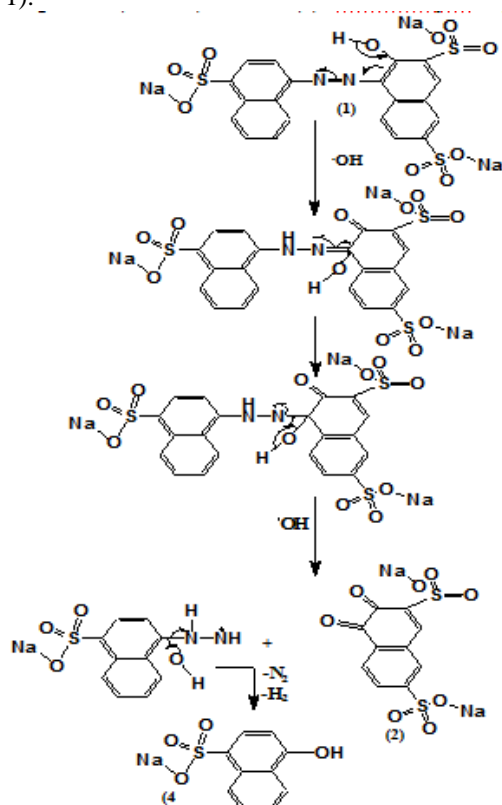


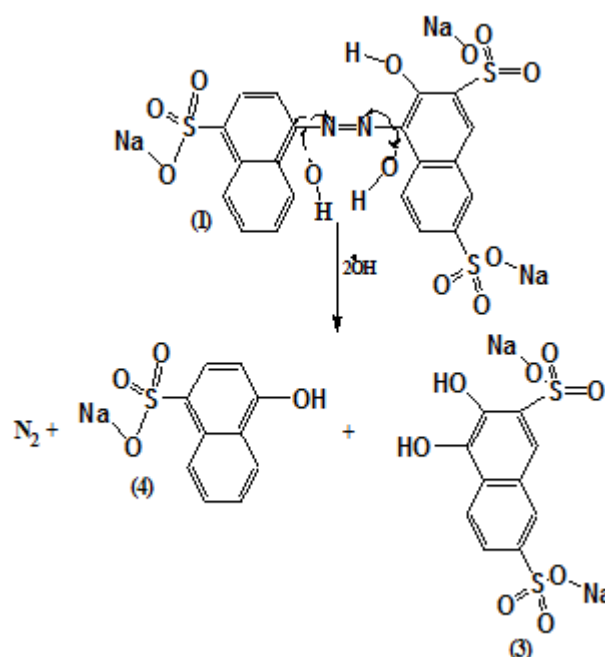
Where (\bar{p}) and (\bar{e}) are the positive hole in the valence band and electron in the conduction band, respectively. Photocatalytic degradation of Amaranth dye proceeds predominantly through oxidation by positive hole (or hydroxyl radical). This type of reaction is activated by absorption of a photon with sufficient energy. The absorption leads to a charge separation due to promotion of an electron (\bar{e}) from the valence band of the semiconductor catalyst to the conduction band, thus generating a hole (\bar{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 \bar{p}^+ , or react with OH^- hydroxyl anion or H_2O 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 Amaranth to form 2 & 4 scheme



Scheme (3): Photocatalytic Degradation of Amaranth 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 $-\text{N}=\text{N}-$ 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.

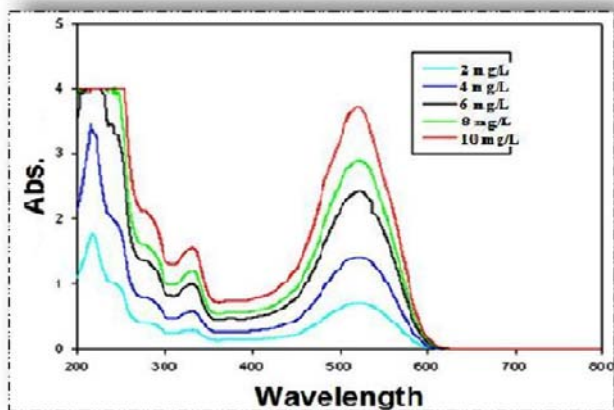


Figure 2: Absorption spectra of Amaranth dye with different Concentration

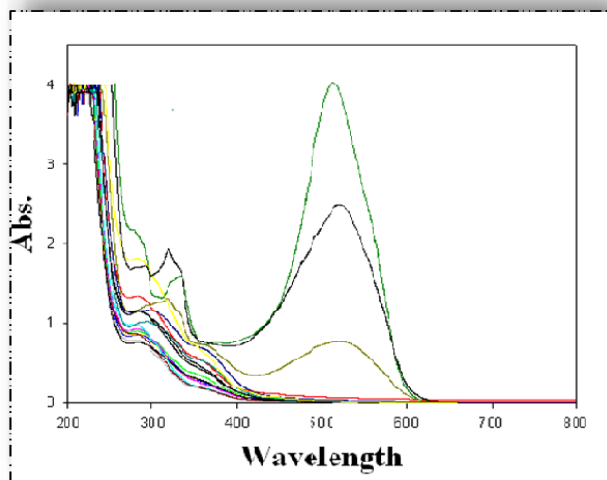


Figure 3: Absorption spectra of Amaranth dye before and after irradiation

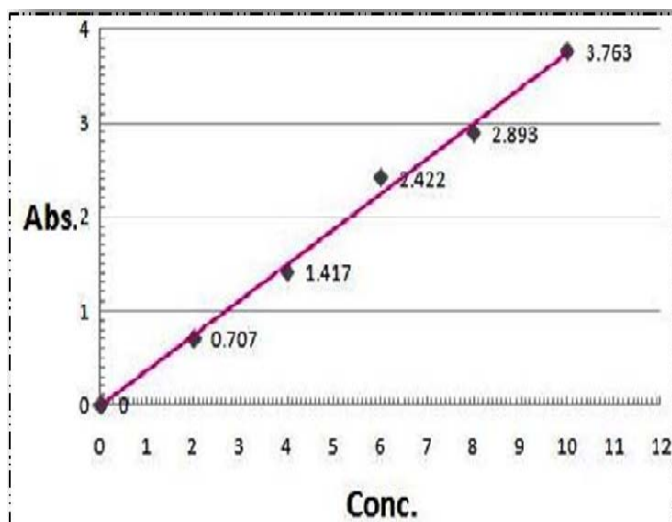


Figure 4: Relation diagram between absorption and concentration of Amaranth dye

Amaranth: Tri sodium salt of 3-hydroxy-4-(4-sulfo-naphthalen-1-ylazo)-naphthalene-2,7-disulfonic acid (1): Purple powder, m.p.: > 350°C, $C_{20}H_{11}N_2Na_3O_{10}S_3$ (M. wt., 604.47). IR (KBr disk) ν , cm^{-1} : 3416.14 (br, OH), 3050 (CH, Str.), 1647.62, 1608.38 (C=C), 1158, 1103 (C-O). 1H -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.: > 300°C, $C_{10}H_4O_8S_2Na_2$ (M. wt., 362). IR (KBr disk) ν , cm^{-1} : 3037.7 (CH, str.), 1693 (C=O), 1500 (phenyl ring), 1184 (C-O). 1H -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.: > 300°C, $C_{10}H_8O_8S_2Na_2$ (M. wt., 366). IR (KBr disk) ν , cm^{-1} : 3529.9, 3394.8 (br, OH), 3000 (CH, str.), 1620 (C=C), 1302, 1114.9 (C-O). 1H -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.: > 300°C, $C_{10}H_7O_4SNa$ (M. wt., 246). IR (KBr disk) ν , cm^{-1} : 3550, 3450 (br, OH), 3000 (CH, Str.), 1600 (C=O), 1100 (C-O). 1H -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

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

5. Acknowledgement

The authors acknowledge and thank the King Abdulaziz City of science and technology for supporting this research project.

References

- [1] Barrows, J.N.; Lipman, A.L. & Bailey, C.J.(2005). Color Additives: FAD's Regulatory Process and Historical Perspectives, *Food Safety Magazine*, Oct./Nov.
- [2] Agostoni, C.; Bresson, J. ; Fairweather-Tait, S.; Flynn, A.; Golly, I.; Korhonen,H.; Lagiou, P.; Løvik,M.; Marchelli,R.; Martin, A.; Moseley, B.; Neuhäuser-Berthold, M.; Przyrembel, H.; Salminen, S.; Sanz, Y.; Strain, S.; Strobel, S.; Tetens, I.; Tomé, D.; van Loveren, H.&Verhagen, H.(2010).Scientific Opinion on the appropriateness of the food azo-colours Tartrazine (E 102), Sunset Yellow FCF (E 110), Carmoisine (E 122), Amaranth (E 123), Ponceau 4R (E 124), Allura Red AC (E 129), Brilliant Black BN (E 151), Brown FK (E 154), Brown HT (E 155) and Litholrubine BK (E 180) for inclusion in the list of food ingredients set up in Annex IIIa of Directive 2000/13/EC1, *European Food Safety Authority*, **8(10)**,1778,1-10.
- [3] Zhang,G & Ma.Y. (2013). Mechanistic and conformational studies on the interaction of food dye amaranth with human serum albumin by multispectroscopic methods, *Food Chemistry*, **136(2)**, 442-449.
- [4] Vig, A.; Sirbiladze, K.; Nagy, H. J.; Aranyosi, P.; Rusznak, I. & Sallay, P. (2007) The light stability of azo dyes and dyeings V. The impact of the atmosphere on the light stability of dyeings with heterobifunctional reactive azo dyes, *Dyes and Pigments*, **72**: 16-22.
- [5] Okada, Y.; Fukuoka, F. & Morita, Z. (1997). Estimation of potential photochemical properties for monochlorotriazinyl reactive dyes and their manifestations in the fading of dyed cellulose, *Dyes and Pigments*, **35 (4)** : 311-330.
- [6] Arana, J.; Diaz, O.G.; Saracho, M.M.; Rodriguez, J.M.D.; Herrera Melian, J.A. & Pena, J.P. (2001). Photocatalytic Degradation of Formic Acid Using Fe / TiO₂ Catalysts : The Role of Fe³⁺ of Fe²⁺ ions in the Degradation Mechanism, *Applied Catalysis*, **B 32**, 49 – 61, Paola, A.D.;G
- [7] Tryba, B.; Morawsk, A.W.; Inagaki,M. & Toyoda, M.(2006). The Kinetic of Phenol Decomposition under UV Irradiation with and ithoutH₂O₂ on TiO₂, Fe-TiO₂ and Fe-C-TiO₂ Photocatalysts, *Applied Catalysis*, **B 63**, 215 – 221.
- [8] Addama, M.; Augugliaro, V.; Garcia-Lopez, E.; Loddo, V.; Marci, G.; Molinari, R.; Palmisano, L. & Schiavello, M. (2004). Preparation, Characterization and Photo-activity of Polycrystalline Nanostructured TiO₂ Catalysts, *Journal of Physical Chemistry B*, **108**, 3303.
- [9] Kansal, S.K.; Singh, M. & Sud, D. (2007). Studies on Photodegradation of two Commercial Dyes in Aqueous Phase Using Different Photocatalysts, *Journal of Hazardous Materials*, **141**, 581.
- [10]Mahmoodi, N.M.; Arami, M.; Limaee, N.Y. & Tabrizi, N.S.(2006). Kinetic of Heterogeneous Photocatalytic Degradation of ReactiveDyesin an Immobilized TiO₂ Photocatalytic Reactor, *Journal of Colloid and Interface Science*, **295**, 159.
- [11]Mahmoodi, N.M.; Liomae, N.Y.; Arami, M.; Borhany, S. & Mohammad – Taheri, M.(2007). Nano – photocatalysis Using Nanoparticales of Titania Mineralization and Finite Element Modeling of Solophenyl Dye Degradation, *Journal of Photochemistry and Photobiology A., Chem.*, **189**, 1.
- [12]Li, F.; Sum, S.; Jiang, Y.; Xia, M.; Sun, M. & Xue, B. (2008). Photodegradation of an Azo Dye Using Immobilized Nanoparticales of TiO₂ Supported by Natural Porous Mineral, *Journal of Hazardous Materials*, **152**, 1037 – 1044.
- [13]Vinodgopal, K. & Peller, J. (2003). Hydroxyl Radical – Medicted Advanced Oxidation Processes for Textile Dyes: A Comparison of the Radiolytic and Sonolytic Degradation of the Mono – azo Dye Acid Orange 7, *Research on Chemical Intermediate*, **29 (3)**, 307 -316.
- [14]Elgendy, E.M. & Khayyat, S.A. (2008). Oxidation reaction of Some natural volatile aromatic compounds: Anethale and Eugenal , *Russian Journal of Organic Chemistry*, **44 (6)** : 823-829.
- [15]Kochevar, I.E. & Dumn, D.A. (1990). Photosensitized Reactions of DNA Cleavage. In M. Morrison (Ed.), *Bioorganic Photochemistry* (**1**, pp. 273). New York: Wiley.
- [16]Hihara, T.; Okada, Y. & Morita, Z.(2007). The Photo – oxidation of Reactive Azobenzene Dyes and an analysis of their Reactivity for the Azo and Hydrazone Tautomers Using the Semiempirical Molecular Orbital PMS Method, *Dyes and Pigments*, **75**, 225 – 245.
- [17]Akpan, U.G. & Hameed, B.H. (2009). Parameters Affecting the Photocatalytic Degradation of Dyes Using TiO₂ – Based Photocatalysts. A Review, *Journal of Hazardous Material*, **170(2-3)**, 520-529.
- [18]Tanaka, K.; Padermpole, K. & Hisanaga, T. (2000). Photocatalytic Degradation of Commercial Azo Dyes, *Water Research*, **34 (1)**, 327 – 333.
- [19]www.mknano.com.
- [20]Epe, B.; Haering, M.; Ramaiah, D.; Stopper, H.; Abou-Elzahab, M. M.; Adam, W. & Saha-Moeller. (1993). DNA damage induced by furocoumarin hydroperoxides plus UV (360 nm), *Carcinogenesis*, **14**: 2271-2276.
- [21]Elgendy, E.M. (2000). Photooxygenation of Visnagin and Xanthotoxin Derivatives, *The Chinese Pharmaceutical Journal*, **52**, 227.