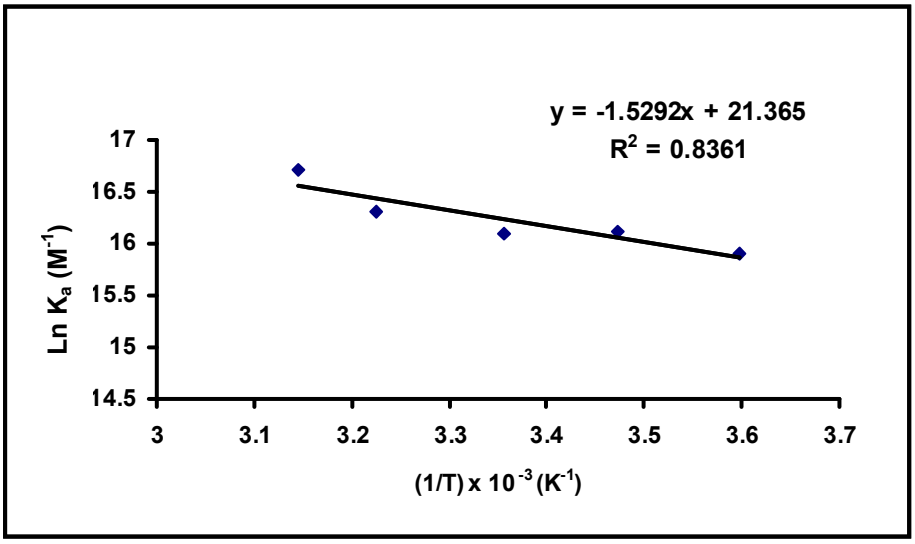

Figure (6): Van't Hoff plot for the binding of ^{125}I-anti CA15-3 antibody to the partially purified CA15-3 in benign breast tumors (Fibroadenoma).

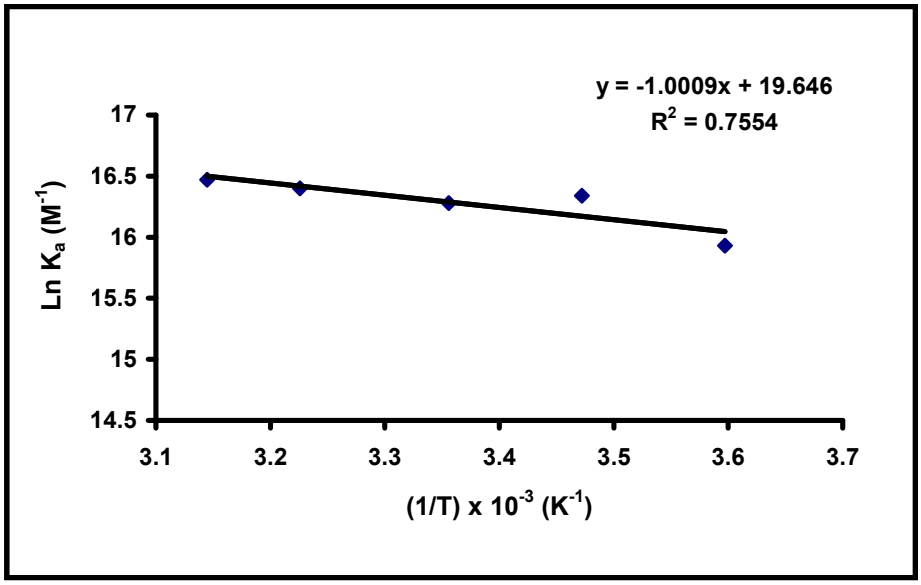

Figure (7): Van't Hoff plot for the binding of ^{125}I-anti CA15-3 antibody to the partially purified CA15-3 in malignant breast tumors (IDC).

Thermodynamic Parameters of Transition State

Transition state theory postulated that the interaction of two substances to form the final product proceeds through the formation of an activated complex (transition state).

Consequently, the association of ^{125}I-anti CA15-3 antibody with its CA15-3 can be represented as follows:

$$\underset{\text{State (A)}}{^{125}\text{I}-\text{antiCA}\ 15-3} + \text{CA}\ 15-3 \rightarrow \underset{\substack{\text{An Activated}\\ \text{Transition State}}}{[^{125}\text{I}-\text{antiCA}\ 15-3/\text{CA}\ 15-3]^{*}} \rightarrow \underset{\substack{\text{Final Product}\\ \text{State (B)}}}{[^{125}\text{I}-\text{antiCA}\ 15-3/\text{CA}\ 15-3]}$$

Thermodynamic parameters (ΔH^*, ΔG^* and ΔS^*) of the transition state were determined from the application of Arrhenius equation to the kinetic data. Figure (8) and (9) show Arrhenius plots for the binding of CA15-3 to its antibody, the slope of the line represents the activation energy (E_a) of the binding reaction, the linear relationship indicates the dependency of the association rate constant of the binding of CA15-3 to its antibody for benign and malignant breast tumors homogenate on temperature. Table (7) and (8) show the values of thermodynamic parameters of the transition state (E_a, ΔH^*, ΔG^* and ΔS^*). The high values of activation energy 9.96 KJ.mol^{-1} and 41.76 KJ.mol^{-1} of CA15-3 partially purified from benign and malignant breast tumors respectively, represents the required energy to overcome the energy barrier of the transition state for the formation of (^{125}I-anti CA15-3 antibody / CA15-3) complex. Also the value of activation energy is in accordance with the high positive values of ΔG^*, which indicates that the formation of the activated complex is a non-spontaneous process and requires a lot of energy (equal to E_a) to overcome the transition state energy barrier and giving the final product, whereas the high negative ΔS^* revealed that the activated complex had a more order structure than the reactants. From the result obtained of the thermodynamic parameters in the transition state, it can be concluded that the positive values of ΔH^* and high positive values of ΔG^* are favorable to overcome the energy barrier of the transition state, the high negative values of ΔG^* is mainly attributed to the decrease in entropy of the transition state ($\Delta S^* < 0$). In addition the positive values of ΔH^* show that the heat content of the activated complex is more than that in isolated species [14, 15]. It is proposed that the formation of a complex occurs in the two steps. The first is the stabilization of the complex by hydrophobic interactions and second is the stabilization by short range interactions , such as electrostatic interaction, hydrogen bonding and Van der Waals interactions [16]. Hydrophobic interactions contribute to the complex stability via high positive entropy change ($\Delta S^* > 0$), while electrostatic interactions, hydrogen bonding and Van der Waals interactions contribute to the stability of the complex via negative entropy change ($\Delta S^* > 0$) [16, 17]. The thermodynamic data indicate that the binding of ^{125}I-anti CA15-3 antibody to partially purified CA15-3 are entropy driven and in agreement with the concept that hydrophobic interaction play an important rote in the formation of (^{125}I-anti CA15-3 antibody / CA15-3) complex and will provide valuable information on the stability [18].

Table (7): Thermodynamic parameters at transition state of ^{125}I-anti CA15-3 antibody to the partially purified CA15-3 in benign breast tumors (Fibroadenoma).

Temp.°C	Ea KJ . mol^{-1}	ΔH^* KJ . mol^{-1}	ΔG^* KJ . mol^{-1}	ΔS^*J .mol^{-1}. K^{-1}
5	9.96	7.65	58.94	-184.50
15	9.96	7.57	60.62	-184.20
25	9.96	7.48	61.72	-182.01
37	9.96	7.38	64.06	-182.84
45	9.96	7.32	68.62	-192.77

Table (8): Thermodynamic parameters at transition state of ^{125}I-anti CA15-3 antibody to the partially purified CA15-3 in malignant breast tumors (IDC).

Temp. °C	Ea KJ . mol^{-1}	ΔH^* KJ . mol^{-1}	ΔG^* KJ . mol^{-1}	ΔS^* J .mol^{-1}. K^{-1}
5	41.76	39.45	59.08	-70.61
15	41.76	39.37	59.09	-68.47
25	41.76	39.28	64.14	-83.42
37	41.76	39.18	66.12	-86.90
45	41.76	39.12	70.00	-97.11

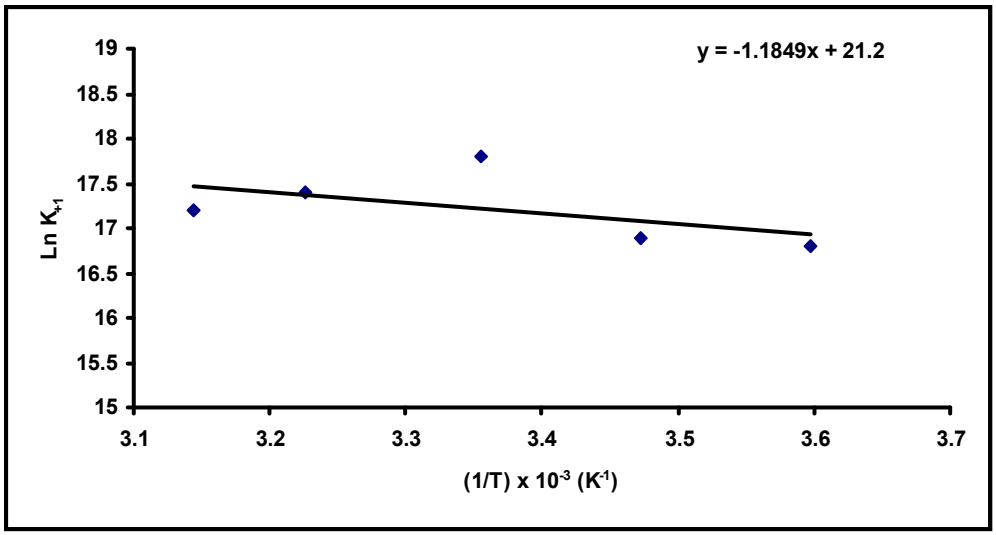

Figure (8): Arrhenius plot for the binding of ^{125}I-anti CA15-3 to the partially purified CA15-3 in benign breast tumor (Fibroadnoma).

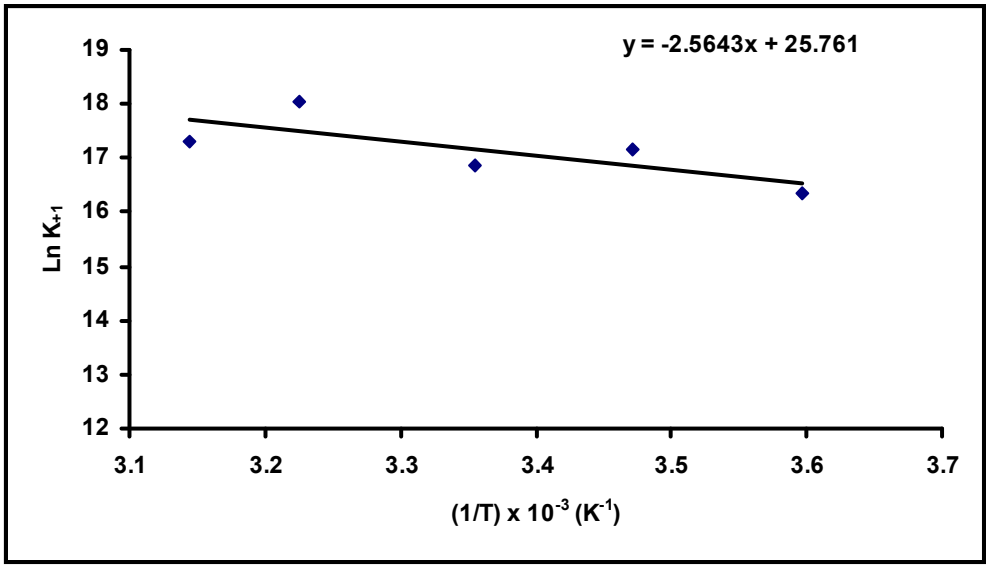

Figure (9): Arrhenius plot for the binding of ^{125}I-anti CA15-3 to the partially purified CA15-3 in malignant breast tumor (IDC).

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