



## Analytical and Theoretical Studies of Some Diazo Dyes

Hanan M. Ali<sup>1\*</sup>, Hasanain AS. A Majeed<sup>1</sup>, Alaa A Hussain<sup>1</sup>, Raghdan Hashim Mohsin<sup>2</sup>

<sup>1</sup> *Depart. of Chemistry, College of Education for Pure Sciences, University of Basrah/Iraq.*

<sup>2</sup> *Animal Production, College of Agriculture, University of Basrah/Iraq.*

\*Corresponding Author: Hanan M. Ali

### Abstract

Analytical studies were carried on diazo dyes that characterized 4,6-*bis*((*E*)-(3-nitrophenyl)diazenyl)benzene-1,3-diol (1), 4-((*E*)-(3-nitrophenyl)diazenyl)-6-((*E*)-(4-nitro phenyl)diazenyl)benzene -1,3-diol (2), 4-((*E*)-(2-nitrophenyl) diazenyl)-6-((*E*)-(3-nitro phenyl)diazenyl)benzene-1,3-diol (3) and 4,6-*bis*((*E*)-(2-nitrophenyl) diazenyl)benzene-1,3-diol (4). The results of the solvents effects and the pH effects were showed high solubility of these dyes in ethanol and the suitable pH values were found to be in the pH 12. Three isopiestic points were gained in the results of the azo dyes (2), (3) and (4). Add to which, the ionization ( $pK_a$ ) and the protonation ( $pK_b$ ) constants were calculated by using the half height method. The results were showed that the  $pK_{a1}$  values were absence in the results of diazo dyes (3) and (4). Theoretical studies also approved the structures of diazo dyes (1), (2), (3) and (4). These studies were focused in the relation between the structure and the properties of these molecules. Due to the internal coordinate mechanics (ICM), the conformational analysis, the molecular mechanics (MM2) and the optimal minimization were attended for each azo dye.

**Keywords:** *Azo dyes, Internal coordinates, Conformational analysis, Molecular mechanics, Ionization constant and Protonation constant.*

### Introduction

Azo dyes contain one or more azo groups ( $-N=N-$ ) which are linked to  $SP_2$  hybridized carbon atoms, based on the number of such groups [1] These compounds contain more than one active group, which is able to formulate chelatic coordination complexes with metal ions distinguished by their color and ability to dissolve in different solvents. [2]. Further, the azo is reactive compound, [3] that was reported for its pharmaceutical importance as anti-diabetic, [4] antineoplastic, [5] antibacterial, [3, 6] and anticancer agent [3]. Add to which, the azo molecules are known to be involved in the inhibition of DNA, RNA, carcinogenesis, and protein synthesis.

The presence of  $-N=N-$  in the molecular structure of azo is responsible for the interaction with the active site of protein. [7] Though, the synthetic 4,6-*bis*((*E*)-(3-nitrophenyl) diazenyl) benzene-1,3-diol (1), [6][2] 4-((*E*)-(3-nitro phenyl) diazenyl)-6-((*E*)-(4-nitrophenyl) diazenyl) benzene -1,3-

diol (2),<sup>6</sup> 4-((*E*)-(2-nitrophenyl) diazenyl)-6-((*E*)-(3-nitrophenyl) diazenyl) benzene-1,3-diol (3) <sup>6</sup> and 4,6-*bis*((*E*)-(2-nitrophenyl) diazenyl) benzene-1,3-diol (4)<sup>6</sup> diazo dyes were characterized using m.p., IR, UV-visible and mass spectrum[2]. Add to which, all the synthetic azo dyes were provided non-toxic effects using different concentrations from each, and didn't show any haemolysis effect in the cells. The results of the cell viability (cytotoxicity assay) were showed well ability to reduce the growth of human breast MDA-MB231 cancer cells, after 24h treatment with 100 $\mu$ M of each dye. The results were also showed well activities of each dye against cell viability in contrast with the control.

Further, the results of human DNA binding of each azo dye were indicated its ability to damage DNA and inhibiting of DNA transcription and replication. Further, the results of the NanoDrop<sup>TM</sup> spectrophotometer were showed increasing in the concentration of the nitrogen bases, which confirmed the

DNA was damaged. Due to recommend the synthetic, non-toxic azo dyes as novel drugs for treatment of human breast MDA-MB231 cancer cells through its ability to destroy the DNA of the cancer cells. Though, the internal coordinates are an attractive alternative to the Cartesian coordinates of each atom when particular degrees of freedom are not of interest. [8] Internal coordinates such as bond lengths, bond angles, and torsion angles are natural coordinates for describing a bonded molecular system. [9] It has recently been suggested by *Mu et al.* [Proteins **58**, 45] to use backbone dihedral angles instead of Cartesian coordinates in a principal component analysis of molecular dynamics simulations. Internal coordinate also have some advantages for suggesting a new derivatives [10].

Dihedral angles may be advantageous because internal coordinates naturally provide a correct separation of internal and overall motion, which was found to be essential for the construction and interpretation of the free energy landscape of a biomolecule undergoing large structural rearrangements. [11] Which describe the essential physics of a biomolecular process such as protein folding or molecular recognition? Molecular modelling can be

considered as a range of computerized techniques based on theoretical chemistry methods and experimental data that can be used either to analyse molecules and molecular systems or to predict molecular, chemical, and biochemical properties. [7] One of the major advantages of molecular mechanics compared to other computational techniques is the relative ease with which structures can be optimized via minimization of the corresponding potential energy functions. [12] The area of molecular mechanics is to study the detailed structure and physical properties of molecules. Molecular mechanics calculates the energy of a molecule and then adjusts the energy through changes in the bond lengths and angles to obtain the minimum energy structure.

## Result and Discussion

Analytical studies were carried on some diazo dyes that characterised 4, 6-*bis*((*E*)-(3-nitrophenyl) diazenyl) benzene-1, 3-diol (**1**), 6 4-((*E*)-(3-nitrophenyl) diazenyl)-6-((*E*)-(4-nitrophenyl) diazenyl) benzene-1, 3-diol (**2**), 6 4-((*E*)-(2-nitrophenyl) diazenyl)-6-((*E*)-(3-nitrophenyl) diazenyl) benzene-1, 3-diol (**3**)<sup>6</sup> and 4, 6-*bis*((*E*)-(2-nitrophenyl) diazenyl) benzene-1, 3-diol (**4**)<sup>6</sup> (Figure 1).

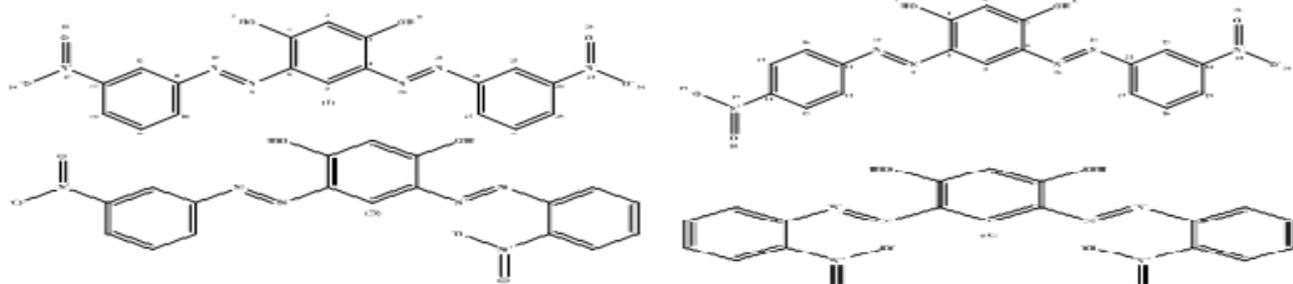


Figure 1: Structure of the synthesis diazo dyes

The effect of the solubility of each synthetic diazo dye was studied in a range of solvents. The results were showed high solubility of each in ethanol. Thus, the pH effect was also

studied for each diazo dye (1), (2), (3) and (4) in a range of buffer solution at pH (1-12) using  $1 \times 10^{-5}$  M concentration, (Figures 2 -5).

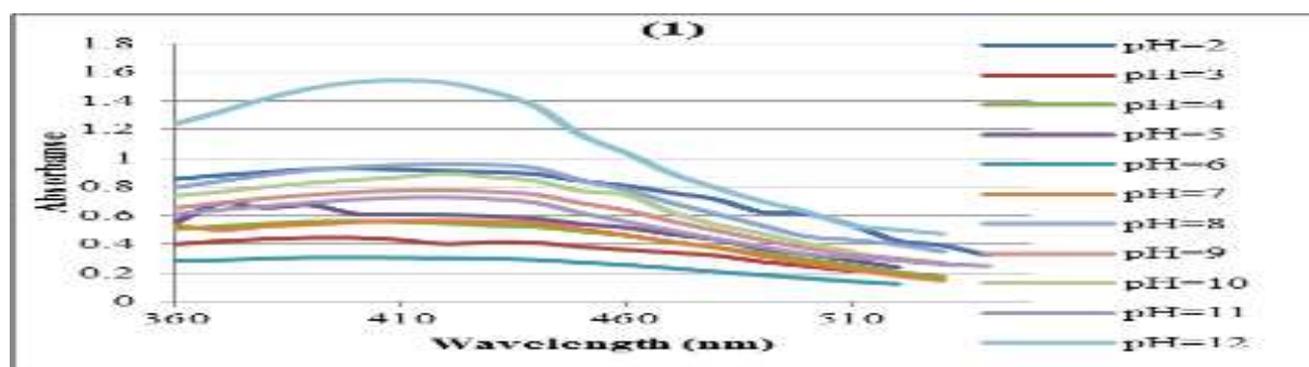


Figure 2: The pH effect of (1)

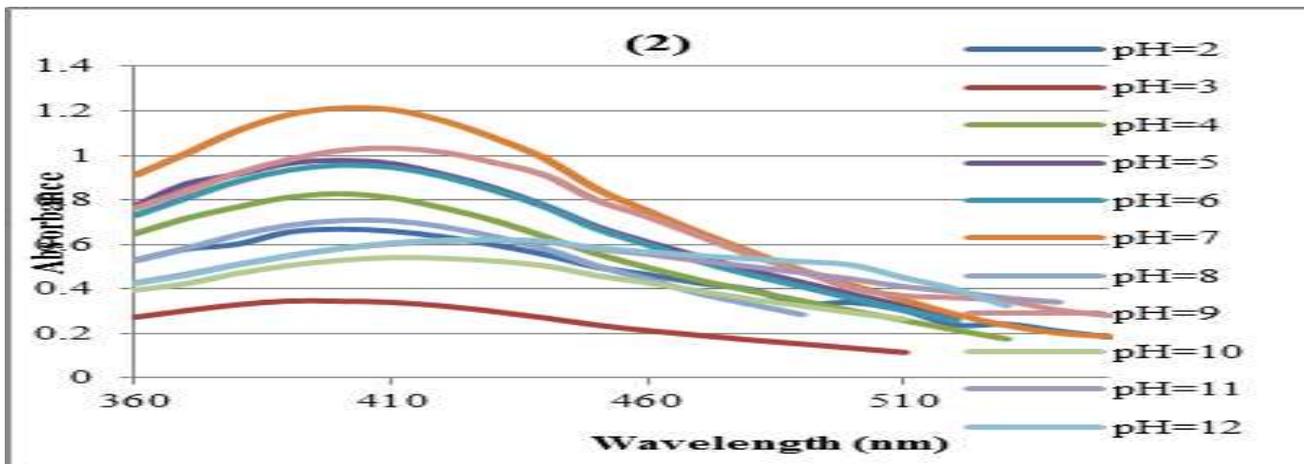


Figure 3: The pH effect of (2)

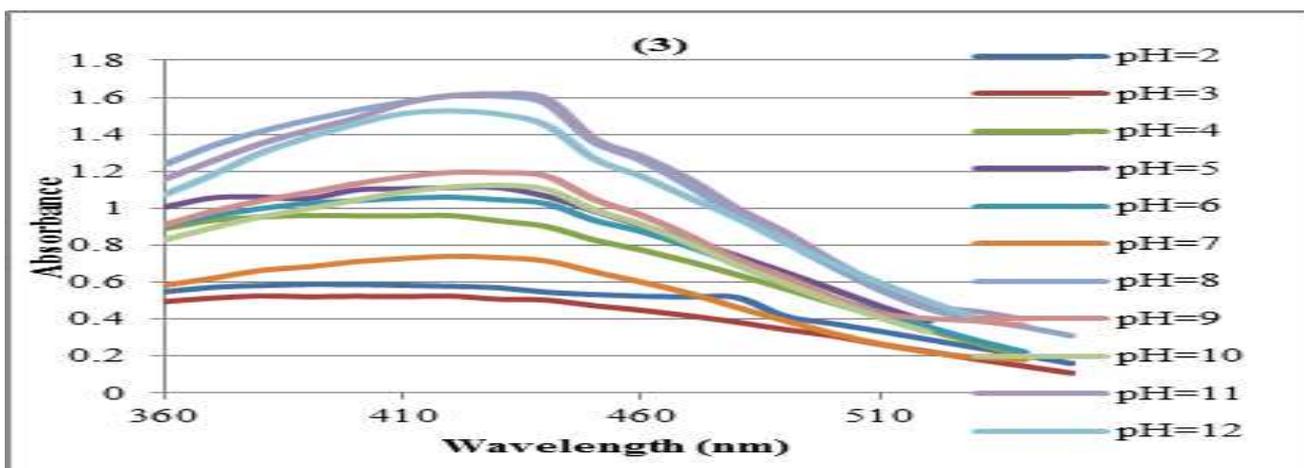


Figure 4: The pH effect of (3)

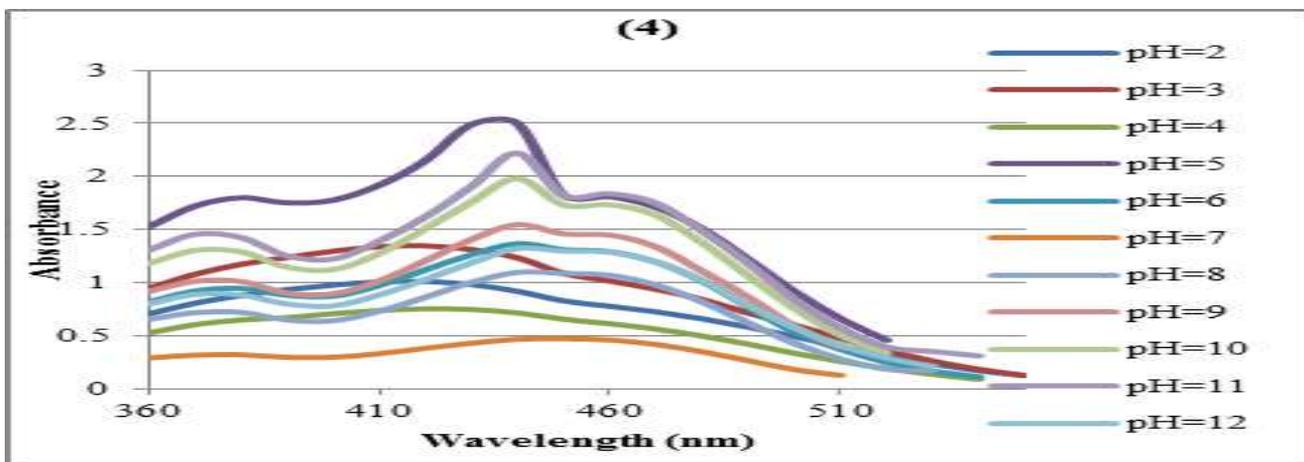


Figure 5: The pH effect of (4)

The results were showed that the suitable pH values were found to be in the pH12 and the three isopiestic points were gained in Figures (3-5). Thus, the  $pK_a$  of hydroxyl group and  $pK_b$  of the nitrogen atom in synthetic diazo dyes (1), (2), (3) and (4) were calculated by applying the half height method [13].

From this method the  $pK$  values were attended using equations (1) and (2) below. This method was depending on the fact that the limiting absorption ( $A_i$ ) represents complete conversion of one form to other.

Since  $pK$  is equal to  $pH$  at which the two forms exist in equivalent amount, then the  $pH$  corresponding to half the height of the absorbance, the  $pH$  curve is equal to  $pK$ .

$$pK = pH \text{ (at } A_{1/2}) \dots\dots\dots (1)$$

$$A_{1/2} = \frac{A_i + A_{min}}{2} \dots\dots\dots (2)$$

The  $pK$  (at  $A_{1/2}$ ) of (1), (2), (3) and (4) were envisioned from the absorbance- $pH$  curve as realized in Figures (4-9) below.

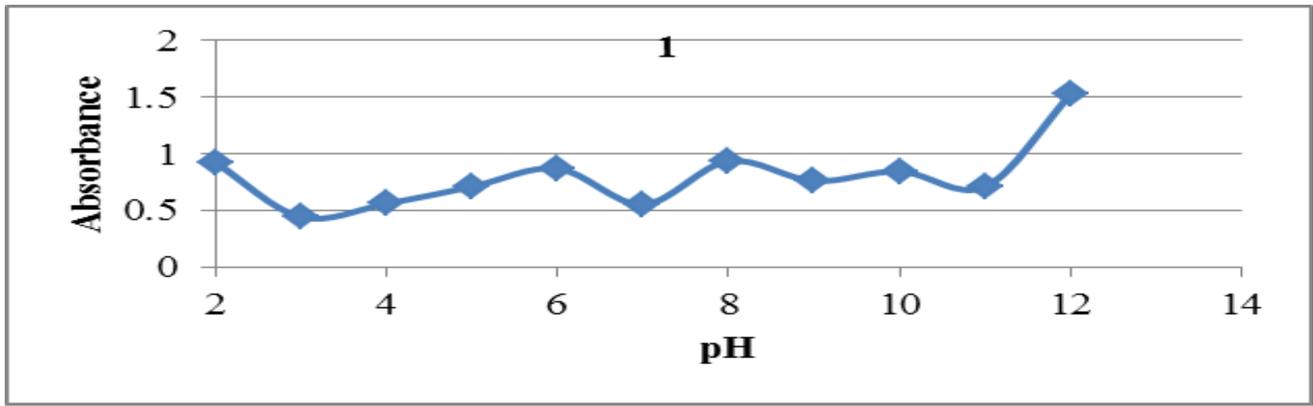


Figure 6: Absorbance-pH effect of (1) at  $\lambda$  400 nm

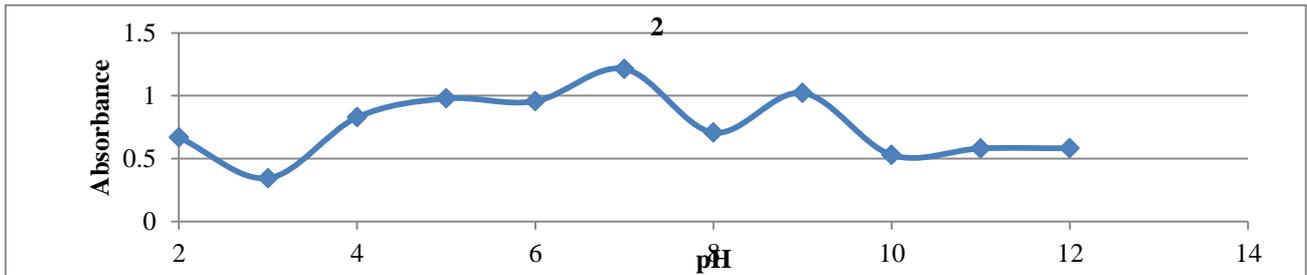


Figure 7: Absorbance-pH effect of (2) at  $\lambda$  400 nm

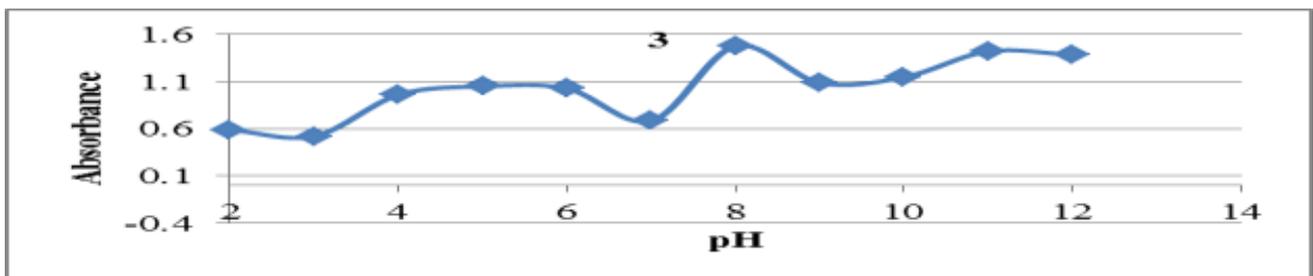


Figure 8: Absorbance-pH effect of (1) at  $\lambda$  390 nm

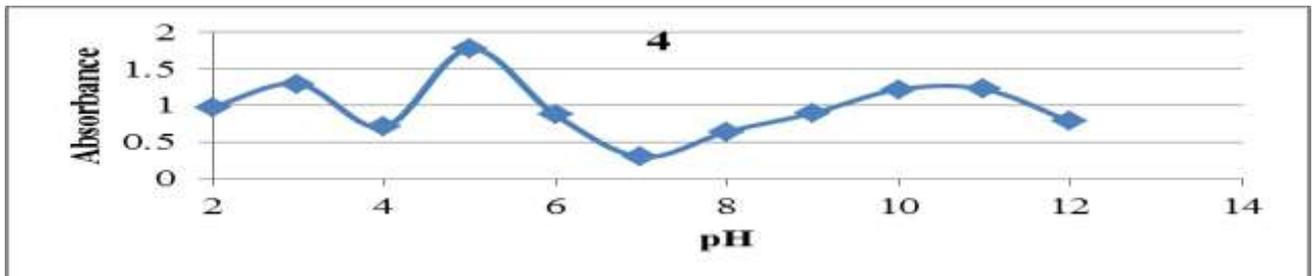


Figure 9: Absorbance-pH effect of (2) at  $\lambda$  400 nm

The results obtained from the absorbance-pH curve in

figures (6-9) above are given in table (1) below.

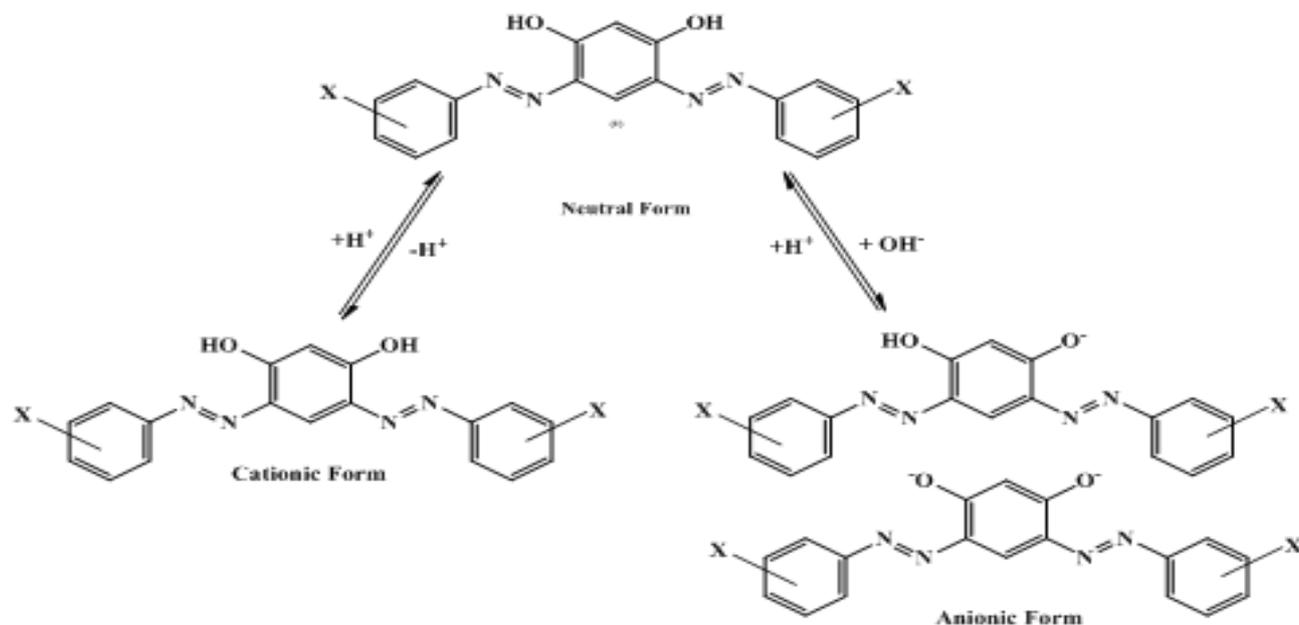
Table 1: Spectrophotometric determination of ionization and protonation constants of the synthetic diazo dyes (1), (2), (3) and (4)

$pK_{p2}$	$pK_{p1}$	$pK_{a2}$	$pK_{a1}$	Diazo dyes	$\lambda$ (nm)
6.5	3.5	8.5	10.5	(1)	400
5.5	3.5	7.5	9.5	(2)	400
6.5	3.0	9	-	(1)	390
3.5	1.5	8	-	(2)	400

$pK_{p1}$ = Protonation of the nitrogen atom.,  $pK_{p2}$ = Protonation of the nitrogen atom  
 $pK_{a1}$ = Ionization of the OH-group.,  $pK_{a2}$ = Ionization of the OH-group

The absorption spectra, (Figures 2- 5) of (1), (2), (3) and (4) in the varying pH values, (1- 12) are explained in the Scheme (1) below.

The results were indicated the existence of the following equilibrium scheme of which displays the suggested ionization of diazo dye in acidic and basic medium.



Scheme 1: The suggested ionization of (1), (2), (3) and (4) in the acidic and basic medium

Theoretical studies also carried on (1), (2), (3) and (4), in order to understand the effect of their structures in their activity.

Due to the ICM was calculated for the synthetic diazo dyes (1), (2), (3) and (4), (Tables 2-5).

Table 2: Internal coordinate (ICM) of (1)

No	Atom	Bond Atom	Bond Length (Å)	Angle Atom	Angle (°)	2 <sup>nd</sup> Angle Atom	2 <sup>nd</sup> Angle (°)	2 <sup>nd</sup> Angle Type
1	C(1)							
2	C(2)	C(1)	1.395					
3	C(3)	C(2)	1.395	C(1)	119.997			
4	H(31)	C(2)	1.1	C(1)	120.001	C(3)	120.002	Pro-S
5	C(4)	C(3)	1.395	C(2)	120	C(1)	-0.006	Dihedral
6	O(8)	C(3)	1.355	C(2)	120	C(4)	120	Pro-R
7	C(5)	C(4)	1.395	C(3)	120.003	C(2)	0.001	Dihedral
8	N(20)	C(4)	1.26	C(3)	119.999	C(5)	119.998	Pro-R
9	C(6)	C(1)	1.395	C(2)	120.003	C(3)	0.006	Dihedral
10	O(7)	C(1)	1.355	C(2)	119.999	C(6)	119.999	Pro-S
11	N(9)	C(6)	1.26	C(1)	120	C(5)	120	Pro-R
12	H(32)	C(5)	1.1	C(4)	120.001	C(6)	120.001	Pro-S
13	N(10)	N(9)	1.248	C(6)	107.5	C(1)	0	Dihedral
14	C(11)	N(10)	1.26	N(9)	107.5	C(6)	180	Dihedral
15	C(12)	C(11)	1.395	N(10)	119.998	N(9)	-180	Dihedral
16	C(16)	C(11)	1.395	N(10)	119.999	C(12)	120.003	Pro-R
17	C(13)	C(12)	1.395	C(11)	119.997	N(10)	-179.994	Dihedral
18	H(35)	C(12)	1.1	C(11)	120.001	C(13)	120.002	Pro-R
19	C(14)	C(13)	1.395	C(12)	120	C(11)	-0.006	Dihedral
20	N(17)	C(13)	1.248	C(12)	120	C(14)	120	Pro-R
21	C(15)	C(16)	1.395	C(11)	120	N(10)	179.999	Dihedral
22	H(36)	C(14)	1.1	C(13)	119.998	C(15)	119.998	Pro-R
23	H(37)	C(15)	1.1	C(14)	120.001	C(16)	120.002	Pro-S
24	H(38)	C(16)	1.1	C(11)	120	C(15)	120	Pro-S
25	N(21)	N(20)	1.248	C(4)	107.5	C(3)	180	Dihedral
26	C(22)	N(21)	1.26	N(20)	107.5	C(4)	180	Dihedral
27	C(23)	C(22)	1.395	N(21)	119.998	N(20)	180	Dihedral
28	C(27)	C(22)	1.395	N(21)	119.998	C(23)	120.003	Pro-S
29	C(24)	C(23)	1.395	C(22)	119.996	N(21)	-179.998	Dihedral
30	H(39)	C(23)	1.1	C(22)	120.002	C(24)	120.002	Pro-S
31	C(25)	C(24)	1.395	C(23)	120	C(22)	-0.006	Dihedral
32	N(28)	C(24)	1.248	C(23)	120	C(25)	120	Pro-R
33	C(26)	C(27)	1.395	C(22)	119.999	N(21)	-179.997	Dihedral
34	H(40)	C(25)	1.1	C(24)	119.999	C(26)	119.998	Pro-R
35	H(41)	C(26)	1.1	C(25)	120.002	C(27)	120.001	Pro-S
36	H(42)	C(27)	1.1	C(22)	120.001	C(26)	120	Pro-R
37	O(18)	N(17)	1.31	C(13)	120.001	C(12)	180	Dihedral
38	O(19)	N(17)	1.31	C(13)	120	O(18)	120	Pro-S
39	O(29)	N(28)	1.31	C(24)	120	C(23)	180	Dihedral
40	O(30)	N(28)	1.31	C(24)	120	O(29)	120	Pro-R
41	H(33)	O(7)	0.972	C(1)	108	C(2)	-180	Dihedral
42	H(34)	O(8)	0.972	C(3)	108	C(2)	180	Dihedral

Table 3: Internal coordinate (ICM) of (2)

No	Atom	Bond Atom	Bond Length (Å)	Angle Atom	Angle (°)	2 <sup>nd</sup> Angle Atom	2 <sup>nd</sup> Angle (°)	2 <sup>nd</sup> Angle Type
1	C(1)							
2	C(2)	C(1)	1.395					
3	C(3)	C(2)	1.395	C(1)	119.997			
4	H(31)	C(2)	1.1	C(1)	120.002	C(3)	120.001	Pro-S
5	C(4)	C(3)	1.395	C(2)	120	C(1)	-0.006	Dihedral
6	O(8)	C(3)	1.355	C(2)	120	C(4)	120	Pro-R
7	C(5)	C(4)	1.395	C(3)	120.003	C(2)	0.001	Dihedral
8	N(20)	C(4)	1.26	C(3)	119.999	C(5)	119.998	Pro-R
9	C(6)	C(1)	1.395	C(2)	120.003	C(3)	0.006	Dihedral
10	O(7)	C(1)	1.355	C(2)	119.999	C(6)	119.998	Pro-S
11	N(9)	C(6)	1.26	C(1)	120	C(5)	120	Pro-S
12	H(32)	C(5)	1.1	C(4)	120.001	C(6)	120.001	Pro-R
13	N(10)	N(9)	1.248	C(6)	107.5	C(1)	0	Dihedral
14	C(11)	N(10)	1.26	N(9)	107.5	C(6)	-180	Dihedral
15	C(12)	C(11)	1.395	N(10)	119.998	N(9)	180	Dihedral
16	C(16)	C(11)	1.395	N(10)	119.999	C(12)	120.003	Pro-R
17	C(13)	C(12)	1.395	C(11)	119.997	N(10)	-179.995	Dihedral
18	H(35)	C(12)	1.1	C(11)	120.001	C(13)	120.002	Pro-R
19	C(14)	C(13)	1.395	C(12)	120.001	C(11)	-0.005	Dihedral
20	H(36)	C(13)	1.1	C(12)	120	C(14)	119.999	Pro-S
21	C(15)	C(16)	1.395	C(11)	120	N(10)	179.999	Dihedral
22	N(17)	C(14)	1.248	C(13)	119.999	C(15)	119.998	Pro-S
23	H(37)	C(15)	1.1	C(14)	120.001	C(16)	120.002	Pro-S
24	H(38)	C(16)	1.1	C(11)	120	C(15)	120	Pro-R
25	N(21)	N(20)	1.248	C(4)	107.5	C(3)	-180	Dihedral
26	C(22)	N(21)	1.26	N(20)	107.5	C(4)	180	Dihedral
27	C(23)	C(22)	1.395	N(21)	119.999	N(20)	180	Dihedral
28	C(27)	C(22)	1.395	N(21)	119.999	C(23)	120.003	Pro-S
29	C(24)	C(23)	1.395	C(22)	119.997	N(21)	-179.999	Dihedral
30	H(39)	C(23)	1.1	C(22)	120.002	C(24)	120.001	Pro-R
31	C(25)	C(24)	1.395	C(23)	120	C(22)	-0.006	Dihedral
32	N(28)	C(24)	1.248	C(23)	120	C(25)	120	Pro-R
33	C(26)	C(27)	1.395	C(22)	119.999	N(21)	-179.997	Dihedral
34	H(40)	C(25)	1.1	C(24)	119.999	C(26)	119.998	Pro-S
35	H(41)	C(26)	1.1	C(25)	120.002	C(27)	120.001	Pro-R
36	H(42)	C(27)	1.1	C(22)	120	C(26)	120.001	Pro-S
37	O(18)	N(17)	1.31	C(14)	120	C(13)	180	Dihedral
38	O(19)	N(17)	1.31	C(14)	120	O(18)	120	Pro-R
39	O(29)	N(28)	1.31	C(24)	120	C(23)	180	Dihedral
40	O(30)	N(28)	1.31	C(24)	120	O(29)	120.001	Pro-R
41	H(33)	O(7)	0.972	C(1)	108	C(2)	180	Dihedral
42	H(34)	O(8)	0.972	C(3)	108	C(2)	180	Dihedral

Table 4: Internal coordinate (ICM) of (3)

No	Atom	Bond Atom	Bond Length (Å)	Angle Atom	Angle (°)	2 <sup>nd</sup> Angle Atom	2 <sup>nd</sup> Angle (°)	2 <sup>nd</sup> Angle Type
1	C(1)							
2	C(2)	C(1)	1.395					
3	C(3)	C(2)	1.395	C(1)	119.997			
4	H(31)	C(2)	1.1	C(1)	120.002	C(3)	120.001	Pro-S
5	C(4)	C(3)	1.395	C(2)	120	C(1)	-0.006	Dihedral
6	O(8)	C(3)	1.355	C(2)	120	C(4)	120	Pro-S
7	C(5)	C(4)	1.395	C(3)	120.003	C(2)	0.001	Dihedral
8	N(20)	C(4)	1.26	C(3)	119.998	C(5)	119.999	Pro-R
9	C(6)	C(1)	1.395	C(2)	120.003	C(3)	0.006	Dihedral
10	O(7)	C(1)	1.355	C(2)	119.998	C(6)	119.999	Pro-R
11	N(9)	C(6)	1.26	C(1)	120	C(5)	120.001	Pro-S
12	H(32)	C(5)	1.1	C(4)	120.001	C(6)	120.001	Pro-R
13	N(10)	N(9)	1.248	C(6)	107.5	C(1)	0	Dihedral
14	C(11)	N(10)	1.26	N(9)	107.5	C(6)	180	Dihedral
15	C(12)	C(11)	1.395	N(10)	119.999	N(9)	-180	Dihedral
16	C(16)	C(11)	1.395	N(10)	119.999	C(12)	120.003	Pro-S
17	C(13)	C(12)	1.395	C(11)	119.997	N(10)	-179.994	Dihedral
18	H(35)	C(12)	1.1	C(11)	120.002	C(13)	120.001	Pro-S
19	C(14)	C(13)	1.395	C(12)	120	C(11)	-0.006	Dihedral
20	N(17)	C(13)	1.248	C(12)	120	C(14)	120	Pro-R
21	C(15)	C(16)	1.395	C(11)	119.999	N(10)	179.999	Dihedral
22	H(36)	C(14)	1.1	C(13)	119.999	C(15)	119.998	Pro-S
23	H(37)	C(15)	1.1	C(14)	120.002	C(16)	120.001	Pro-R
24	H(38)	C(16)	1.1	C(11)	120	C(15)	120.001	Pro-R

25	N(21)	N(20)	1.248	C(4)	107.5	C(3)	-180	Dihedral
26	C(22)	N(21)	1.26	N(20)	107.5	C(4)	-180	Dihedral
27	C(23)	C(22)	1.395	N(21)	119.999	N(20)	180	Dihedral
28	C(27)	C(22)	1.395	N(21)	119.998	C(23)	120.003	Pro-S
29	C(24)	C(23)	1.395	C(22)	119.997	N(21)	-179.999	Dihedral
30	N(28)	C(23)	1.248	C(22)	120.001	C(24)	120.002	Pro-S
31	C(25)	C(24)	1.395	C(23)	120	C(22)	-0.006	Dihedral
32	H(39)	C(24)	1.1	C(23)	120	C(25)	120	Pro-R
33	C(26)	C(27)	1.395	C(22)	120	N(21)	-179.997	Dihedral
34	H(40)	C(25)	1.1	C(24)	119.998	C(26)	119.998	Pro-S
35	H(41)	C(26)	1.1	C(25)	120.001	C(27)	120.002	Pro-R
36	H(42)	C(27)	1.1	C(22)	120	C(26)	120	Pro-S
37	O(18)	N(17)	1.31	C(13)	120	C(12)	180	Dihedral
38	O(19)	N(17)	1.31	C(13)	120	O(18)	120	Pro-R
39	O(29)	N(28)	1.31	C(23)	120	C(22)	180	Dihedral
40	O(30)	N(28)	1.31	C(23)	120	O(29)	120	Pro-R
41	H(33)	O(7)	0.972	C(1)	108	C(2)	-180	Dihedral
42	H(34)	O(8)	0.972	C(3)	108	C(2)	180	Dihedral

Table 5: Internal coordinate (ICM) of (4)

No	Atom	Bond Atom	Bond Length (Å)	Angle Atom	Angle (°)	2 <sup>nd</sup> Angle Atom	2 <sup>nd</sup> Angle (°)	2 <sup>nd</sup> Angle Type
1	C(1)							
2	C(2)	C(1)	1.395					
3	C(3)	C(2)	1.395	C(1)	119.997			
4	H(31)	C(2)	1.1	C(1)	120.001	C(3)	120.002	Pro-R
5	C(4)	C(3)	1.395	C(2)	120	C(1)	-0.006	Dihedral
6	O(8)	C(3)	1.355	C(2)	120	C(4)	120	Pro-R
7	C(5)	C(4)	1.395	C(3)	120.003	C(2)	0.001	Dihedral
8	N(20)	C(4)	1.26	C(3)	119.998	C(5)	119.999	Pro-R
9	C(6)	C(1)	1.395	C(2)	120.003	C(3)	0.006	Dihedral
10	O(7)	C(1)	1.355	C(2)	119.999	C(6)	119.998	Pro-S
11	N(9)	C(6)	1.26	C(1)	120	C(5)	120.001	Pro-S
12	H(32)	C(5)	1.1	C(4)	120.001	C(6)	120.001	Pro-S
13	N(10)	N(9)	1.248	C(6)	107.5	C(1)	0	Dihedral
14	C(11)	N(10)	1.26	N(9)	107.5	C(6)	180	Dihedral
15	C(12)	C(11)	1.395	N(10)	119.999	N(9)	180	Dihedral
16	C(16)	C(11)	1.395	N(10)	119.999	C(12)	120.003	Pro-R
17	C(13)	C(12)	1.395	C(11)	119.997	N(10)	-179.994	Dihedral
18	N(17)	C(12)	1.248	C(11)	120.001	C(13)	120.002	Pro-S
19	C(14)	C(13)	1.395	C(12)	120	C(11)	-0.006	Dihedral
20	H(35)	C(13)	1.1	C(12)	119.999	C(14)	120	Pro-R
21	C(15)	C(16)	1.395	C(11)	119.999	N(10)	179.999	Dihedral
22	H(36)	C(14)	1.1	C(13)	119.999	C(15)	119.998	Pro-R
23	H(37)	C(15)	1.1	C(14)	120.001	C(16)	120.002	Pro-R
24	H(38)	C(16)	1.1	C(11)	120	C(15)	120.001	Pro-R
25	N(21)	N(20)	1.248	C(4)	107.5	C(3)	180	Dihedral
26	C(22)	N(21)	1.26	N(20)	107.5	C(4)	180	Dihedral
27	C(23)	C(22)	1.395	N(21)	119.999	N(20)	-180	Dihedral
28	C(27)	C(22)	1.395	N(21)	119.999	C(23)	120.003	Pro-S
29	C(24)	C(23)	1.395	C(22)	119.997	N(21)	-179.999	Dihedral
30	N(28)	C(23)	1.248	C(22)	120.001	C(24)	120.002	Pro-S
31	C(25)	C(24)	1.395	C(23)	120	C(22)	-0.006	Dihedral
32	H(39)	C(24)	1.1	C(23)	120	C(25)	120	Pro-R
33	C(26)	C(27)	1.395	C(22)	120	N(21)	-179.997	Dihedral
34	H(40)	C(25)	1.1	C(24)	119.998	C(26)	119.998	Pro-S
35	H(41)	C(26)	1.1	C(25)	120.002	C(27)	120.001	Pro-R
36	H(42)	C(27)	1.1	C(22)	120	C(26)	120	Pro-R
37	O(18)	N(17)	1.31	C(12)	120	C(11)	-180	Dihedral
38	O(19)	N(17)	1.31	C(12)	120.001	O(18)	119.999	Pro-S
39	O(29)	N(28)	1.31	C(23)	120	C(22)	-180	Dihedral
40	O(30)	N(28)	1.31	C(23)	120	O(29)	120	Pro-R
41	H(33)	O(7)	0.972	C(1)	108	C(2)	180	Dihedral

42	H(34)	O(8)	0.972	C(3)	108	C(2)	-180	Dihedral
----	-------	------	-------	------	-----	------	------	----------

The conformational analysis in (1), (2), (3) and (4), was also studied theoretically. The

energies of the conformers that generated by rotation around single bond in each side of the azo group in (1) were presented in Figure 10 (a & b) below.

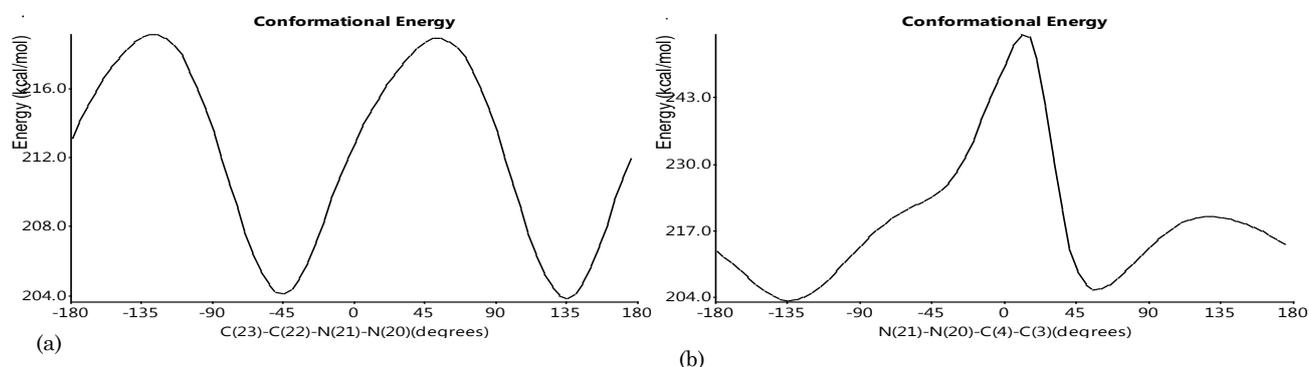


Figure 10: The conformational energy around one side in diazo dye (1)

The results were showed that each rotation can generate eclipsed and staggered conformers as seen in Figure 10) above. The results of figure 10a) were showed that the eclipsed E for E (-130°), E (55°) and E (180°), which equal to 219.14, 218.89 and 213.13 kcal/ mole respectively. But, the staggered conformers for E (-180°), E (-45°) and E (135°) were equal to 213.12, 204.11 and 203.99 kcal/ mole respectively. But, the results of figure (10b) were showed that the eclipsed E for E

(-180°), E (10°) and E (135°), which equal to 213.11, 255.38, 205.08 and 219.69 kcal/ mole respectively. But, the staggered conformers for E (-135°), E (55°) and E (180°) were equal to 203.63, 205.59 and 213.11 respectively. The symmetry in azo dye (1) was observed. However, the results of (2) were showed that each rotation can generate eclipsed and staggered conformers as seen in Figure 11 (a- d) below.

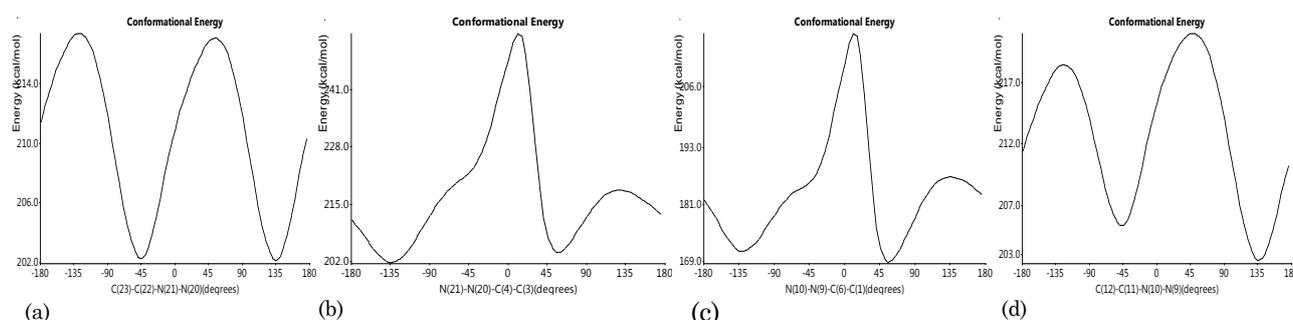


Figure 11: The conformational energy around each side in diazo dye (2)

The results of Figure 11a) were showed that the eclipsed E for E (-130°), E (50°) and E (180°), which equal to 217.40, 217.07 and 211.41 kcal/ mole respectively. But, the staggered conformers for E (-180°), E (-45°) and E (135°) were equal to 211.41, 202.26 and 202.14 kcal/ mole respectively.

But, the results of figure 11b) were showed that the eclipsed E for E (-180°), E (10°) and E(135°), which equal to 211.41, 253.82 and 218.20 kcal/ mole respectively. But, the staggered conformers for E (-135°), E (55°)

and E (180°) were equal to 201.80, 204.01 and 211.41 respectively. But, the results of other side of azo group in (2) (figure 11c and 11d) were showed that the eclipsed E for E (-180°), E (10°) and E(135°), which equal to 181.93, 217.14 and 186.85 kcal/ mole respectively. But, the staggered conformers for E (-135°), E (55°) and E (180°) were equal to 171.19, 168.92 and 181.93 kcal/ mole respectively.

But then, the eclipsed E for E (-125°), E (50°) and E (180°), which equal to 218.48, 221.01 and 211.41 kcal/ mole respectively. But, the

staggered conformers for E (-180°), E (-45°) and E (135°) were equal to 211.41, 205.42 and 202.51 respectively.

The conformational analysis in (3) as seen in Figure (12 (a-c) below.

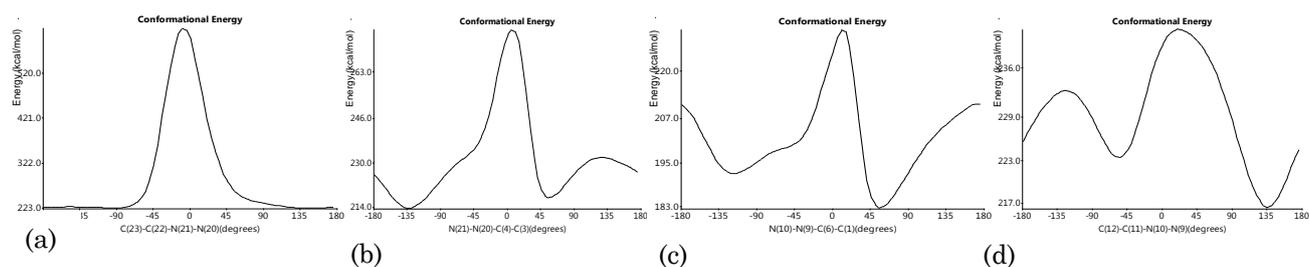


Figure 12: The conformational energy around each side in diazo dye (3)

The results were showed that each rotation can generate eclipsed and staggered conformers as seen in Figure (12) above. The results of figure (12a) were showed that the eclipsed E for E (-10°), which equal to 618.18 kcal/ mole. But, the staggered conformers for E (-180°) and E (180°) were equal to 225.68 kcal/ mole in each. But, the results of figure (12b) were showed that the eclipsed E for E (-180°), E (5°) and E (125°), which equal to 225.68, 278.21 and 232.00 kcal/ mole respectively. But, the staggered conformers for E (-135°), E (55°) and E (180°) were equal to 213.68, 217.35 and 225.68 respectively. The results of figure (12c) were showed that the eclipsed E for E (-180°), E (-10°) and E

(170°) were equal to 210.73, 231.37 and 211.10 kcal/ mole. But, the staggered conformers for E (-120°), E (-55°) and E (180°) were equal to 192.22, 183.50 and 211.10 kcal/ mole. But, the results of figure (12d) were showed that the eclipsed E for E (-125°), E (25°) and E (180°), which equal to 232.76, 241.27 and 225.68 kcal/ mole respectively. But, the staggered conformers for E (-180°), E (-55°) and E (135°) were equal to 225.68, 223.41 and 216.42 respectively. However, the results of (4) were showed that each rotation can generate eclipsed and staggered conformers as seen in Figure (13 (a- d)) below. The figure was also showed the symmetry in diazo dye (4).

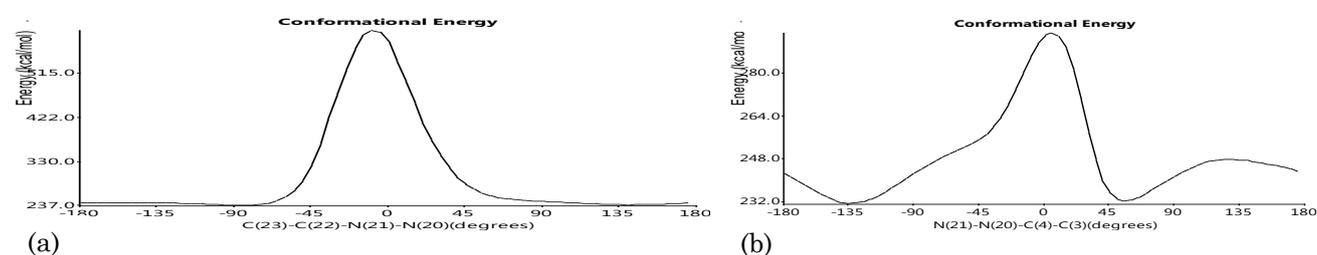


Figure 13: The conformational energy around one side in diazo dye (4)

The results of figure (13a) were showed that the eclipsed E for E(-10°), which equal to 606.43 kcal/ mole. But, the staggered conformers for E(-180°) and E(180°) were equal to 242.34 kcal/ mole in each. But, the results of figure (13b) were showed that the eclipsed E for E (-180°), E(5°) and E(125°), which equal to 242.34, 295.08 and 247.74 kcal/ mole respectively. But, the staggered conformers for E (-135°), E(55°) and E(180°) were equal to 231.33, 232.26 and 242.34 respectively. Furthermore, the MM2 properties was intended for (1), (2), (3) and (4), the results were revealed that the stretch, bend, stretch-bend, torsion, non-1,4 VDW, 1,4 VDW, dipole/ dipole and the total energy were equal to (37.0896, 10.3015, -

0.2998, 73.8864, 12.5744, 33.5215, -5.6528 and 156.3964 kcal/ mol.), (37.0888, 10.3016, -0.2998, 73.8861, 13.2814, 34.0355, -5.5887 and 156.2758 kcal/ mol.), (37.0864, 10.3015, -0.2998, 73.8862, 39.2271, 36.3271, -7.4107 and 190.4949 kcal/ mol.) and (37.0881, 10.3015, -0.2997, 73.8863, 39.0772, 36.3275, -7.7732 and 185.4072 kcal/ mol.) respectively. Further, the MM2 minimization for (1), (2), (3) and (4) were studied. The results were showed that the (stretch, bend, stretch-bend, torsion, non-1,4 VDW, 1,4 VDW, dipole/ dipole and the total energy) were equal to (1.6859, 12.3234, 0.1553, -16.5493, 2.2187, 29.1708, -0.9341 and 23.0124 kcal/mol.), (1.6206, 12.2983, 0.1517, -16.5650, 2.2192, 29.1042, -1.1970 and 21.4161 kcal/ mol.),

(2.3344, 14.4662, 0.2090, -16.7015, 3.6754, 29.1462, 1.0400 and 36.1303 kcal/mol.) and (2.6864, 15.9677, 0.2321, -16.1161, 5.4563, 29.0966, 2.9063 and 34.6947 kcal/mol.) respectively. The MMFF94 total energy, MMFF94 minimization iteration energy and MMFF94 minimization/ sampling energy were equal to (283.827 kcal/mol., 109.332 kcal/mol. and 105.386 kcal/mol.), (295.842 kcal/mol., 121.283 kcal/mol. and 117.472 kcal/mol.), 292.159 kcal/mol., 112.245 kcal/mol. and 105.873 kcal/mol.) And (322.891 kcal/mol., 114.006 kcal/mol. and 104.234 kcal/mol.) respectively. All these results were showed that the minimization was attended successfully and displayed the action of the structure and the position of the substituted group in the properties and biological activity of each dye.

## References

- Zollinger H (1991) "Color chemistry; synthesis, properties and Application of organic Dyes and Pigments", VCH.
- Eady SJ, Ali HM, Ali A Al-Shawi (2019) Preparation of novel Azo Dyes as a new anti-Human Breast Cancer MDA-MB231 Cells and study its association with DNA, Journal of Basrah Researches/Sciences.
- Farghaly TA, Abdallah ZA (2008) "Synthesis, azo-hydrazone tautomerism and antitumor screening of N-(3-ethoxycarbonyl 4, 5, 6, 7-tetrahydro-benzo [b]thien-2-yl)-2-arylhydrazono-3-oxobutanamide derivatives," Archive for Organic Chemistry, 17: 295-305.
- Garg HG, Praksh C (1972) "Preparation of 4-arylazo-3, 5-disubstituted-(2H)-1, 2, 6-thiadiazine 1, 1-dioxides," Journal Medicinal Chemistry, 15: 435-439.
- Child RG, Wilkinson RG, Tomcu-Fucik A (1977) "Effect of substrate orientation of the adhesion of polymer joints," Journal of Chemical Society, 87: 6031-6038.
- Ali H, Majeed H, Al-Asadi I, Abdulredha A, Hussain A (2018) Structures effect of two azo dyes associated with their antimicrobial activity, Journal of Chemical, Biological and Physical Sciences, 8: 171-185.
- Mechanics M (2002) Molecular modelling: molecular mechanics, An Introduction to Computational Biochemistry, 285-314.
- Schwieters CD, Clore GM (2001) Internal Coordinates for Molecular Dynamics and Minimization in Structure Determination and Refinement, Journal of magnetic resonance, 152: 288-302.
- Vaidehi N, Jain A (2015) Internal coordinate molecular dynamics: A foundation for multi-scale dynamics, Journal of Physical chemistry B, 119: 1233-1242.
- Ali HM (2016) Synthesis, theoretical studies of N1, N2, 1,2-tetraphenylethane-1,2-diimine and their Derivatives, The International Institute for Science, Technology and Education (IISTE).
- Alexandros A, Phuong HN, Rainer H, Gerhard S (2007) Dihedral angle principal component analysis of molecular dynamics simulations, The Journal of chemical physics, 126: 244-111.
- Ponder J, Richards F (1987) An efficient Newton-like method for molecular mechanics energy, Journal of Computational Chemistry, 8, 1016-1024.
- Issa RM, Zewail AH (1971) J. Chem. U.A.R., (Egypt J. Chem.), 14, 461.

## Summary

This study focused on analytical and theoretical studies of the synthetic diazo dyes with different position of substituted group. This position may affect the MM2 of the dye, which study the detailed structure and physical properties of the molecule. MM2 calculates the energy of a molecule and then adjusts the energy through changes in the bond lengths and angles to obtain the minimum energy structure.

The rotation of atoms about the single bonds is the subject of conformational analysis. This can affect the internal coordinate due to give some advantages for suggesting new derivatives.

