Comparative Study of Natural and Synthetic Food Additive Dye “Amaranth” through Photochemical Reactions

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Abstract: ***Food additive Amaranth dye (1) was isolated from amaranth plant (Amaranthaceae). Natural and synthetic ones were subjected to photo-catalyzed reaction using titanium dioxide (in micro and nano particles size) as heterogeneous singlet oxygen sensitizer. or Rose Bengal as homogeneous singlet oxygen sensitizer under irradiation of mercury lamp. Synthetic Amaranth dye (1) gave disodium 3,4-dioxo-3,4-dihydro-naphthalene-2,7-disulfonate (2), disodium 3,4-dihydroxy-3,4-dihydro-naphthalene-2,7-disulfonate (3) and sodium 4-hydroxy-naphthalene-1-sulfonate (4) in the case of TiO_2. Whereas photo-oxidation of 1 with Rose Bengal, which gave 2 and 4. Toxicological examination of the photo-degraded product 3 on DNA, was investigated showing a high degree of DNA degradation. Natural Amaranth dye (1) showed high stability towards this reaction; only decolorization has been done to it.***

Keywords: Food additive, Photooxygenation, Titanium dioxide.

1. Introduction

Color additive, as defined by regulation, is any dye, pigment, or other substance that can impact color to food, drug, cosmetic or human body. Color additives are important components of many products, as they make them attractive appealing, appetizing, and informative. Added color serves as a kind of code that allows us to identify products on sight, like candy flavors, medicine dosages, and left or right contact lenses. One of the U.S. Food and Drug Administration’s (FDA) rules is to assure that color additives are safely and appropriately used [1]. The U.K. Food additives and contaminants committee recommend less than 15 synthetic dyes for general or restricted use, among which was Amaranth [2].

Amaranth is a common azo dye colorant and is implicated in adverse reactions, such as chronic urticaria and angio-edema in adults and children [3,4] Studies on the light stability of food additives colorants with heterobifunctional reactive azo dyes as well as with their partly and fully deactivated derivatives, respectively, have been published earlier. The earlier studies mentioned have been performed mainly in open air. Among many other studies on the subject, the work of [5] has to be mentioned. Titanium dioxide TiO_2 has been widely used to prepare reduction / oxidation and catalytically active nanoparticles for the reductive dehalogenation of chloroinated organic compounds in aqueous solutions [6,7]. The practice of photocatalytic oxidation with TiO_2 in waste water treatment has only been successful in the bench test but not widely applied in the field by [8-13] reported that advanced oxidation processes (AOPs) using hydroxide radicals as the primary oxidant have emerged as a promising new technology for the degradation of organic pollutant. Our research scoped on studying the photocatalytic reaction of food additive azo dye, Amaranth, using TiO_2 in micro and nanoparticles. In addition, the photooxidation reaction of Amaranth in presence of Rose Bengal as a singlet oxygen sensitizer was also discussed.

2. Materials and Methods

Amaranth dye was isolated from Amaranth plant (*Amaranthaceae*) which grows in glass house in the Kingdom of Saudi Arabia. Synthetic Amaranth dye was supplied from sigma –Aldrich. IR spectra were performed on a Perkin-Elmer 16 FPC FT-IR spectrophotometer as thin films. ^{1}H-NMR and ^{13}C-NMR spectra were obtained in [D_6] DMSO solution on a Brucker AVANCE D.P.X. 400 MHz apparatus. GCMS were determined by Joel JMS 600H, GC Hewlett Packerd, HP 6890 Series, with capillary column (30m x 0.32 mm x 0.25 µm) HP-5 cross linked 5% dimethyl polysiloxane. Mass spectra were determined by Varian MAT 112 spectrometer. A sodium lamp (Phillips G/5812 SON**)** was used for photo-irradiation reactions with DNA. A mercury lamp (Phillips 1000 watt**)** was used for photo-catalytic reactions. Thin layer chromatography (TLC) and preparative layer chromatography (PLC): Polygram SIL G/W 254, Mecherey-Nagel. A rotatory evaporator (at 80°C/15 torr) was used to remove the solvents. Spectrophotometric measurement was carried out using ultraviolet photometric apparatus SHIMADZU U.V. Probe.

2.1 Extraction of Natural Amaranth dye

Amaranth dye was extracted as in fig 1.

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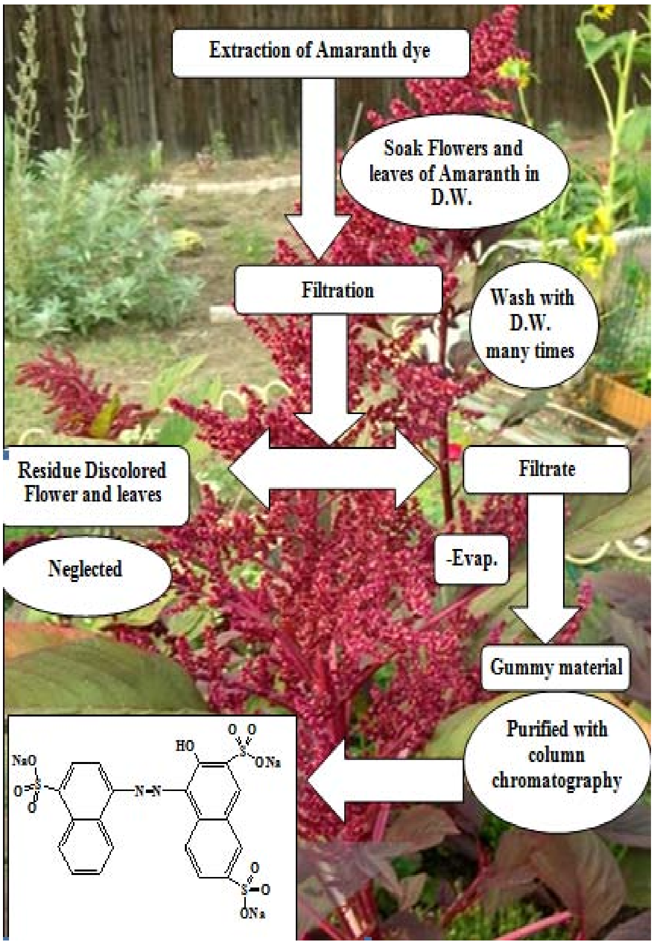

Figure 1: Extraction of natural Amaranth dye

2.2: General photocatalytic degradation procedures of Amaranth azo dye (1)

A solution of H_2O_2 (2.5 ml, 50 %) was added cautiously drop wise over 5 min to a stirred mixture of **1** (5 mmol) in C_2H_5OH (25 ml) and TiO_2 in different particle size (0.5 gm to 142 nm and 0.25 gm to 50 nm) at room temperature and a small amount of sodium bicarbonate was added to the mixtures before irradiation. The mixtures were irradiated using mercury lamp (1000 watt) in an atmosphere of nitrogen. The reaction mixtures were filtered off, the filtrate was collected and evaporated under reduced pressure at room temperature to give gummy material. Chloroform (25 ml) was added to the crude products. The combined extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give crude products, which were purified by column chromatography on silica gel adsorbent. Elution of the column with the solvent mixture showed in Table 1.

Table 1: photocatalytic degradation of Amaranth dye

Comp No.	**Time Hours**	**Sensitizer**	**Start Color**	Yield Gm	Yield %	**Photo Products:Isolated Yield gm Distribution %**	Elution system
1	25	TiO_2 142 nm	purple	0.756	%76	**2**= (0.24) 32% **3**=(0.21) 28% **4**=(0.305) 40%	CH_3COOH 7.5 $CH_3COOC_2H_5$ 2.5 NH_4OH 0.5
	12	50 nm		0.850	% 85	**2**= (0.283) 33% **3**=(0.252) 30% **4**=(0.315) 37%	

2.3: General photooxygenation of Amaranth azo dye (1)

A solution of **1** (10 mmol) in C_2H_5OH was irradiated externally by means of mercury lamp at -5ºC. During the irradiation a continuous stream of dry oxygen gas was allowed to pass through the reaction mixture at a slow rate to avoid evaporation of solvent. The solvent was evaporated at 60ºC/15 Torr [14]. The crude products were purified by column chromatography on silica gel adsorbent by eluting with the solvent mixture showed in Table 2.

Table 2: Photooxygenation of Amaranth dye

Comp No.	Time Hours	Sensitizer	Start Color	Yield Gm	Yield %	Photo Products:Isolated Yield gm Distribution %	Elution system
1	150	Rose Bengal	purple	0.68	%68	2= (0.52) 76% 4=(0.16) 24%	CH_3COOH 7.5 $CH_3COOC_2H_5$ 2.5 NH_4OH 0.5

2.4: DNA alkylation by photodegraded compound 3

To a solution of **3** in ethanol (1mg/5 ml) was added 1 ml. of DNA in saline solution. The reaction mixture was irradiated at 0ºC, using a sodium lamp, for 8 hours. Samples were taken at different times to determine the damaging effects of **3** on DNA using the gel electrophoresis technique [15]. The photographs of the gel were taken under U.V. light (365 nm). The results showed high degree of alkylation after sixty hours in Table 3.

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Table 3: the effect of photodegraded compound **3** on DNA

Sample	Time of irradiated (h)	Degree of DNA alkylat.
DNA	-------------	--------------
3 + DNA	0	None
3 + DNA	10	None
3 + DNA	20	Poor
3 + DNA	30	Moderate
3 + DNA	35	Moderate
3 + DNA	40	Moderate
3 + DNA	45	High
3 + DNA	50	High
3 + DNA	55	High
3 + DNA	60	Very High

3. Results and Discussion

3.1: Extraction of Amaranth dye

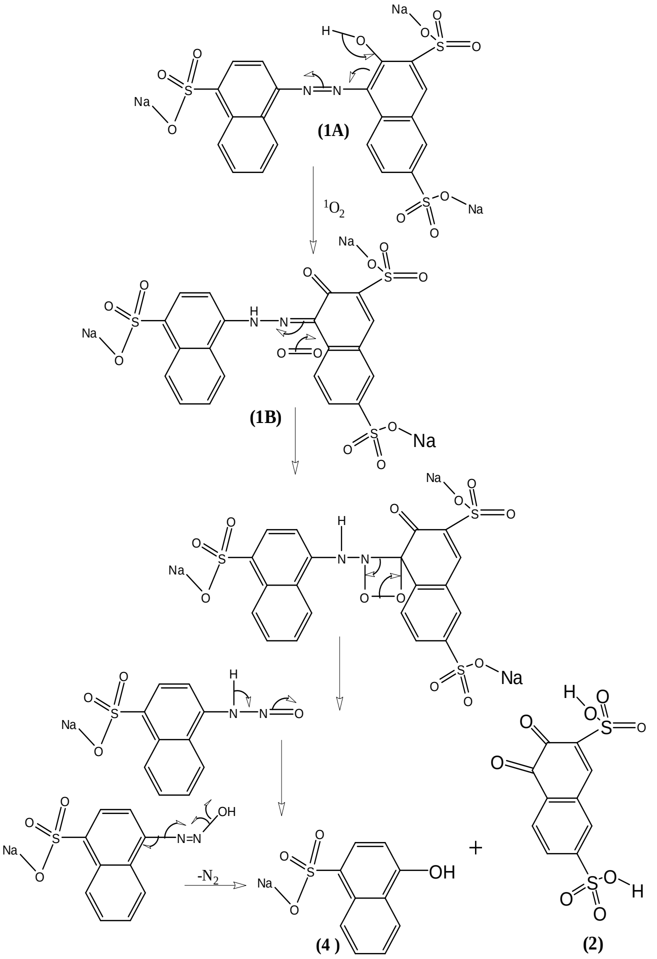
The Amaranth dye [trisodium (4E)-3-oxo-4-[(4-sulfonato-1-naphthyl) hydrazono] naphthalene-2, 7- disulfonate] (**1**) was isolated from Amaranth plant (*Amaranthaceae*). The chemical structure of **1** was confirmed by its spectral data. The ^{1}H-NMR spectrum of **1** showed singlet at δ 4.4 ppm for proton of hydroxyl group, two double doublets at δ 7.7 ppm for protons in position 6$^-$ -H,7$^-$ -H and five doublet signals at δ 7.85 , 8.14 , 8.24 , 8.55 and 9 ppm for protons in position 8$^-$ -H,5-H, 6-H, 3$^-$ -H, and 2$^-$-H respectively and two broad singlets at δ 7.99 and 8.34 ppm for protons in position 5$^-$-H and 1-H,8-H respectively. The mass spectrum of **1** exhibited a molecular ion peak at m/z = 604 $[M]^+$.

3.2: Photooxygenation of Amaranth dye

Interestingly, the photo induced oxygenation of the synthetic compound **1** in the presence of Rose Bengal (RB), as a singlet oxygen sensitizer led to the formation of disodium 3,4-dioxo-3,4-dihydro-naphthalene-2,7-disulfonate **(2)** together with sodium 4-hydroxy-naphthalene-1-sulfonate **(4)** (scheme 1). Compound **2** showed in the NMR spectrum a double doublet signal at δ 7.68 ppm due to proton H-6, two doublets at δ 8.1 and 8.41 ppm for protons H-5 and H-8 respectively & a broad singlet at δ 8.36 ppm for H-1. In the mass spectrum of **2** the molecular ion peak was observed at m/z 362.

Compound **4** showed in the ^{1}H-NMR spectrum a broad singlet at δ 5.6 ppm for hydroxyl proton, two doublets at δ 7.09 and 7. 9 ppm due to H-3 and H-2 respectively and four double doublet signals at δ 7.50 , 7.51 ,7.78 and 7.93 ppm for protons H-6, H-7, H-8 and H-5 respectively. The mass spectrum of **4** showed the molecular ion peak at m/z 246$[M]^+$.

A probable mechanism for the formation of **2** and **4** in photooxygenation reaction is shown in (Scheme 1). According to the frontier orbital theory, the double bond of C=N in azo – hydrazone - tautomers of Amaranth **(1A and 1B)** attacked by singlet oxygen,to give dioxetane intermediate via ene and or [2+2] cycloaddition which decomposes spontanously to form **2** and **4,** this result is in line with the work of [16], on the photo-oxidation of azobenzene dyes.



Scheme (1) : Photooxygenation of Amaranth using Rose Bengal

3.3 photocatalytic degradation of Amaranth dye

The photocatalytic reaction of **1** with hydrogen peroxide in presence of TiO_2 in two different particle size [50 nm,142 nm] under irradiation with mercury lamp 1000w [irradiation time 12: 2 hrs respectively] gave disodium 3,4-dioxo-3,4-dihydro-naphthalene-2,7-disulfonate **(2)** , disodium 3,4-dihydroxy-3,4-dihydro-naphthalene-2,7-disulfonate **(3)** and sodium-4-Hydroxy-naphthalene-1-sulfonate **(4)** **(**schemes 2-3). Though TiO_2 has the disadvantage of not being activated by visible light, but by ultra violet (UV) light , it is advantageous over the others especially with nano particle size (50 nm) than micro particle size (142 nm) in that it is chemically and biologically inert, photocatalytically stable, relatively easy to produce and to use, able to efficiently catalyze reactions, cheap and without risks to environment or humans.

The structure of **3** was established by spectra measurements, its ^{1}H-NMR spectrum showed a double doublet at δ 7.68 ppm (H-6), two doublets at δ 8.1 ppm and δ 8.41 ppm (H-5 and H-8) respectively and a broad singlet at δ 8.36 ppm (H-1). In the mass spectrum of **4** the molecular ion peak was observed at m/z 366. A probable mechanism of the photocatalytic reaction is shown in the following equations.

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$$TiO_2 \xrightarrow{h\nu} \overset{-}{e} + \overset{+}{p}$$

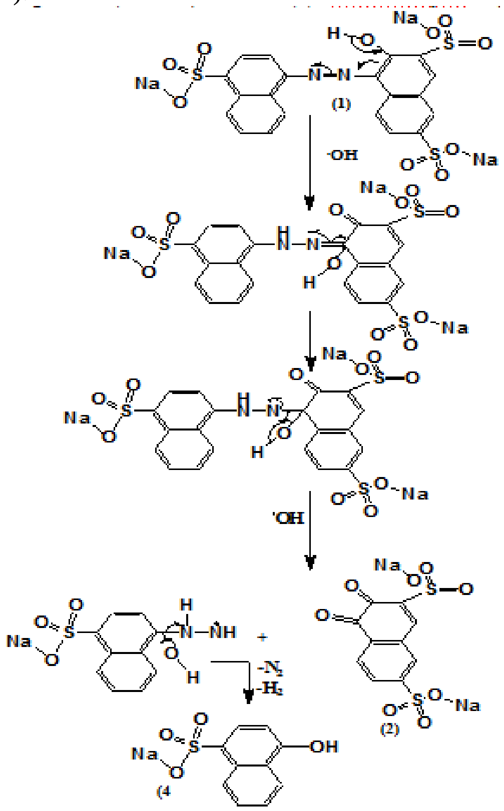
$$P^+ + O\overset{-}{H}\,(\text{water}) \longrightarrow {}^{\cdot}O\,H + TiO_2$$

$$\text{Dye} + p^+(\text{or})\ {}^{\cdot}OH \longrightarrow \text{Dye}_{ox}$$

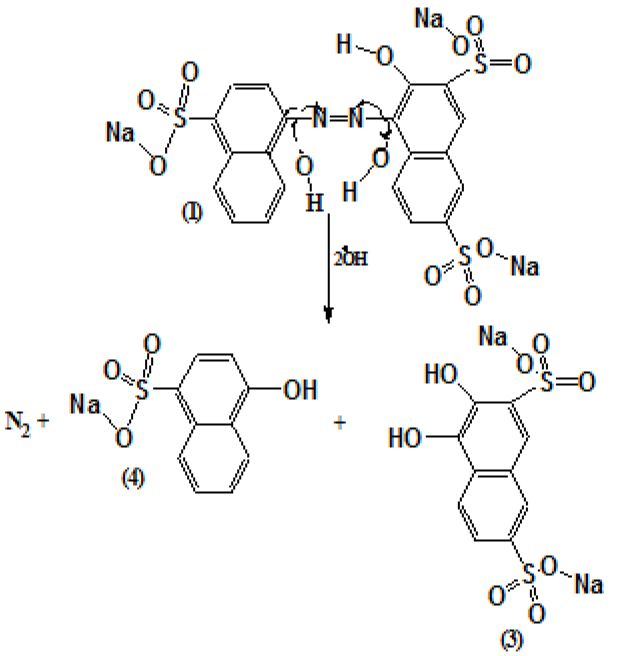
Where (p^+) and (e^-) are the positive hole in the valence band and electron in the conduction band, respectively. Photocatalytic degradation of Amaranth dye proceeds predominantly through oxidation by positive hole (or hydroxyl radical). This type of reaction is activated by absorption of a photon with sufficient energy. The absorption leads to a charge separation due to promotion of an electron (e^-) from the valence band of the semiconductor catalyst to the conduction band, thus generating a hole (p^+) in the valence band. The recombination of electron and the hole must be prevented as much as possible if a photocatalyzed reaction must be favored [17].

The ultimate goal of the process is to have a reaction between the generated holes with a reductant to produce an oxidized product, the photogenerated holes can oxidize the organic molecule to form p^+ ,or react with OH^- hydroxyl anion or H_20 oxidizing them into $OH^{\cdot}$ hydroxyl radical together with other highly oxidant species (peroxide radicals), they are reported to be responsible for the heterogeneous TiO_2 photodecomposition of organic substrates as dyes [17]. According to this, the degradation of dyes can be carried out as in the last equations [18].

Nanoscaled TiO_2 particles have high photocatalytic degradation than microscaled TiO_2 [13]. This result is due to increasing the surface area of the nano molecules and decreasing the area which electrons moved in it so the electron gain more energy [19]. The results are given in (Table 1).



Scheme (2): Photocatalytic Degradation of Amarnath to form 2 & 4 scheme



Scheme (3): Photocatalytic Degradation of Amarnath to form 3 & 4 scheme

On the other hand, the natural Amaranth azo dye [which was extracted] was subjected to photocatalytic degradation process using nanoscaled TiO_2 . It showed high stability towards this reaction; only decolorization has been done to it. This indicates that the Amaranth azo dye was converted from the hydrazine tautomer to hydrazone without any damage of dye molecule.

3.4 DNA Damaging properties of photodegraded compound 3

The present work included also a study on the effect of compound **3** on DNA, which indicated damage to DNA. It is known that some photochemical hydroperoxide compounds cause photochemical DNA damage [20,21]. Therefore, a sample of DNA was mixed with a solution of product **3.** The resulting solution was irradiated using a sodium lamp. The experimental data clearly indicates that compound **3** gave a positive result showing a high degree of DNA degradation, when the irradiation time was prolonged for 60 hours.

3. 5 Spectrophotometric Measurement

It was carried out using ultraviolet photometric apparatus to determine the absorption spectra of Amaranth azo dye before and during the irradiation. The results of UV-spectra of Amaranth are shown in fig 2- 4. It was observed that, the UV-spectrum changed greatly and had a weak absorbance at 520 nm when the time of irradiation prolonged. It is confirmed that the cleavage of –N=N- was complete and the absorbance at 320 and 230 nm also decreased much. λ_{max} was shifted from visible region to UV region, after irradiation; this means that the dye molecule was completely damaged.

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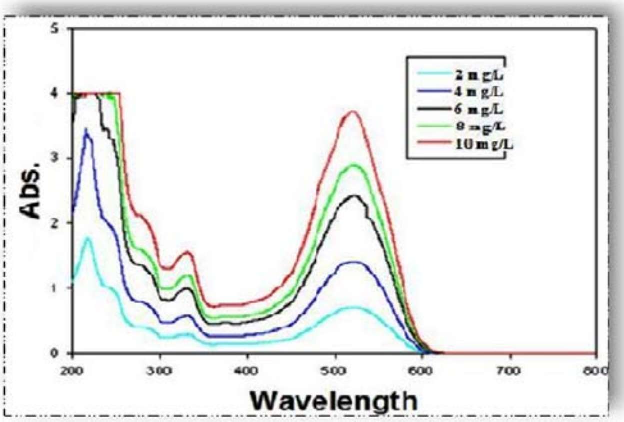

Figure 2: Absorption spectra of Amaranth dye with different Concentration

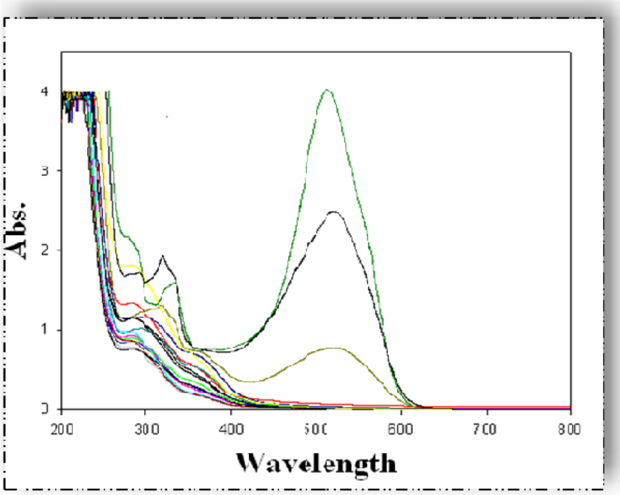

Figure 3: Absorption spectra of Amaranth dye before and after irradiation

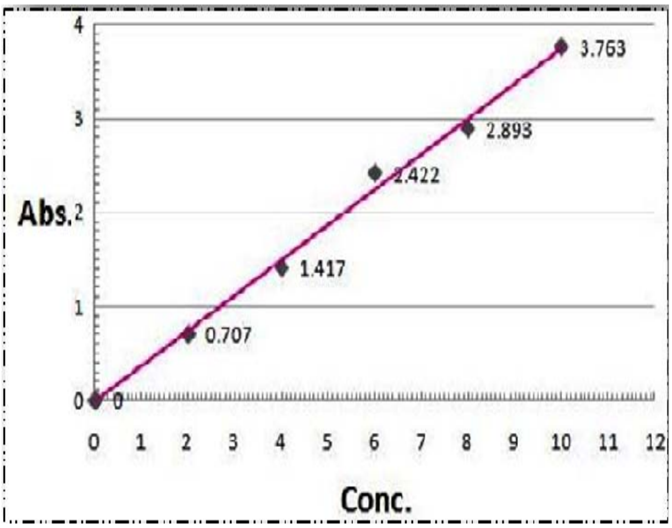

Figure 4: Relation diagram between absorption and concentration of Amaranth dye

Amaranth: Tri sodium salt of 3-hydroxy-4-(4-sulfo-naphthalen-1-ylazo)-naphthalene-2,7-disulfonic acid (1):

Purple powder, m.p.: > 350ºC, $C_{20}H_{11}N_2Na_3O_{10}S_3$ (M. wt., 604.47). IR (KBr disk) ύ, cm^{-1}: 3416.14 (br, OH), 3050 (CH, Str.), 1647.62, 1608.38 (C=C), 1158, 1103 (C-O). ^{1}H-NMR ([D_6]DMSO) δ, ppm : 4.4 (s, 1H, OH), 7.7 (dd, 1H, J = 8،8 Hz, H-6^{-}), 7.78 (dd, 1H, J = 8،8 Hz, H- 7^{-}), 7.85 (d, 1H, J = 8Hz, H-8^{-}), 7.99 (brs, 1H, H-5^{-}), 8.14 (d, 1H, J = 8Hz, H-5), 8.24 (d, 1H, J = 8Hz, H-6), 8.34 (brs, 2H, H-1,8), 8.55 (d, 1H, J=8 Hz, H-3^{-}), 9.00 (d, 1H, J = 8Hz, H-2^{-}). MS, m/z : 604.47 (M^+, $C_{20}H_{11}N_2Na_3O_{10}S_3$) (24.5 %), 500 ($M^+$-$HSO_3Na$) (27%), 377 ($M^+$-$C_{10}H_4SO_3Na$) (23.5%), 229 ($C_{10}H_6SO_3Na$) (26 %), 128 ($C_{10}H_8$) (33 %), 103 ($SO_3Na$) (30 %), 77 ($C_6H_5$) (40 %), 57 ($C_4H_9$) (100 %).

Disodium 3,4-dioxo-3,4-dihydro-naphthalene-2,7-disulfonate (2) :

Colorless powder, m.p.: > 300ºC, $C_{10}H_4O_8S_2Na_2$ (M. wt., 362). IR (KBr disk) ύ, cm^{-1}: 3037.7 (CH,str.), 1693 (C=O), 1500 (phenyl ring), 1184 (C-O).^{1}H-NMR ([D_6]DMSO) δ, ppm : 7.68 (dd, 1H, J =2, 8 Hz, H-6), 8.1 (d, 1H, J = 8Hz, H-5), 8.36 (brs, 1H, H-1), 8.41 (d, 1H, J=2 Hz, H-8). ^{13}C-NMR, (D_2O) δ, ppm : 124.3 (C-8), 125.8 (C-2,6), 127.6 (C-5), 137.7 (C-1^a,4^a), 140.8 (C-1), 142 (C-7), 175.6 (C-3), 176.6 (C-4). MS, m/z : 362 (M^+, $C_{10}H_4O_8S_2Na_2$), 340 (M^++1-Na) (36 %), 284 ($C_8H_5O_6S_2Na_2$) (20 %), 178 ($C_8H_2O_3S$) (31 %), 177 (C_8HO_3S) (100 %), 161 (C_8HO_2S) (80 %), 137 (C_6HO_2S) (13 %).

Disodium 3,4-dihydroxy-3,4-dihydro-naphthalene-2,7-disulfonate (3):

Colorless powder, m.p.: > 300ºC, $C_{10}H_8O_8S_2$ Na_2 (M. wt., 366). IR (KBr disk) ύ, cm^{-1}: 3529.9, 3394.8 (br, OH), 3000 (CH, str.), 1620 (C=C), 1302, 1114.9 (C-O). ^{1}H-NMR (D_2O) δ, ppm :5.55 (s, 1H, OH), 7.4 (d, 1H, J = 8Hz, H-5), 7.63 (d, 1H, J = 8Hz, H-6), 7.72 (brs, 1H, OH), 8.16 (s, 1H, H-1), 8.28 (s, 1H, H-8). MS, m/z : 366 (M^+, $C_{10}H_8O_8S_2Na_2$), 364 (M^+-2) (1.5 %), 336 ($C_9H_6O_7S_2Na_2$) (12 %), 306 ($C_8H_4O_6S_2Na_2$) (1.5 %), 281 ($C_8H_2S_2O_6Na$) (2%), 267 ($C_8H_6O_5S_2Na$) (1%), 154 ($C_4H_3O_3SNa$) (4%), 128(C_2HO_3SNa) (6.5 %), 113 (C_4HO_2S) (3.5 %).

Sodium 4-hydroxy-naphthalene-1-sulfonate (4):

Colorless powder, m.p.: > 300ºC, $C_{10}H_7O_4SNa$ (M. wt., 246). IR (KBr disk) ύ, cm^{-1}: 3550,3450 (br, OH), 3000 (CH, Str.), 1600 (C=O), 1100 (C-O). ^{1}H-NMR (D_2O) δ, ppm : 5.6 (br.,1H,OH), 7.09 (d, 1H, J=8Hz, H-3), 7.50 (dd, 1H, J=8Hz, H-6), 7.51 (dd, 1H, J= 8،8 Hz, H-7),7.78 (dd, 1H, J =8, 2Hz, H-8), 7.9 (d, 1H, J = 8Hz, H-2), 7.93 (dd, 1H, J=8,2Hz, H-5). MS, m/z : 246 (M^+, $C_{10}H_7O_4SNa$) (5 %), 245 (M^+-1) (2 %), 223 (M^+-Na) (5 %), 207 (M^+-NaO) (3 %), 191 (M^+-$HNaO_2$) (10 %), 126 ($C_{10}H_6$) (5 %), 76 (C_6H_4) (10 %).

4. Conclusion

It has been **concluded** that the photo-catalytic and photo-oxidation reactions cause degradation of food additive dyes and gave degraded products. It has been found that adding TiO_2 nano particles into the photo-catalytic reaction accelerated the rate of degradation. It is plausible that such degraded products are produced in situ when these dyes are treated with DNA under irradiation conditions and they might be responsible for some adverse effects in cells. It should, therefore, be relevant to elucidate the biological consequence of these products with DNA and other cell compounds. The genotoxicity of photo-binding DNA

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intercalators, which possess additionally oxidative potential, has so far not been investigated.

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