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STUDY ON THE INTERACTION OF DODECYL TRIMETHYL AMMONIUM BROMIDE (DTAB) WITH ALCOHOL DEHYDROGENASE (ADG) ENZYME

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ABSTRACT

We can calculate the surfactant concentration on interaction with enzyme by UV-VIS method, also we can construct and implement ion selective electrode, which is sensitive to surfactant. In this research stability and thermodynamic properties of enzyme have been investigated. In order to understand the quality of enzyme stability, study of complex is necessary. In this research, at first, ion selective membranous electrode was constructed, so an electrochemical cell was designed to attain the potentiometer data of binding of surfactant to alcohol dehydrogenase enzyme (ADG). Potentiometer reply was used to attain binding isotherms of binding of surfactant to ADG with doing essential calculations. Using calculated Gibb's free energy change of binding (ΔGv), we can discuss about binding thermodynamic of these materials to ADG. Investigation the effect of the environmental conditions change, such as pH, ionic strength, enzyme concentration and presence of chemical denaturant of urea on the binding process of surfactants to ADG are important purposes of this research. At the end we analyzed the ADG structure considering the number of site sets, sites affinity and the number of binding places in each site in experimental conditions, using binding data and calculating the Wyman binding potential (Δ), binding valence (Δ) and Scatchard plots. Finally we could interpret the results and investigate the effect of DTAB on ADG enzyme by UV-VIS spectrometry technique.

Keywords: Ion Selective Membranous Electrode, Alcohol Dehydrogenase (ADG) Enzyme, Dodecyl Trimethyl Ammonium Bromide (DTAB)

1. INTRODUCTION

Interaction of surfactants with proteins has been investigated vastly in recent decades [Bordbar et al., 2003; 2004]. Conformation and regular structure of protein that is a polyamino acid can be destroyed through interaction with some surfactants [Hall et al., 1981; Wright and Thompson, 1975]. This issue is important because the action of each protein in creatures' body is influenced by its special structure. Enzymes are organic catalysts, which are made by live creatures and activate a lot of chemical reactions in organism. Enzyme activity is influenced by different factors such as pH, temperature, ionic strength, chemical nature of buffer, concentration of substrate and etc [Figgeet al.1986; Makino et al., 1973].

Our purposes in this research are at first construction of ion selective membranous electrode of surfactant. In this regard an electrochemical cell is designed to attain the potentiometery data of binding of surfactant to ADG enzyme. Potentiometer reply is used to attain the binding isotherms. Using calculated amounts of Gibb's free energy change of binding (ΔG_V), we will be able to discuss about thermodynamic of binding.

Investigation of environmental conditions change such as pH, ion strength, enzyme concentration and presence of urea as a chemical denaturant on binding process are important purposes of this research. Finally using binding data and calculating the Wyman binding potential (π), binding valence (θ) and constructing the Scatchard plots we analyze the ADG structure in order to determine the number of site sets, affinity of each site and the number of bound places in each site in experimental conditions. And by UV-VIS spectrometry technique we can interpret the results and investigate the effect of DTAB on the ADG enzyme.

2. Experimental

Materials

(ADG) enzyme, nicotine amide denucleotide triphosphate, carboxylated polyvinyl chloride (PVC) with high molecular mass, (DTAB), (THF) solvent, acetone, nitric acid, chloridric acid, sodium bromide, pentaoxidephosphorus, ethanol, sodium hydroxide and urea were obtained from Merck. Deoctyle phthalate (DOP) was obtained from Aldrich. Silver wire and reference electrode of sodium was obtained from Metrohm Company.

Equipments

a) Potentiometer and pH-meter

All potentiometery and pH-metry determinations were carried out on

ΩMetrohm-744 pH-meter and potentiometer. Because of electrode sensitivity to temperature, all experiments were done under the temperature controlling of apparatus.

UV-VIS spectroscopy

b) The absorption of interaction of ADG with DTAB was recorded on a carry-100 UV-VIS spectroscopy.

c) Heater-stirrer

HT-202 Heater-stirrer was used to homogenize the solutions.

METHODOLOGY

Preparing the membrane and selective electrode of surfactant

In order to obtain a suitable membrane for making the selective electrodes, that act reversible for cationic surfactant ions of DTAB we used carboxylated (PVC) with high molecular mass. 0.5gr PVC was dissolved in 20 ml (THF), this solution was added drop wise to 50 ml surfactant solution (3mM), and was stirred calmly to attain a fibrous white precipitate, that was filtered and washed with distilled water. Then we put it on a watch glass and transferred it into a desiccators containing, $P_2O_5$for 24 hours to be desiccated completely.

In order to prepare plasticizer solution, 0.18gr (DOP) was dissolved in 3-4 ml (THF) solvent. 0.12gr of desiccated membrane was added to (DOP) solution; it took 4 to 6 hours to attain a limpid and homogenized gel, in effect of vaporizing the (THF). In order to obtain a complete smoothness on the surface of glass tubes we used emery and then they were washed and dried for binding the membrane to them. In order to prevent the air current interference and smoothing the basic layer thickness of membrane, we closed the tube mouth with forefinger and then put it into the membrane gel. After emitting, we put them vertically exposed to the air for at least 12 hours.

Coating the surface of silver wire

The surface of silver wire should be coated by precipitate of silver bromide. We used a saturated solution of sodium bromide and a dilute solution of nitric acid.

At first we cleaned the surface of silver wire by emery and washed it with water and ethanol. Then 3 to 4 cm of this wire was entered into the nitric acid solution. Surface of silver wire was oxidized in a short time less than 1 minute, so a thin layer of Ag^+ ions were formed on the wire surface that compose with bromide ions after transferring to the saturated solution of sodium bromide and precipitated again on the silver wire surface.

Conditioning solutions

This solution is 1mM related to the surfactant and 0.1mM related to the NaBr. The prepared glass electrode in previous stage was put inside this solution for preparing the membrane. The membrane was placed exposed to the solution from both inner and outer part. It took 24 hours to prepare the membrane surface of electrode. After these stages we could use the surfactant electrode for basic determination with entering a coated silver wire into the standard solution inside the tube.

Determination method

All potentiometery experiments were carried out using a 10 ml beaker as determination cell. Initial tests were done on electrode. 5ml buffer of NaBr (10-4M) was entered to the cell and ion selective electrode of surfactant was placed in solution next to a reference electrode of sodium. Connective wires of electrode were connected to the potentiometer (to show potential difference between two electrodes).

Equal volumes of 10ml surfactant were added to the test cells by micropipette, and potential difference was recorded. Finally we constructed amounts of obtained potential difference versus logarithm of surfactant concentration. Linearity of curves indicates the correctness of electrode reply. After confidence of correct reply we did experiment with a specified concentration of ADG in presence of NaBr (10-5M) and in different conditions about pH and ionic strength but using previous method.

Investigating the effect of ADG concentration on the surfactant binding

We chose concentrations of 0.5, 1.1, 3.2 and 41mg/ml of ADG, pH=6.5, NaBr (0.1mM), and about each surfactant, we used its special ion selective electrode.

Investigating the effect of pH on the interaction of surfactant with ADG

From previous section we concluded that concentration of 1mg/ml is the best concentration for quantitative experiments. In this section, experiments were carried out at pH=6.5 and 9.5. In order to adjust the pH, we used NaOH solution (0.5M).

Investigating the effect of ionic strength on the binding of surfactant to ADG

In this regard solutions of ADG enzyme with NaBr concentrations of 10^{-5}, 10^{-4}, $10^{-3}$and 10^{-2} M were prepared. All experiments were done at pH=6.5.

Investigating the effect of chemical denaturant

Urea is one of the important denaturants of proteins. Urea and hydrochloride guanidine cause to unfolding the protein through

hydrogen bond, which is stronger than water-protein binding. In other hand urea solution is not stable so decomposes to ammonium and cyanine ions. Urea solution should be freshly prepared and used because of interaction between cyanine ions and ADG enzyme.

Measuring the concentration of enzyme solution

For measuring the concentration of enzyme stock solution we used absorption coefficient based on its weight percentage at 270nm. Calculated molar absorption coefficient was about 12.6. The solution of enzyme (1mg/ml) was prepared and it's absorption at 270nm was recorded and then absolute concentration of enzyme solution was determined using Beer rule.

Determination of the thermal denaturation plots of enzyme

For determining the thermal denaturation plots, we used determined absorption amount of protein solution at 270nm as a function of temperature. In this regard at first ,200µl of enzyme stock solution (1mg/ml) was placed in a spectrophotometer determination cell, then appropriate amounts of DTAB 0.020 stock solution and buffer were added to it based on determination conditions. These amounts were placed in reference cell, but instead of 200µl of enzyme stock solution, 200µl buffer was added to it, then put them in spectrophotometer and the thermal probes were installed on them.

Thermal denaturation experiment was carried out using related software. Temperature range was 20 to 90°C and measurement rate was 0.50°C/min. Finally, amount of absorption difference at 270nm with respect to reference solution was recorded based on a function of temperature. Amount of absorption coefficient in each temperature was calculated through division of absorption to enzyme concentration. The plot of variations of enzyme absorption coefficient at 270nm versus T is thermal denaturation normalized plot of enzyme that can be determined in each condition. Considering the molecular mass of 141000 Dalton for enzyme, we can calculate the molar absorption coefficient and construct it versus temperature. Denaturation plots were recorded at concentrations of 0.013, 0.014, 0.015, 0.016 and 0.017 M.

3. RESULTS AND DISCUSSION

Designed electrochemical cell for determining the surfactant concentration, contains a reference sodium electrode and an ion selective electrode sensitive to surfactant. A special volume of buffer solution consists of NaBr (10-4M) and protein (1mg/ml) is used. After turning the potentiometer on, absolute

volumes of surfactant are added gradually and potential difference is recorded. The obtained information will be investigated using Excel software. The plot of potential difference versus logarithm of surfactant concentration shows that in starting point, that binding process has not been occurred, potentiometer reply is nearly independent of protein presence. Relation of potential to surfactant concentration is expressed by equation stated as below:

$$Emf = E° + m \log[s]f$$

Where Emf is, obtained potential from potentiometer, E° is intercept of plot in initial part and m is slope, which is attained between 7 to 61mv, and is listed in Table 1. Concentration of free surfactant is calculated using equation mentioned above. We can determine the number of bound surfactant moles to enzyme from difference of total and free surfactant concentration. Then we can attain the proportion of overage bound surfactant moles to total existent enzyme moles, and calculate the binding potential, appearance binding constant and molar Gibb's free energy change from binding isotherms plot.

Calibration plot of potentiometer reply

The plot of potential difference variation versus log [s]f shows three distinct regions that are shown in Fig.1. Initial part of plot is a straight line with nurnstian slope, indicates that in very low concentration of surfactant, binding has not been started. This part is used as standard reply and obtained equation will be basic reply of electrode for next parts. The middle part is the start point of binding process and forming the surfactant-protein complex. The end part is the sign of approaching to the critical micelle concentration (CMC) region, so with increasing the monomer concentration in solution and aggregation incidence, actually reduction of concentration in solution or reduction of potential difference will be observed.

Investigation of binding isotherms plots and comparison of results

Fig.2 shows the binding isotherms plot of interaction between DTAB and ADG enzyme at different concentration of protein. It seems that these curves in the limit of measurement uncertainty conform on each other in concentrations of 1 and 2 mg/ml, and in higher concentration, curves show relatively high difference. This manner is due to the ADG enzyme phenomenon in higher concentration, in fact aggregation increases upon increasing the concentration.

Plot shows that with ADG enzyme aggregation, binding of surfactant to ADG (Vapp) decreases, so we can claim that in higher concentration of ADG, resistance of ADG to DTAB increases because of ADG aggregation. Based on results, concentration of 1mg/mL of ADG is the most suitable concentration; because it is the highest concentration that aggregation phenomenon has not been occurred.

Effect of pH on the interaction of DTAB with ADG

Negative charge density on ADG enzyme increases upon increasing the pH, so interaction of the cationic surfactant with DG increases. Shifting of binding isotherms to fewer concentrations at higher pH indicates that electrostatic effects increase upon increasing the pH. These results confirm the results obtained from previous investigations about ADG structure.

Effect of ion strength on the interaction of DTAB with ADG

Fig.3 shows ΔG_V `variations for binding of DTAB to ADG enzyme versus log [s]f, at different ionic strength. At first interaction of DTAB with ADG decreases upon increasing the ionic strength from10-4 to 10-3 M, but hydrophobic forces show more strong role in interaction at higher ionic strengths.

Effect of chemical denaturant of urea

Binding isotherms are placed in higher states in absence of urea compare to binding isotherms with urea concentration of 3M. In these states urea concentration isn't enough for denaturizing the ADG, so cause to decrease hydrophobic interactions and decreases the binding of DTAB to ADG. Slight difference and shifting are due to the hydrophobic tails difference of DTAB, so interactions of DTAB are more predominant because of longer hydrophobic tail of DTAB. So curves appear at fewer concentrations.

Overlapping of binding isotherms at concentrations higher than 5M indicates denaturizing of ADG enzyme in this range of urea concentration. Binding isotherms have been shifted towards the fewer concentrations because of unfolding of ADG enzyme and destruction of its compact structure and increasing the connection surface and Probability of connecting of surfactant to binding sites of ADG enzyme.

Fig.4 shows the Scatchard plot for interaction of DTAB with ADG at various concentration of ADG enzyme, unusual state in all concentration of ADG is observed except in concentration of 3 mg/ml, which is defined for state of one site set with positive cooperation.

States of simple two site sets

Fig.5 shows variations of binding valence for interaction of DTAB with ADG at pH=9.5. It indicates that ADG is a two site set structure with specified values of coefficients and defined sites, which are listed in **Table 2**. Obtained values of nH for first and second site sets show that affinity of first site set is much more than second site set and this is a reason for complete separation of two site set. In this case Scatchard plot didn't show difference between pH=9.5 and 6.5, so we can compare preference of using the binding data with Scatchard method.

UV-VIS spectra changes of interaction between DTAB and ADG

UV-VIS spectrum of ADG has a maximum at 270nm. Absorption in this wavelength is related to tryptophan, phenylalanine and tyrosine groups, which are inside the protein. Absorption changes in this wavelength are related to the polarization change around these groups due to the interaction with DTAB. Binding of DTAB ions to groups of aspirate and glutamate of ADG at first disarrays the charge balance on the protein surface and cause to change ADG conformation. The plot of A270nm changes versus DTAB concentration shows a sigmoid process, which indicates cooperative unfolding of protein in effect of denaturizing by DTAB. These results indicate high strength of DTAB for ADG denaturation. Process of changes at various wavelengths is shown in Fig.6 and total processes of changes of all natural regions are shown in Fig.7 and 8.

Table 1: Characteristics of prepared DTAB electrode

	Surfactant	[Pr],mg/ml	pH	[NaBr] 10-5M	[urea], M	Slope	R^2
1	DTAB	1	6.5	1	0	58.9	1
2		2	6.5	1	0	60.4	0.998
3		3	6.5	1	0	58.3	0.998
4		1	95	1	0	58.2	0.999
5		1	6.5	1	1	60.4	0.996
6		1	6.5	1	3	60.3	0.997
7		1	6.5	1	5	58.3	0.996
8		1	6.5	1	7	59.4	0.997
9		1	6.5	1	9	58.3	0.994
10		1	6.5	1	0	58.3	0.999
11		1	6.5	10	0	58.3	0.998
12		1	6.5	100	0	57.3	0.999
13		1	6.5	1000	0	57.8	0.996

Table 2: Structural analysis of ADG enzyme using binding valence plots

Experiment conditions	g_1	nH_1	g_2	nH_2
pH=9.5, DTAB	27.15	4.42	437	1.66
9M urea, DTAB	63.3	8.8	369	2.2
10-4M(NaBr), DTAB	49	3.81	189	1.25
PH=6.5, DTAB	70	1.7	340	1.72
10-3M(NaBr), DTAB	45	2.4	320	1.3

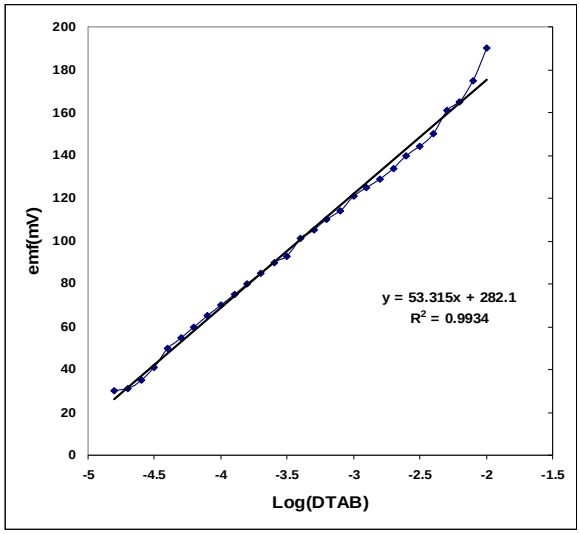

Fig.1. The plot of emf versus log [DTAB] at 25°C, pH=6.5, 1 mg/ml ADG enzyme and 0.1mM NaBr

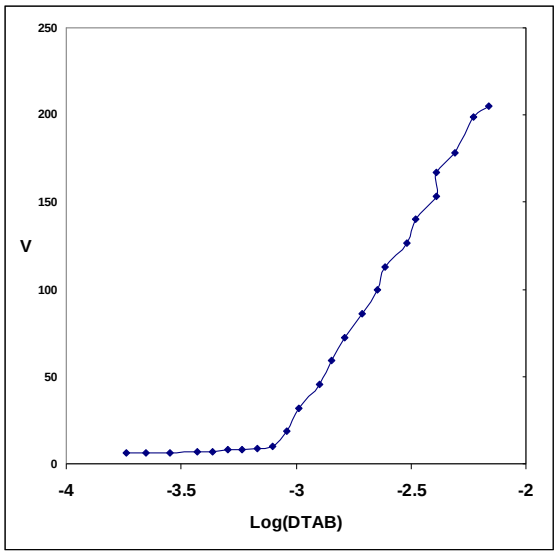

Fig.2. Binding isotherm plot of interaction of DTAB with ADG enzyme at 25°C, pH=6.5 and 0.1mM NaBr in various concentrations of DTAB

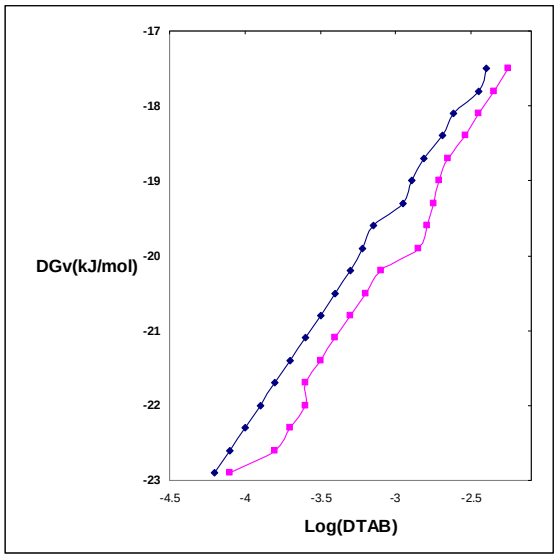

Fig.3. Variations of molar Gibb's free energy versus log [DTAB] in various concentration of NaBr at 25°C, pH=6.5

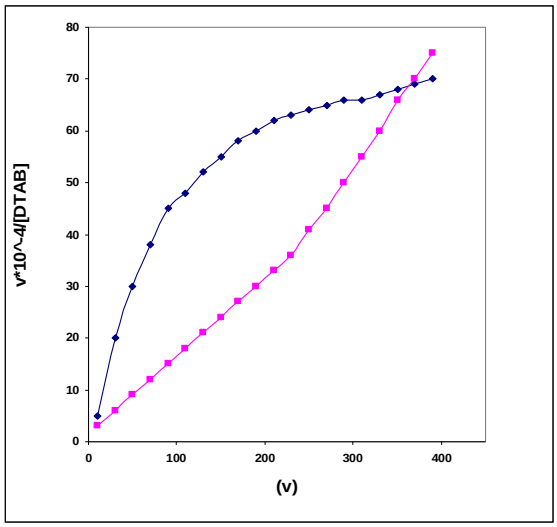

Fig.4. The Scatchard plot of interaction of DTAB with ADG enzyme at 25°C, pH=6.5 and 0.1mM NaBr

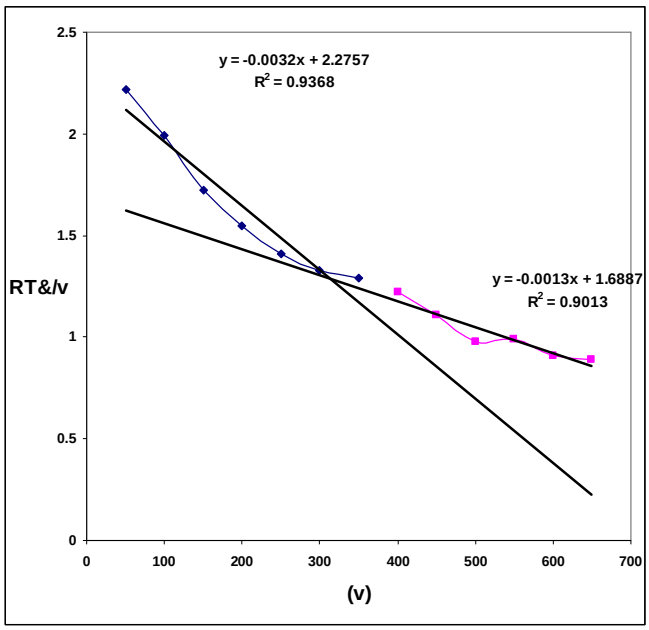

Fig.5. Variations of binding valence (θ) in interaction of DTAB with ADG at 25°C and pH=9.5

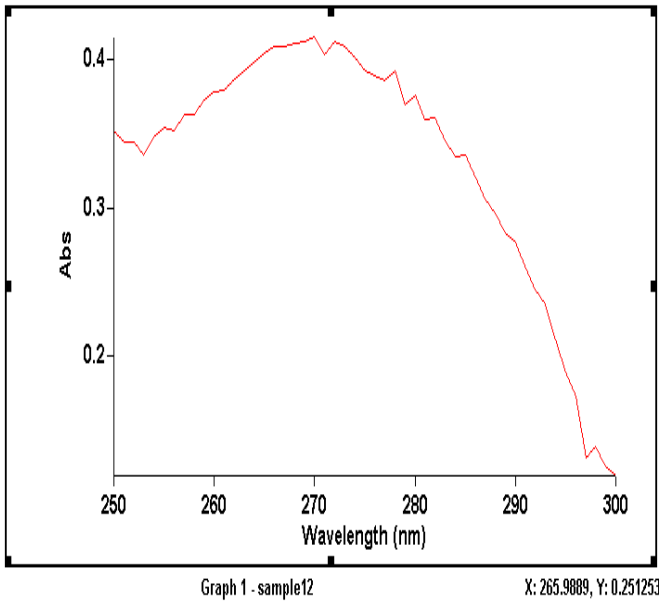

Fig.6. Absorption spectrum of ADG enzyme at 25°C, pH=6.5 and 0.1mM NaBr (maximum absorption is in 270nm)

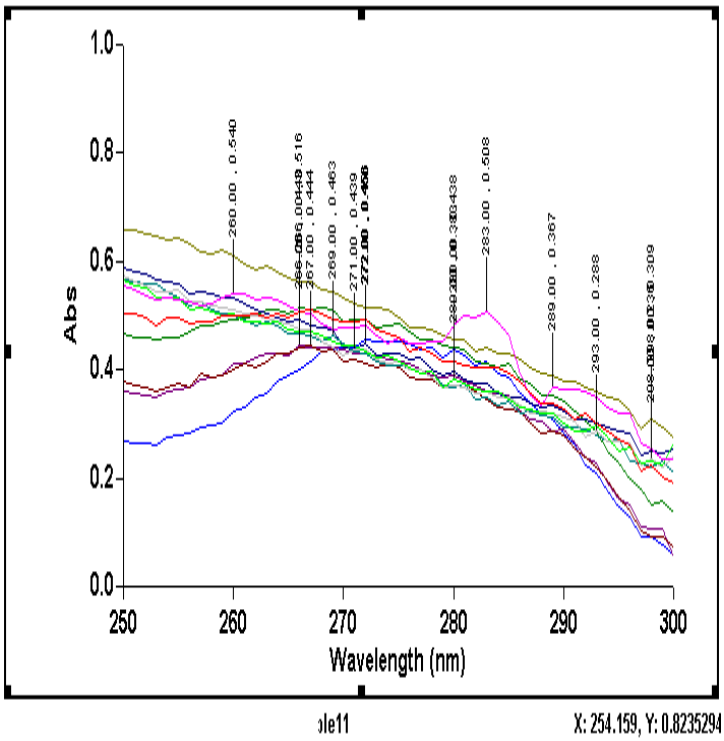

Fig.7. Absorption spectrum of interaction of DTAB with ADG enzyme in various concentrations of DTAB at 25°C, pH=6.5 and 0.1mM NaBr

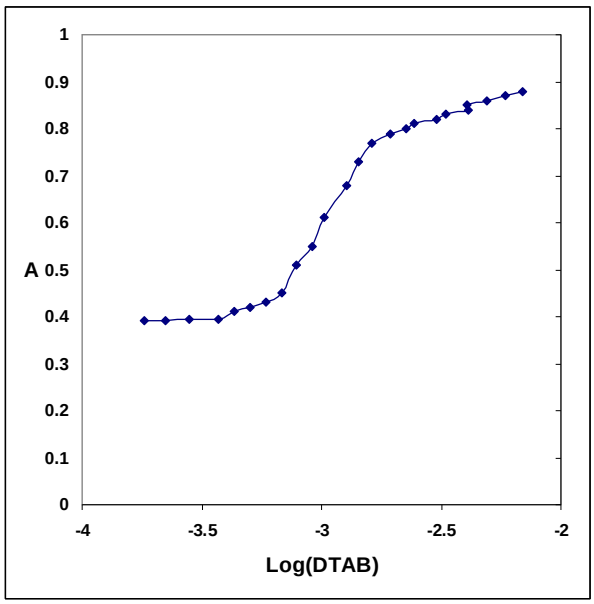

Fig.8. The plot of UV-VIS absorption of interaction of DTAB with ADG enzyme versus log[DTAB] at 25°C, pH=6.5 and 0.1mM NaBr

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