

Radiation Degradation and Hemolytic Toxicity Evaluation of Mono Azo Reactive Dyes

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Summary: Monoazo reactive dyes have been synthesized and subjected to degradation before their application. Advanced oxidation process has been recognized as a promising radiation technology for the remediation of hazardous organic compounds. Radiation induced degradation of two mono azo reactive dyes have been tried at different absorbed dose, 5 kGy, 10 kGy and 15 kGy. Aqueous solutions of these dyes were treated with gamma radiation using Cs ¹³⁷ radiation source at Nuclear Institute of Agriculture and Biology (NIAB) Faisalabad. Dyes were evaluated spectrophotometrically by UV-visible and fourier transform infra red (FT-IR) spectroscopic techniques before and after irradiation to analyse their percentage decolorization and degradation. Maximum percentage decolorization of 93% and 63% was achieved for mono azo dyes D₁ and D₂ at 15 kGy absorbed dose. Toxicity study of these dyes was also tested by haemolytic activity assay. Percentage haemolytic activity of untreated dyes was found within permissible limit showing non toxicity of dye solutions.

Keywords: Mono azo reactive dyes; Irradiation; Percent decolorization; Degradation; Haemolytic activity

Introduction

Dyes from textile industries are released to the environment through wastewater. A large amount of dye is utilized in the textile industry and new dyes are also being developed for better fixation onto the fabric [1]. Every year more than 100,000 kinds of synthetic commercial dyes (~700,000 tons) are used in the world. Up to 15% of the dyes find its way to the wastewater streams [2]. Dyes are very reactive chemically and much harmful to the components of ecosystem both at macro and molecular levels [3]. Dyes are also known as carcinogens [4]. Furthermore, degraded products from dyes may be toxic to aquatic life and even carcinogenic to human life [5]. Most textile dyes are stable to thermal and photolysis and do not degrade with ease in the environment.

Dyes from textile, printing industries and other commercial activities have been a focus of environmental remediation in the last two decades [6-7]. Dyes containing waste has been considered as an important source of water pollution because dyes are highly toxic and colored water is undesirable.

Azo dyes are much stable residual surfactants give fine, deep and shocking shades to the textiles but are toxic and textile wastewater contains harmful additives also part from these dyes. High concentration of azo dyes is resistant to the conventional biological treatment. The common biological processes of degradation and discoloration

on modern dyes are ineffective because of high degree of aromaticity in dye molecule. The conventional physical methods such as using active carbon, filtration, reverse osmosis and coagulation are costly; moreover these methods do not degrade the dye completely and just change its phase from one to another [8]. In recent years, advanced oxidation processes (AOPs) have been developed to deal with the problem of destruction of dyes in aqueous systems. Reactive dyes are harmful to the environment, to resolve this problem, several advanced oxidation processes have been investigated, such as TiO₂-mediated photocatalytic oxidation, UV/H₂O₂ process, fenton process and ultrasonic irradiation etc [9]. Radiation and photochemical methods also fall in AOP's.

Radiation technology has been recognized as a promising tool for the treatment of hazardous organic residues present in wastewater. Most of the colored materials undergo bleaching or decoloration when exposed to ionizing radiation. Therefore, high energy radiation such as gamma rays and electron beam irradiation can be considered suitable for the treatment of waste water from textile industries.

The present work aimed at investigating the decoloration and degradation of mono azo reactive dyes. Two mono azo reactive dyes have been synthesized and their aqueous solutions have been exposed to radiation induced degradation.

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Spectroscopic techniques i.e. UV-Visible and FT-IR have been employed in order to characterize the un-irradiated and irradiated aqueous solutions of dyes. Toxicity study of these synthesized mono azo reactive dyes has also been performed by evaluating the haemolytic activity.

Experimental

Two mono azo reactive dyes were synthesized at Radiation Chemistry Lab, Department of Chemistry, University of Agriculture, Faisalabad and their aqueous solutions were prepared to study the degradation effect of gamma radiation. UV/Visible spectroscopy and FT-IR spectroscopy were used to study the percent decolorization and degradation. Dyes were also evaluated for toxicity to human blood. Chemicals used were of analytical grade and triply distilled water was used for the preparation of sample dye solutions.

Irradiation

Aqueous solutions of dyes D₁ and D₂ were irradiated to 5, 10 and 15 kGy absorbed doses at Nuclear Institute for Agriculture and Biology (NIAB) Faisalabad by gamma ray source Cs¹³⁷. The gamma ray source was calibrated by Frickey dosimeter. Samples were irradiated at ambient temperature and immediately stored at 4°C in dark.

Spectrophotometric Analysis

The dye solutions were analyzed spectrophotometrically before and after treatment using double beam UV-Visible Spectrophotometer (U-2001 Hitachi, Japan), triply distilled water was used as a blank. The percentage of decolorization was calculated from the difference between initial and final values of absorbance at λ_{\max} using the following formula [10]:

Decolorization(%)=

$$\frac{\text{Initial absorbance value} - \text{Final absorbance value}}{\text{Initial absorbance value}} \times 100$$

For fourier transform infrared analysis dye powder was ground with Potassium bromide (KBR powder), pressed to form a uniform disk [11] and then analyzed by FT-IR (Bruker Tensor 27) instrument at Pakistan Council for Scientific and Industrial Research (PCSIR) Laboratories Complex Lahore.

Toxicity Test

Fresh heparinized blood was subjected to washing three times by centrifugation in isotonic Phosphate buffer (PBS) at room temperature keeping pH 7.0. After centrifugation upper buffy coat was removed and red blood cell (RBC) suspension of the pellet was prepared PBS. Aliquots of RBC suspension plus dye solution were shaken in dye bath at 37°C. After that centrifugation was done in tabletop Remi-centrifuge to allow broken and unbroken cells to settle down. For standard 100% hemolysis, Triton (in PBS) was added to red blood cell suspension. The supernatant was removed and the liberated hemoglobin in the supernatant was measured spectrophotometrically as absorbance (ABS) at their specific λ_{\max} [12]. The experiment was carried out in triplicate and mean \pm S.D. was calculated. Percentage hemolysis was calculated by using the following formula:

$$\text{Hemolysis (\%)} = \text{HbABS} / \text{Hb100\%} \times 100$$

Statistical Analysis

All the experiments were conducted individually three times. Data for decolorization and toxicity assessment of mono azo dye 1 and mono azo dye 2 are presented as Mean \pm SD (standard deviation) of three individual determinations.

Results and Discussion

Two mono azo reactive dyes D₁ and D₂ were subjected to gamma radiation treatment for degradation. Structures of reactive dyes are shown in Fig. 1 and Fig. 2.

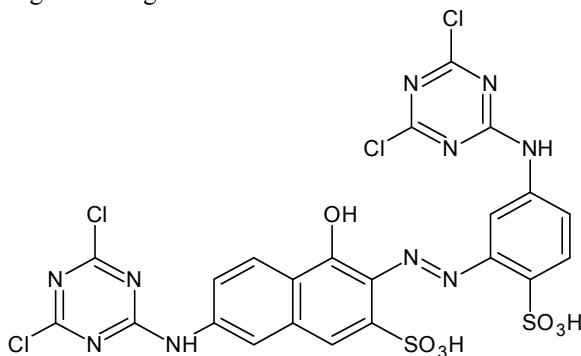


Fig. 1: Structure of mon azo reactive dyes D₁

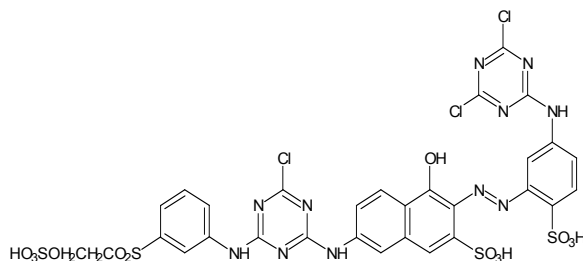


Fig. 2: Structure of mon azo reactive dyes D₂

FT-IR and UV-Visible spectroscopic methods were employed for the characterization of the synthesized dyes and for the evaluation of their degradation through radiation.

UV/Visible Spectroscopic Analysis

UV/Visible Spectroscopy was used for the study of percentage decolorization after radiation treatment. It was clearly observed that absorption maxima (λ_{max}) were shifted to the ultraviolet region after irradiation. It means that interaction of radiation lead to breakdown of colouring chromophoric azo group and new products were formed via degradation. The degraded product might be any reactants or intermediates or new products formed during oxidation process such as lower molecular mass carboxylic acids or aldehydes. Spectra showed λ_{max} value of dye D₁ and D₂, at 488.8 nm and 489.6 nm, which is in the visible region of spectrum, after irradiation this maximum wavelength, was shifted to the ultraviolet region after irradiation of dye samples at 5, 10 and 15 kGy absorbed doses. When dye D₁ was irradiated at 5 kGy, 10 kGy and 15 kGy, percentage decolorization calculated was found to be 55%, 69% and 93% respectively, as shown in Fig. 3. In another study monoazo dye Alizarin Yellow GG was degraded by gamma radiations and it was found that decolorization was increased by increasing dose and maximum value (65%) was obtained at 9 kGy absorbed dose [13].

UV/visible absorption spectrum of mono azo dye D₂ showed λ_{max} at 489.6 nm, while after irradiation breakdown of dye molecule took place and maximum absorption was shifted towards lower wavelength region. The absorption at 200 nm region corresponds to the absorption of fatty acids, it meant dyes were oxidized by oxidative species and then converted to lower molecular weight products and finally to carbon dioxide [14]. Percentage decolorization was found to be 50%, 64% and 69 % at 5 kGy, 10 kGy and 15 kGy absorbed dose, respectively, as shown in Fig. 4.

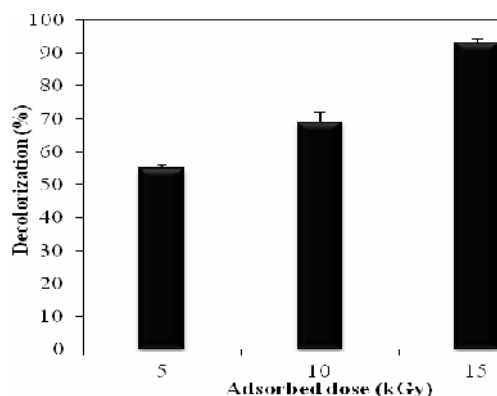


Fig. 3: Decolorization (%) of mono azo dye D₁ at absorbed dose of 5 kGy, 10 kGy and 15 kGy absorbed dose.

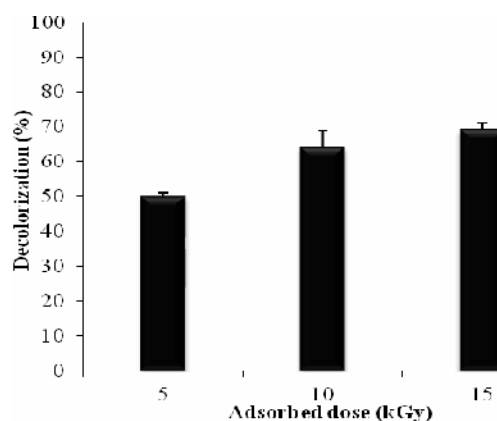


Fig. 4: Decolonization (%) of mono azo dye D₂ at absorbed dose of 5 kGy, 10 kGy and 15 kGy absorbed dose

Fourier Transform Infra Red Analysis

Fourier transform infra red analysis of dyes was carried out before and after irradiation. Fig. 5 shows FT-IR spectrum of synthesized non-degraded dye D₁ and degradation effect of radiation at 5, 10 and 15 kGy absorbed doses. Non degraded dye sample shows many characteristic peaks of different functional groups, the most important of our concern are imino and azo group peaks.

Different peaks observed were 3741.90 cm^{-1} (-NH), 2875.86 cm^{-1} (-CH₂), 2310.72 cm^{-1} , 1747.51 cm^{-1} (-C=O), 1558.48 cm^{-1} (-N=N-), 1479.40 cm^{-1} (aromatic C=C double bond), 1394.53 cm^{-1} (-S=O), 1138.00 cm^{-1} (-S=O), 1012.63 cm^{-1} (-C-Cl), 950.91 cm^{-1} (aromatic C=C double bond), 798.53 cm^{-1} (aromatic C=C double bond), 761.88 cm^{-1} (aromatic C=C double bond) and 677.01 cm^{-1} (aromatic C=C

double bond). It was observed that after radiation treatment (5 kGy), main peaks of non degraded dye sample were vanished and functional peaks developed were 3346.50 cm^{-1} and 1635.64 cm^{-1} . FT-IR spectra of radiated dye sample at 10 kGy showed peaks at 3348.42 cm^{-1} and 1635.64 cm^{-1} and after irradiation at 15 kGy characteristic peaks observed were 3348.42 cm^{-1} and 1637.56 cm^{-1} , attributing to the breakdown of main dye molecule. It is very clear that after degradation a new peak was observed at 1635.64 cm^{-1} which is in accordance with already reported results [15], they studied the degradation of methyl orange dye by gamma rays and reported the appearance of new peak at 1647 cm^{-1} . It was observed after treatment (5kGy) that main peaks of non degraded dye samples were vanished and new characteristic peaks of -C=O group were developed. However during oxidation process low molecular mass carboxylic acids were formed and same kind of degradation trend was observed for the dye solutions irradiated to 10 and 15. FT-IR profile showing functional group peaks of dye D₂ such as imino, hydroxyl, azo, sulfonic, aromatic double bond groups and background peaks and after irradiation are shown in Fig. 6.

After irradiation, few peaks were observed including hydroxyl and carboxylic groups characteristic of carboxylic acids which are formed during degradation. Mono azo reactive dye D₂ was characterized by FT-IR before and after treatment to study the effect of gamma radiations. Before radiation treatment peaks were observed at 3741.90 cm^{-1} (-NH), 2927.94 cm^{-1} (-CH_2), 1745.58 cm^{-1} , 1558.55 cm^{-1} (-N=N-), 1479.40 cm^{-1} , 1388.75 cm^{-1} (-S=O), 1138.00 cm^{-1} (-S=O), 1014.56 cm^{-1} (-C-Cl), 950.91 cm^{-1} (aromatic C=C double bond) and 754.17 cm^{-1} (aromatic C=C double bond) corresponding to hydroxyl, amino, azo, sulfo and substituted benzene ring peaks. When dye solution was exposed to 5 kGy absorbed dose, FT-IR spectrum exhibited bands at 3346.50 cm^{-1} and 1637.56 cm^{-1} , which might be attributed due to the presence of hydroxyl, carbonyl group. FT-IR spectrum of irradiated dye displayed peaks at 3346.50 cm^{-1} and 1635.64 cm^{-1} when irradiated at 10 kGy absorbed dose. When dye was irradiated to 15 kGy peaks observed were 3348.42 cm^{-1} and 1635.64 cm^{-1} . Therefore, FT-IR pattern clearly depicts that color imparting group (azo) has been disappeared and new group which is not present in dye has been developed.

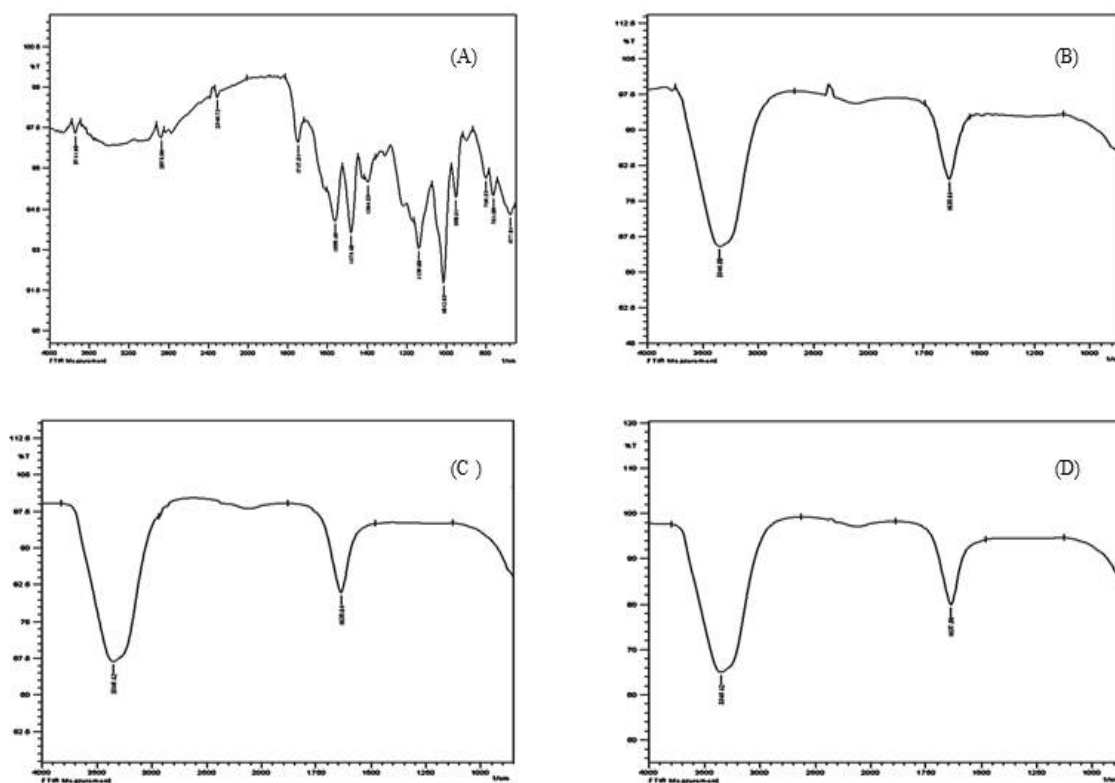


Fig. 5: FT-IR spectrum of dye D₁ before degradation (A) after irradiation at 5kGy (B),10 kGy (C) and 15 kGy (D) absorbed dose.

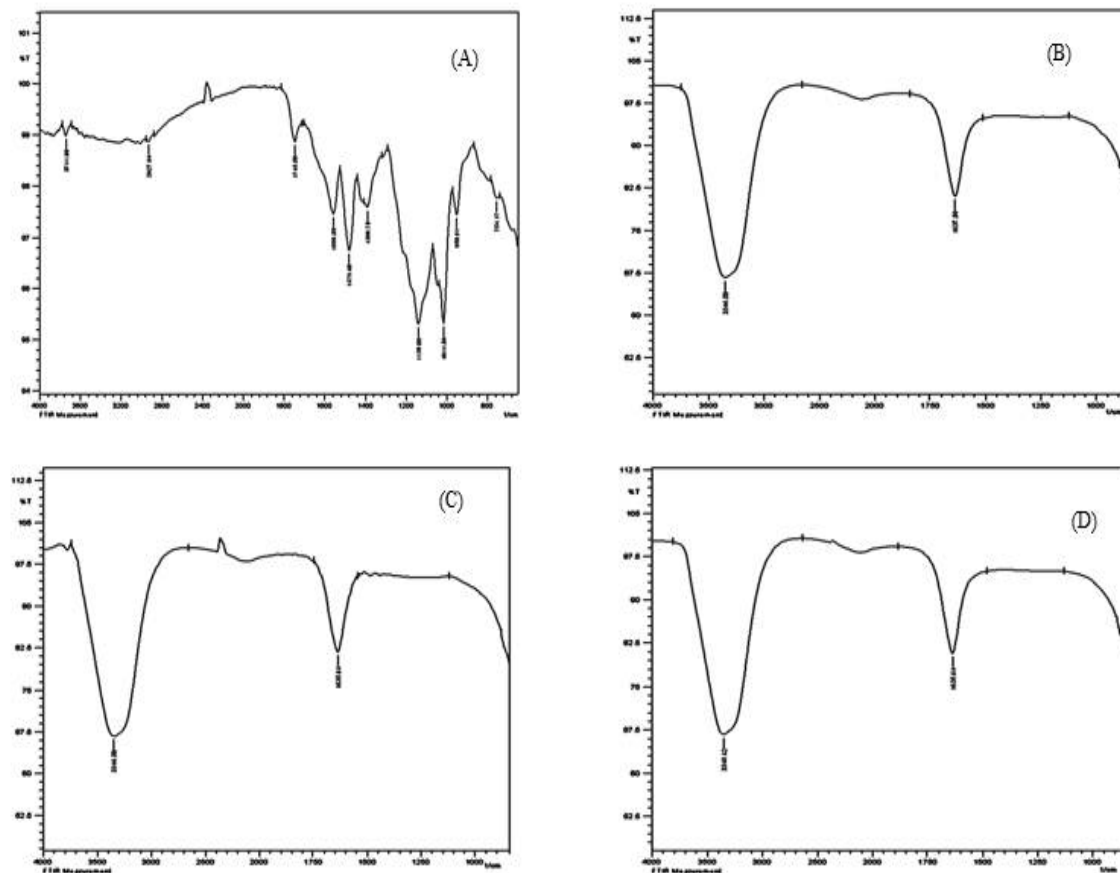


Fig. 6: FT-IR spectrum of dye D₂ before degradation (A) after irradiation at 5kGy (B), 10 kGy (C) and 15 kGy (D) absorbed doses

Toxicity Study

Synthesized dyes were tested for extent of toxicity on hemolytic activity and results were compared with hundred percent hemolysis. Results indicated that dye D₁ and D₂ have only 1.31 and 1.42 percentage lysis of human erythrocytes respectively with mean and standard deviation of ± 0.23 and ± 0.13 . When these results are compared with permissible limit for hemolytic toxicity which is 5.0% we can conclude that dyes may have only minor toxicity to human cells [16].

Conclusions

Both synthesized mono azo reactive dyes were decolorized and degraded by gamma ray treatment to the absorbed doses of 5, 10 and 15 kGy. UV/visible and fourier transform infra red analysis confirmed the decoloration and degradation of the dyes. It was found that λ_{max} was shifted and the colour of dye was removed. FT-IR study indicated that characteristic peaks of synthesized dyes appeared in

the spectra before treatment were vanished after irradiation. Value of percentage hemotoxicity was also much low which indicated that dyes did not exhibit toxicity. Since monoazo reactive dyes can be effectively degraded and decolorized by gamma ray treatment therefore, advanced oxidation process is a promising technique that could be used for the treatment of dye effluents.

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