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Assessment of the breakdown products of solar/UV induced photolytic degradation of food dye tartrazine

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ABSTRACT

The food dye tartrazine (CI 19140) was exposed to UV irradiation from an artificial source, a mercury vapor lamp, and a natural one, sunlight. It was observed that conditions such as energy dose, irradiation time, pH and initial dye concentration affected its discoloration. There was 100% of color removal, after 30 min of irradiation, when a dye solution 1×10^{-5} mol L^{-1} was submitted to an energy dose of 37.8 J cm^{-2}. Liquid Chromatography coupled to Diode Array Detection and Mass Spectrometry confirmed the cleavage of the chromophore group and the formation of five by-products at low concentration. Although by-products were formed, the *Salmonella/microsome* mutagenicity assay performed for both, the dye solution at a dose of 5.34 mg/plate and the solutions obtained after exposure to UV irradiation, did not present mutagenic activity for TA98 and TA100 with and without S9.

1. Introduction

It is one of nature's inevitabilities that all colored objects dyed with organic dyes will fade upon exposure to light. In general, the sun's energy is made up of three distinct spectral components: ultraviolet radiation, visible radiation, and near-infrared radiation and all of them are capable of promoting reactions that lead to fading. Fading is influenced by many environmental factors such as the dye chemical structure, chemical environment, environmental temperature, humidity and radiation.

Usually, the UV radiation has enough energy to cause unstable bonds to break or transform the dye structure. Putt et al. (2012) showed in their study that some food, drug and cosmetic (FD&C) dyes, including the azo dye Yellow 5 (also known as tartrazine), are suitable for degradation upon irradiation with UV light. Furthermore, they reported dyes were degraded showing simple decay curves.

The change in the chemical molecule can lead to the formation of new compounds, which can either be more or less harmful to humans. According to Oancea and Meltzer (2013), the lower decrease on the total organic carbonic (TOC) percentage when compared to tartrazine's degradation percentage upon the Photo-Fenton process, 43% and 98% respectively, suggested that the dye is degraded to small molecules, such as aldehydes and organic acids. Even when the irradiation time was prolonged to 120 min there was only a partial degradation of those molecules once TOC decrease did not exceed 80%. Therefore, the understanding of the light effect on dyes, present in the aquatic environment or in food stuff, is important to predict chemical alterations and toxicological properties (Neppolian et al., 2002).

The interest of this work is to study tartrazine (CI 19140) as a model of azo dye widely used in food industry. There is a controversy about its genotoxic potential and other adverse effects (Hutchinson et al., 1992; Pestana et al., 2010; Lockey, 1959). Watabe et al. (1980) showed that primary metabolization mechanism of ingested tartrazine in man is bacterial azo reduction, generating mainly sulphanilic acid. Amin et al. (2010) observed that rats exposed to high dosage of tartrazine (500 mg/kg bw) exhibited hepatocellular damage. The cytotoxicity study of tartrazine, reported by Mpountoukas et al. (2010), indicates that tartrazine at higher concentrations (4 and 8 mM) has significant toxic effect on the quality of chromosomes, probably by affecting the condensation of chromosomes in mitosis. In addition, the acute oral exposure to tartrazine induced DNA damage in mouse colon as observed in the in vivo comet assay (Sasaki et al., 2002). Some studies indicated that tartrazine induced chromosomal aberrations

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Abbreviations: MR, mutagenic ratio; MW, molecular weight.

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in fibroblast cells of *Muntiacus muntjac* (Chung et al., 1981), on bone marrow cells of mice and rats (Giri et al., 1990) and on chromosomes of *Allium cepa* (Roychoudhury and Giri, 1989). Nevertheless, literature also reports studies indicating negative results for genotoxicity/carcinogenicity tests (Kada et al., 1972; Ishidate et al., 1984; Combes, 1986). Therefore, its clear that discussion regarding the toxic potential of tartrazine started long ago and, as it can be seen in the studies cited above, even today there is no clear consensus on this matter. In addition, along with the complexity of the by-products generated due azo dye degradation, few studies are focusing on the influence of light on dye residues present in the wastewater and surface water, due to limitation of the available analytical methods.

Thus, the aim of this work is to investigate the tartrazine degradation under UV and solar light irradiation by using High Performance Liquid Chromatography coupled to Diode Array Detection (HPLC–DAD) and Liquid Chromatography coupled to Mass Spectrometry (LC–MS–MS), mimicking experimental situations where tartrazine is submitted to artificial and sunlight irradiation. Our particular interest was to focus on evidence of harmful by-products generation for a food dye exposed to different irradiation conditions. Understanding the dye biotransformation route under light influence can help us to predict the toxicity of by-products generated. The original dye solution and the breakdown products generated from photolysis were evaluated by using *Salmonella typhimurium* strains TA98 (*his*D3052, *rfa*, *Δbio*, *Δuvr*B, and pKM101) and TA100 (*his*G46, *rfa*, *Δbio*, *Δuvr*B, pKM101) with and without exogenous metabolic activation.

2. Material and methods

2.1. Apparatus and procedure

The photolytic experiments were performed using indoor and outdoor reactors. In the indoor equipment was used a 300 mL single compartment reactor, maintained at 20 °C using a thermostatically controlled water bath (Nova Técnica, Brazil). The dye solution was illuminated using a 125 W Philips medium pressure mercury lamp ($I = 21$ mW/cm^2), without the glass tube and inserted into a quartz bulb, as the UV light source (315–400 nm). The solution was constantly stirred.

The outdoor photolytic experiments were carried out using a solar photochemical reactor, previously described in literature (Trovo et al., 2008). Aliquots were taken after 0, 1, 2, 3, 4, 5, 6 h from the beginning of photochemical treatment. The reaction vessels were held in a rack at a 22° angle from horizontal at the Chemistry Institute, UNESP (Araraquara, São Paulo, 21°50′S, 48°00′W). The experiments were performed in sunny days of October, between 10 am and 4 pm. The ambient temperature ranged between 28 and 36 °C.

UV irradiation, from both artificial and solar source, was measured with an UV-A radiometer Solar Light Co (PMA2100). For comparison of solar and artificial light test results, the UV radiation was evaluated in terms of irradiance, which is defined as the rate at which radiant energy is incident on a surface per unit area of surface (W cm^{-2}) and the energy dosage, received on the illuminated reactor surface (J cm^{-2}) (Eq. (1)) (Trovo et al., 2005):

$$Energy\ dose\ (\mathrm{J\ cm^{-2}}) = \int_{t1}^{t2} Irradiance\ (\mathrm{W\ cm^{-2}}) \times dt(s) \qquad (1)$$

The solar irradiance measured was 0.95 mW cm^{-2} and the artificial light irradiance was 21 mW cm^{-2}.

All pH measurements were taken using a Corning 555 pH meter. In experiments where the pH was to be kept constant, 0.1 mol L^{-1} NaOH solution or H_2SO_4 solution was added to the cell in order to control the pH.

2.2. Analytical methods

2.2.1. UV–Vis

The UV–Vis absorption was recorded with a Hewlett Packard 8453 spectrophotometer operating from 200 to 800 nm and a quartz cell. The discoloration percentage of tartrazine ($\lambda = 427$ nm) was calculated using Eq. (2):

$$\%\ Discoloration = \frac{(A_{t0} - A_{t1})}{A_{t0}} \times 100 \qquad (2)$$

where A_{t0} is the absorbance of the initial tartrazine solution and A_{t1} the absorbance measured at different photolysis time intervals.

2.2.2. HPLC–DAD determination

The High Performance Liquid Chromatography with photodiode array detector (HPLC–DAD) was carried out using a Shimadzu SCL-10AVP equipped with an autosampler. Analysis was performed in a reversed-phase column Shimadzu CLC-ODS (C18) (25 cm × 4.6 mm i.d. × 5 μm, 100 Å). All solutions were previously filtered through a 0.45 μm PTFE filter. The best experimental conditions optimized were mobile-phase consisting of 50 mmol L^{-1} aqueous solution of ionic liquid tetrafluoroborate 1-butyl-3-metylimidazolium, BMIm-BF_4, and acetonitrile (80:20) v/v, flow rate of 0.4 mL min^{-1} and a column temperature of 40 °C. The chromatograms were investigated between 200 and 800 nm, and the maximum wavelengths selected to analyze the tartrazine was 427 nm.

Tartrazine concentration was determined using a calibration curve constructed in the linear range of concentrations between 1 and 9 μmol L^{-1}. Analysis were performed using the HPLC–DAD optimized condition, and each peak area was related to the respective concentration, at 427 nm, through the following equation: $Area = 1.03 \times 10^5 + 2.06 \times 10^{10}$ concentration, $n = 7$, $r = 0.9962$, with detection and quantification limits equal 0.12 and 0.39 μmol L^{-1}, respectively.

2.2.3. LC–ESI–MS/MS

All experiments using Liquid Chromatography coupled to Mass Spectrometry were recorded in an electrospray ionization tandem Mass Spectrometry (ESI–MS/MS) QTrap system (Applied Biosystems 3200 QTrap). The LC system was Agilent 1200 equipped with an autosampler. The Mass Spectrometry measurements were performed in negative ionization mode, ESI(−). Nitrogen was the collision gas. Ion electrospray voltage, ion source temperature and desolvation potential were −4.5 kV, 650 °C and −50 V, respectively. The scan rate was set at 4000 UMA s^{-1} for 400 ms and collision energy of 10, 30 and 50 V. Entrance potential was 8 V and only fragments with m/z between 50 and 600 Da were taken. The previous separations were performed using an Agilent Zorbax C_{18} column (150 × 4.6 mm i.d., 5 μm). The mobile phase system consisted of methanol and an aqueous solution 1 mmol L^{-1} ammonium acetate 70:30 (v/v). The elution was on isocratic mode, the flow rate 0.5 mL min^{-1}, the injection volume 10 μL and the oven temperature 40 °C. After elution, samples were introduced directly into the mass spectrometer without flow splitting. All samples were prepared by dilution in the mobile phase and filtered through a 0.22 μm membrane.

2.3. Chemicals and reagents

The tartrazine dye 85% (CI 19140) was obtained from Sigma–Aldrich and used without purification. HPLC grade methanol and acetonitrile were purchased from Tedia and ultrapure water was prepared by the Milli-Q system (Millipore). For the irradiation experiments tartrazine was dissolved only in ultrapure water.

2.4. The Salmonella/microsome mutagenicity assay

The mutagenicity of the dye and its by-products, obtained after exposure to UV irradiation, was evaluated using the *Salmonella/microsome* assay according to the ISO 16240:2005 (2005) with the *S. typhimurium* strains TA98 (*his*D3052, *rfa*, *Δbio*, *Δuvr*B, and pKM101) and TA100 (*his*G46, *rfa*, *Δbio*, *Δuvr*B, pKM101) with and without exogenous metabolic activation. The metabolic activation was provided by Arochlor 1254 induced Sprague Dawley rat liver S9 mix (MolTox, Boone, NC), which was prepared at a concentration of 4% (v/v). For TA98 and TA100, the positive controls were 4-nitroquinoline-1-oxide (4NQO) (Sigma–Aldrich) at 0.5 μg plate^{-1} and 2-aminoanthracene (2AA) (Sigma–Aldrich) at 2.5 μg plate^{-1}, both dissolved in dimethylsulfoxide. Sterile water was used as negative control. The test was conducted using a single dose for each treatment. For each plate, 0.5 mL of sodium phosphate buffer or S9 mix and 1 mL of sample solution were added. Sample solutions were sterilized by filtration (0.45 μm). The test was performed in single doses of 5.34 μg per plate using two replica plates. After 66 h incubation at 37 °C, the colonies were manually counted under a stereoscope. Before each test, samples were checked for contamination using nutrient agar plates. Single maximum doses were tested in duplicates because the volumes of the treated solutions were not enough for dose response experiments.

Toxicity was also evaluated by visual observation under a stereoscope. Results are expressed as the number of revertants μg^{-1} equivalent of the sample per plate. The ratio mutagenic was calculated for each sample, as the average number of revertants per plate divided by the average number of revertants per plate in the negative control. A sample was considered positive when the ratio mutagenic was ⩾2.

3. Results and discussion

3.1. Dye discoloration

Fig. 1a shows the absorption spectra obtained for 1×10^{-5} mol L^{-1} dye solution before and after exposure to UV irradiation for 4 h (302.4 J cm^{-2}). The characteristic absorption bands of the