Chemistry and Materials Research
ISSN 2224- 3224 (Print) ISSN 2225- 0956 (Online)
Vol.8 No.9, 2016

The Effect of NADH(H+) in the Oxidization of Benzidine in the H_2O_2 / Horseradish Peroxidase System

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Abstract
The aromatic amines metabolized under the effects the enzymes by different path way and the peroxidase path is one of these methods, in Which aromatic amines changed from non-active into active form, Which can interact with the larges biological molecules as nucleic acids DNA and inhibited it . Intra cellular components performed multi-functional such as Glutathione G-SH , NADH(H^+) and NADP. It was chosen because of the high damper NADH concentration in the cell (10-3M)) and its spread in all types of tissues.NADH(H^+) inhibit large number of products of oxidation reactions of the amino compounds enzymatically carcinogens, such as substrate benzidine and its derivatives such as 3,3',5,5' tetra methyl benzidine in Competitive path way.NADH(H^+): Nicotinamide adenine dinucleotide(reduced form)
Keywords: Benzidine, , Peroxidase, inhibition , Nicotinamide adenine dinucleotide

1. Introduction

Enzymes Contribute to activate aromatic amines (AA), including substance benzidine according to the different Figure (1), and is the result of the oxidation of aromatic amines constitute the electrophilic compounds (an electrons passionate) carcinogenic because they interact easily with the metabolic nucleophilic outputs in a living cell (Figure 2) [1]. The latter comprising nucleophilic vehicles large group of compounds each of which has its own chemical structure, and differ mutually exclusive in terms of function performed, and there nucleophilic compounds in cells with different concentrations.

Benzidine (BD) is one of aromatic compounds(AA), which is oxidized in two phases.

At each phase of the loss of an electron and a turn as a result of this amino group turned into azo methane group and turn it benzidine material to the active form, which can interact with DNA (Figure 3) [6].

The living cells containing many anti-oxidants such as phenols, and a number of steroidal hormones and organic acids (citric acid, ascorbic acid), and a range of antibiotics, and glutathione compound NADH (H^+) [8, 9].

Nicotine amide adenine nucleotide dual-NAD $^+$:
A chemical compound found in all living cells and plays a role coenzyme composite molecular structure $C_{21}H_{27}N_7O_{14}P_2$(663.44 g / mol), and dissolved in water, and consists of two units of oligonucleotide: the first contains a loop adenosine and contains a second amide nicotine, linked through a set phosphate. Have a nitrogen atom in the ring nicotine amide additional positive charge. Was chosen damper NADH (H +) due to its spread in all types of tissues and high concentration in the cell (10-3M)) [8].
The figure (4) shows chemical structure of the compound NADH (H^+).

Peroxidase enzyme (EC1.11.1.7):
It is one of oxidation and reduction enzymes (Oxidoradoctaz), are widely spread in plants (Horseradish, mustard, figs, smoke leaves ..), were obtained enzyme is crystalline, it has a molecular weight of 44,000 and the hematin represents 1.84% of weight.

It is the properties of reaction the enzyme is its ability to bind with hydro peroxide to composition of an intermitted compound is characterized by the properties of different spectral and discovered four types of complexes, and found to be the previous four complexes regard to the concentration of hydro peroxide, it was noted that two complexes III and IV formed in the presence of increase of hydro peroxide, and have no catalytic activity, and leads formation to stop the activity of the enzyme, Figure (5) shows of peroxidase enzyme reaction mechanism with of S1 S2 and [4].

Complex I consists directly sell add hydro peroxide a complex weak green and soon changes to complex II red color, illustrated in Figure (6) peroxidase enzyme reaction with hydro peroxide mechanism. It can peroxidase enzyme interaction stimulates the oxidation of materials using molecular oxygen in the absence of H_2O_2, and the act oxide of enzyme peroxidase are working under air conditions. It is supposed to oxide function and peroxidase of enzyme peroxidase performed the same reaction center of the enzyme [10].

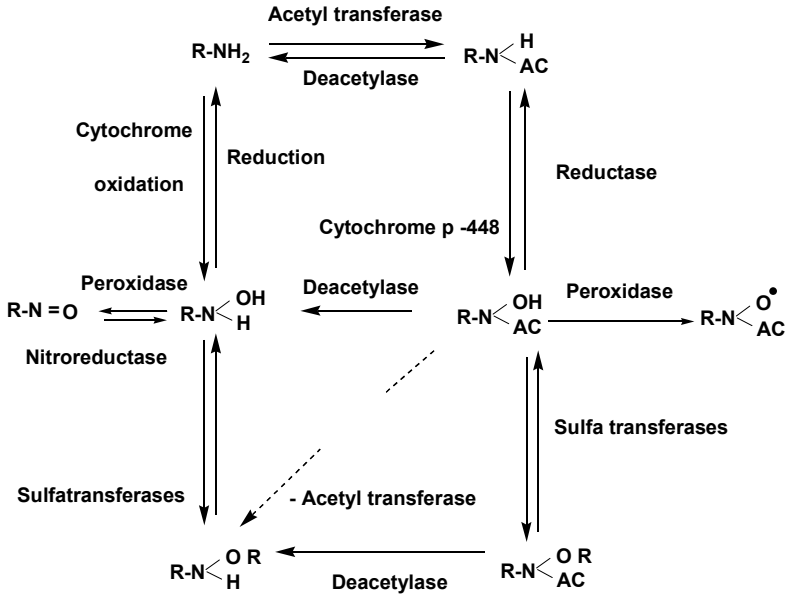


Figure 1: Methods of transformation and revitalization of aromatic amines by enzymes

Figure (1) Shows the aromatic amino compounds transformation when entering the living body through the mouth (food intake or medications) or through the nose (breathing) or through the skin (as hair dye) under the influence of enzymes into other compounds are carcinogenic such as aromatic amines shift to carcinogenic azo compounds [6].

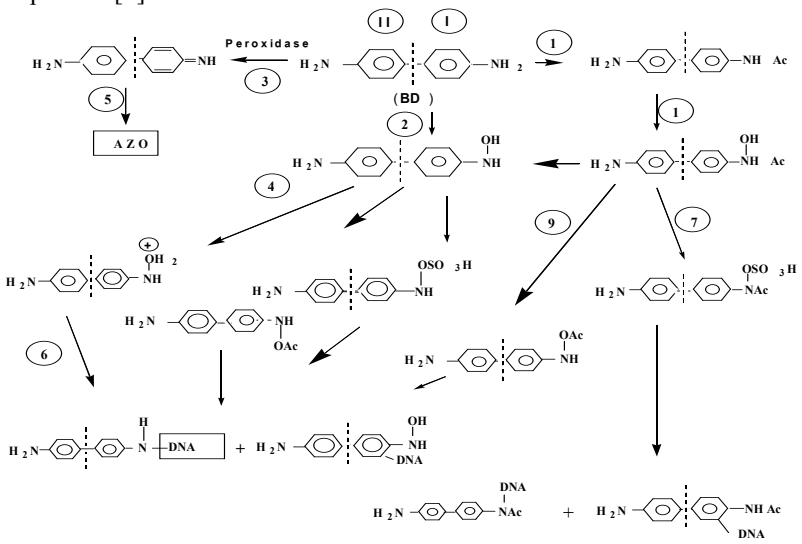


Figure 2: Methods of metabolism of aromatic amines

The figure (2) shows ways metabolism of various items of benzidine, which indicates the number (1) in Fig.

The interaction (2) of acylation atom of nitrogen and then enter a group hydroxyl, or enter clique hydroxyl atom nitrogen directly figure (2) and can last for a compound that protonation (H^+) and turns into a electrophilic compound doting electrons (4), which can easily interact with DNA disincentive his movement in the transfer of genetic traits from parents to children.

Of the Ways of metabolism benzidine the peroxidase road (3) which amino group turn NH_2 - related to the episode aromatic Aimneh clique (NH =), mutant later to carcinogenic azo compounds [7].

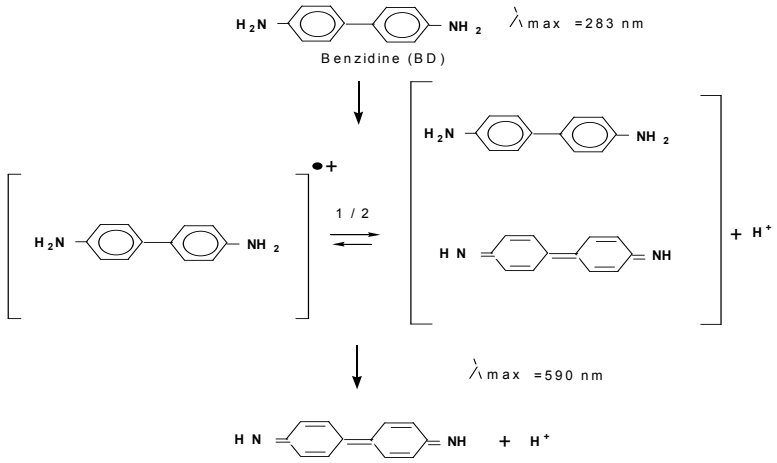


Figure 3: The Different metabolic forms of benzidine مادة

The figure (3) shows the method of metabolism of benzidine according the beroxidase way in which give up two electrons of the reactive material (-2e), and the transformation of reactive material to form active through amino group (-NH_2) turned into emine group(= NH).

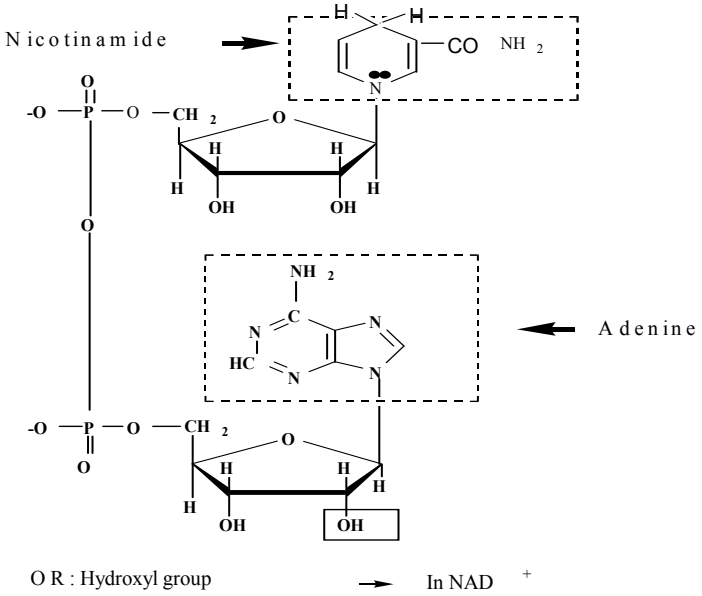


Figure 4: Chemical structure for NADH (the reduction form)

The effect of the enzyme mechanism scheme with H_2O_2 and peroxidase (AA), which put the researcher 's Chance [4].

$$E + S_1 \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} E_1 + H_2O \quad (1)$$

$$E_1 + S_2 \xrightarrow{K_2} E_2 + \tilde{P} \quad (2)$$

$$E_2 + S_2 \xrightarrow{K_3} E + \tilde{P} \quad (3)$$

$$2\,\tilde{P} \xrightarrow{K_4} P \quad (4)$$

Figure 5: The effect mechanism of the enzyme peroxidase EC (1.111.7)

S1: hydro peroxide , E: enzyme, K: the fast constant of interaction
S2: the reactive material is donor of hydrogen, P: the reaction product
E1: a compound average represents the first complex.
E2: average vehicle represents the second complex.

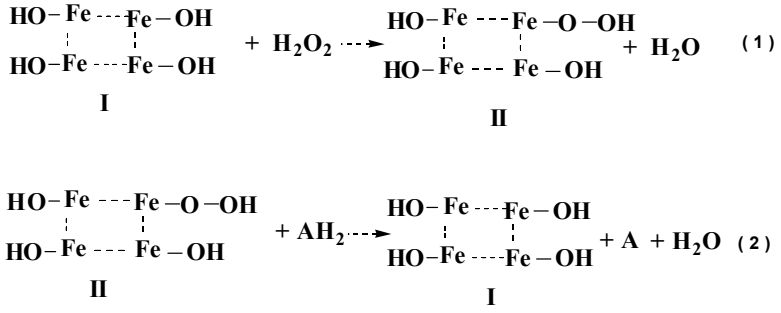


Figure 6: the reaction mechanism of peroxidase enzyme with hydro peroxide

The research aims to: study the kinetics of enzyme reaction peroxidase presence of reactive substance benzidine and H_2O_2 oxidized material which has been studied:

- the effect of pH in the fast reaction of enzymatic.
- the effect of the concentration of reactive materiale benzidine [BD] in the fast of enzymatic interaction.
- Identification of damping through the curves of Lineweaver-Burk.
- Find the kinetic constants in the absence and presence of inhibitors.
- Find the concentration of the inhibitor that lead to the reduction of the fast of enzymatic interaction to 50%.
- Find a constant inhibiting compound NADH (H^+).

2. Experimental section

2.1. Materials and Methods:

UV/Vis spectroscopy (model: Hitachi U-1900), the apparatus optical density
Peroxidase from Horse Radish, Ortho phosphoric acid, citric acid, sodium hydroxide , glycerol, phosphate citrate and solvents / Merck/.

2.2. Preparation of the solution phosphate - citrate:

We dissolve in a flask containing 0.5 L distilled water following materials:
3.2 L Ortho phosphoric acid, 11.9 g citric acid, 3.54g fallow acid, adds to the previous mix 343 ml of sodium hydroxide solution 0.1 N, and attend the previous solution different solutions of PH.
The solution used is a solution of citrate phosphate (5.5 pH).
The source of the peroxidase enzyme is from wild Horse Radish

2.3.Calculate the concentration of the enzyme solution peroxidase:

Using UV device, the concentration of the enzyme peroxidase is calculated by taking 2 ml of the enzyme peroxidase, added to 2 ml glycerin concentration of 25%, in a bowl containing glycerin, was saved enzyme solution refrigerator temperature (10-15ºC) below zero, and it has admeasured optical density Δ D.
We has been the focus of an enzyme peroxidase account when the wavelength 403nm using a molar absorption coefficient of 109000 M^{-1}. cm^{-1}, and the following relationship:
Peroxidase] = Δ D / ξ L [
Δ D: Scouts optical, L: display glass cell to a UV
ξ: absorption coefficient
We save the enzyme solution in a refrigerator fridge temperature (-15 °C) .
volume of the reaction mixture 2ml in the presence of inhibitors and absence it includes the following materials:
A. In the absence of inhibitors: reactive substance, an enzyme, the solution citrate phosphate, H_2O_2 .
B. In the presence of inhibitors: reactive substance, an enzyme, inhibitor, the solution citrate phosphate, H_2O_2.
The tubes of the reactants mixture have put in the thermostat at 30º C.
It is expressed of the speed of the speed of interaction form the oxidation products.
V = + d C / d A, where A: the material compound, C: the resulting compound
The speed of calculation constitute the outputs of the oxidation of the compound BD presence of peroxidase enzyme using the following table(1):

table(1)

The oxidation result		The amino compound		The interact material
E.10-4 $M^{-1}.cm^{-1}$	λ_{max}	E.10-4 $M^{-1}.cm^{-1}$	λ_{max}	
0.31	590	2.10	283	BD

3.Result and discussion:

3.1. the effect of various factors on the speed of enzymatic reaction

3.1.1. the effect of PH on the effectiveness of the oxidation of benzidine (BD).

All experiments were conducted at the class 30˚C, and through the amount of two minutes.
Shown in Figure (7) the effect of pH on the effectiveness of the enzymatic reaction.
In this experiment was to determine the optimum PH value of the work of the enzyme peroxidase, which amounted to 5.5 value.

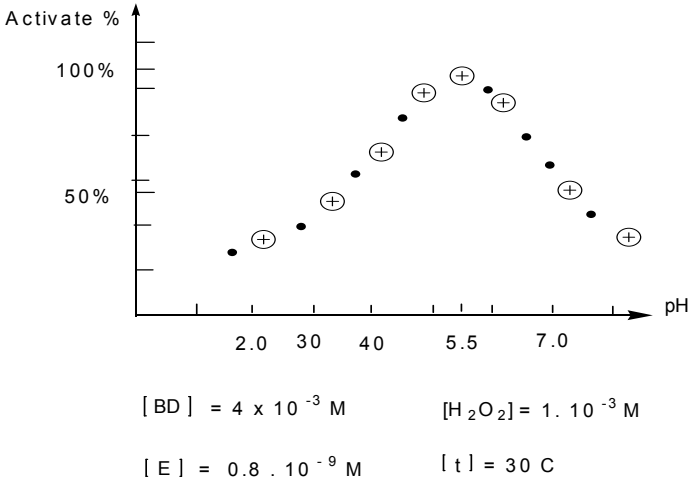


Figure (7): The Effect of PH at the speed of oxidation of benzidine.

3.1.2. The effect of different concentrations of NADH (H^+) at the speed of interaction Enzyme in inverted coordinates.

Deduce from the figure(8) Next:

- The compound NADH (H^+) inhibits oxidation reaction wholesale benzidine hydro peroxide / peroxidase enzyme in a competitive manner.
- The great speed of enzymatic reaction value $1.31x10^{-7}$ MS^{-1}
- The value of the Makaels constant K_m with a reactive material benzidine 1.4×10^{-4} mol/l , and in the case not to use any inhibitor.
  In terms of a constant value Makaels in presence of the inhibitor NADH (H^+) of the following values:
  mol / L mol / L, 5.5 X 10^{-4} 2.3 X 10^{-4} ، mol / L 3.3 X 10^{-4} ، mol / L 4.5 X 10^{-4}

when using the concentrations of the inhibitor following:
0.5X10^{-5}, 0.75 X 10-^{4}, 1 X 10^{-4}, 1.5 X 10^{-4} mol / L, This means that a Makaels constant value in presence of the inhibitor NADH (H^+) increased.
And a stimulus constant was value 1.37 x 10^{+2} S^{-1}.
The account of concentration of enzyme E by using 2 ml of the solution:

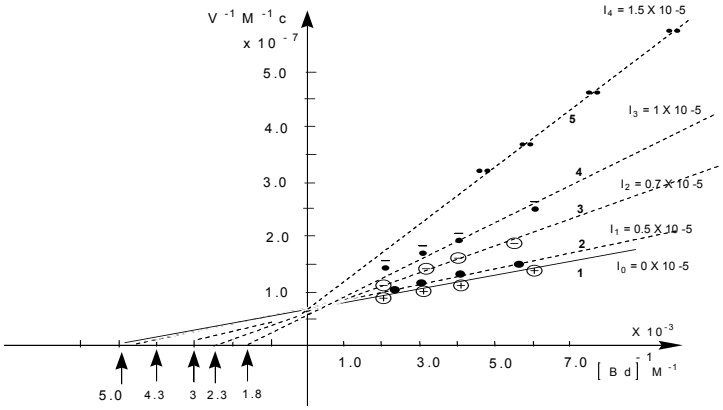
$$V_1 N_1 = V_2 N_2 \Rightarrow 0.04 \text{ X } 0.8 \text{ X } 10^{-9} = 2 \text{ X } N_2 \Longrightarrow$$

$$N_2 = 0.016 \text{ x } 10^{-9} \Longrightarrow N_2 = 1{,}6 \text{ x } 10^{-11} \text{ Eq/ liter}$$

$$K_{kat} = \frac{V_{max}}{[E]} = \frac{1.37 \text{ X } 10^{-7}}{1.6 \text{ X } 10^{-11}} = 0.85 \text{ X } 10^{+4} \text{ S}^{-1}$$

$$K_{kat} / K_m = 0.98 \text{ x } 10^{+6}$$

$$\frac{K_{kat}}{K_M} = \frac{0.85 \times 10^{+4}}{1.4 \times 10^{-4}} = 0.61 \times 10^{+8}\ M^{-1}\ S^{-1}$$



Form (8): the effect of different concentrations of NADP(H^+) at the fast of interaction Enzyme in inverted coordinates.

1 - NADH (H^+) = 0 X 10^{-5} M

2 - NADH (H^+) = 0.5 X 10^{-5} M

3 - NADH (H^+) = 0.75 X 10^{-5} M

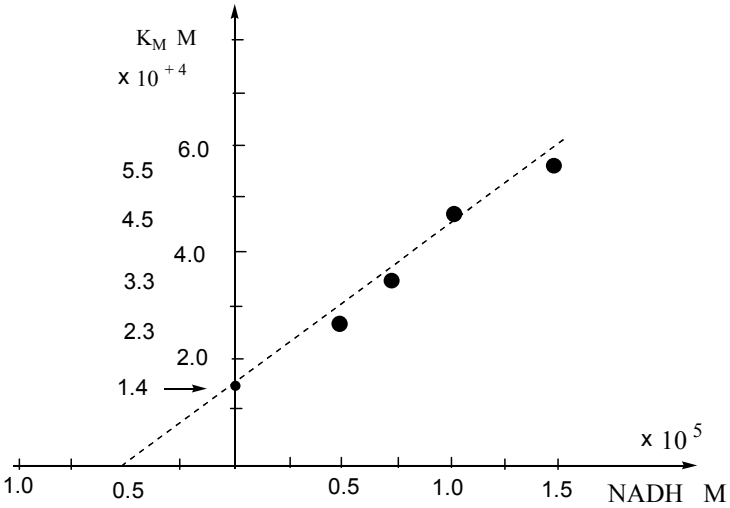
4 - NADH (H^+) = 1 X 10^{-5} M

5 - NADH (H^+) = 1.5 X 10^{-5} M

3.1.3.A constant inhibition damper NADH (H^+) the existence of benzidine (BD): The table (2) shows the value of fixed Makaels - Mintn in the absence of the inhibitor and values Different damping constants when increasing concentrations of Article inhibitory NADH (H^+).

Table (2)

[NADH (H^+)] M	The value of inhibitor constant
0 x 10^{-5}	1.4 x 10^{-4}
0.5 x 10^{-5}	2.3 x 10^{-4}
0.75 x 10^{-5}	3.3 x 10^{-4}
0.1 x 10^{-5}	4.5 x 10^{-4}
1.5 x 10^{-5}	5.5 x 10^{-4}

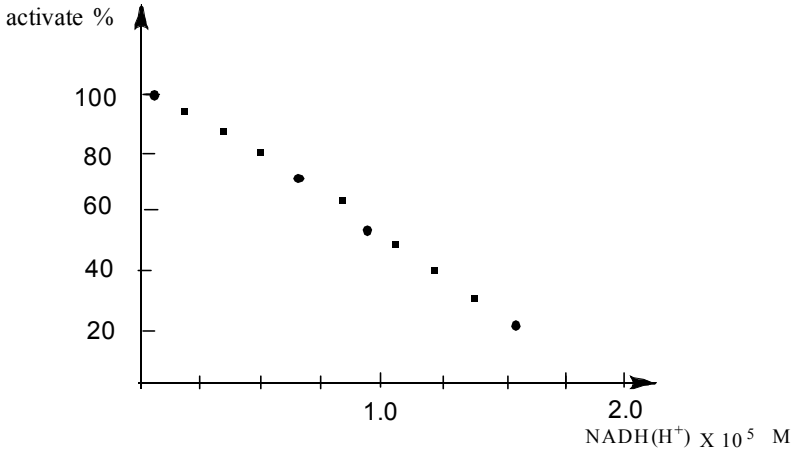


Form (9): a constant inhibition of NADH (H^+) the existence of benzidine

Table (3) a constant oxidation inhibition peroxidase of the reactive substance benzidine

K_i	Reactive material
NADH(H^+)	
5.0×10 6 M	بنزيدين(BD)

3.1.4.the effect of increasing concentration NADH (H^+) in reducing the effectiveness of the interaction enzymatic:



[BD] = 4X10^{-3} M $[H_2O_2]$ = 1X10^{-4} M

[E] = 0.8 X 10^{-9} M pH= 5.5 t = 30 C°

Form (10): the effect of increasing the concentration of NADH (H^+) to reduce the fast of enzymatic reaction.

Conclusions and suggestions:

In summary, we can be concluded from the above figures the following results:

1. The effective peroxidase enzyme extracted from the radishes is the very greatest in the acidic solution, reaching pH value ideal for the act of the enzyme with benzidine reactive material 5.5, as is evident from (Figure 7).
2. The compound NADH (H^+) inhibits oxidation reaction of benzidine in presence of peroxidase enzyme and hydro peroxide in a competitive manner (Figure 8).
3. The great fast amounted of interaction enzymatic value 1.31x10-7 MS^{-1} And it amounted to a constant value Makaels Km with a reactive substance benzidine 1.4 × 10^{-4} mol / L, and in the case not to use any inhibitor. The increased value of the constant presence of the inhibitor Makaels NADH (H^+) in the following amounts: 1.64, 2.35, 3.21, 3.92, and this shows that the affinity between the benzidine material reaction and enzyme peroxidase waned estimator almost four times.

Acknowledgment

The author would like to thank D Dr. Thanaa shriteh for her useful remarks.

4. Reference

[1].Seince E, F, and Knyazov P,.G , 1986-Molecular Oncologia Leningrad,Medicine p.349.
[2].Dixon, M, Webb, E .C. 1964- Enzymes . Longmas,London,2nd ed
[3].Bernhard,S . 1968- The Structure and Function of Enzymes. W.A Benjamin lnc ., New York
[4].Andre,V, A,1988- Peroxidase Enzyme. Science, MoscowP.127.
[5].Keleti T. 1990-Basic Enzyme Kinetics. MIR , Moscow , p.348.
[6].Belad F.A.,KadlubarF.F. Formation and persistence of arylamine DNA adducts in Vivo .Environmental Health perspectives.-1985.V.62 N1.P.19-30.
[7].Kennelly J.C.,Beland F.A.,Kadlubar F.F., Marting.C.N.Binding of N-acetyl benzidine and N,N'-diacetyl benzidine to hepatic DNA of rat and hamster in vivo and in vitro., Carcinogenesis.-1984,N,5, P.407-412.
[8].French I.S.,Coon M.I.Properties of NADPH-cytochrome p-450 reductase purified from rabbit liver microsomes . Arch . Biochem. Biophys.-1979.-V.195,N2. P.565-577.
[9].Enoch H.G., Stritmatter p. Cytochrome b-450 reductase .J.Biol. Chem. -1979. –V.254, N.18,P. 8976 -8981.
[10]. Mustafa, M., Ain Shams University, 2003, the oxidation and reduction enzymes, P.306.