



Technical Note

Liquid chromatography tandem mass spectrometry analysis of photodegradation of a diazo compound: A mechanistic study

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ABSTRACT

The photolytic degradation of the diazo dye, Amido Black, using UV/H₂O₂ has been carried out experimentally and parameters for most efficient dye degradation have been determined. The degradation of the dye was followed by UV–Vis spectroscopy, HPLC, and LC–MS and is proposed to be initiated by ·OH radicals formed by the photolysis of H₂O₂. A detailed study was also carried out using LC–MS and LC–MS/MS to determine the degradation pathway of the dye as well as to identify some of the intermediate products formed. Our results suggest that Amido Black degradation occurs preferentially by ·OH radical attack at the more electron rich diazo functionality of the molecule. Furthermore, evidence is presented that subsequent steps in this diazo dye degradation pathway include radical denitration, radical desulfonation and radical diazotization. This report is one of the very few studies that have proposed possible mechanistic pathways for the degradation pathways of a diazo compound.

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1. Introduction

Advanced oxidation process (AOP) based on UV/H₂O₂ photolytic decoloration is a well documented simple method which has also shown promising results in degrading many other organic compounds (DeLaat and Gallard, 1999; Lee et al., 2003; Rauf et al., 2008). The process offers a highly reactive, non-specific oxidant namely ·OH radicals in solution in the presence of UV light. These radicals are capable of destroying organic pollutants by reacting with them to undergo a series of reactions in which the organic molecule is finally decomposed or converted into simple harmless compounds. The degraded products in the case of many organic compounds have been identified by employing various analytical techniques (Baiocchi et al., 2002; Gosetti et al., 2008; Prevot et al., 2008). Amongst these techniques, HPLC and LC/MS are most useful and widely used because of their sensitivity. LC/MS technique is versatile and has been used extensively for various product analyses such as environmental pollutants including dyes (Holčápek et al., 2007; Sakalis et al., 2007; Mai et al., 2008; Rauf et al., 2010). Characterization of products formed during various AOPs by using LC–MS technique has been employed in many cases (Schröder, 1996; Epolito et al., 2005; Wu et al., 2007; Zhang and Laursen, 2009). For example, LC–MS has been used for the separation, identification and characterization of the photocatalytic

degradation products of Victoria Blue, a triphenylmethane dye (Mai et al., 2008). The identified degradation intermediates were used to propose a degradation mechanism which involves free ·OH radicals. We have previously studied the photocatalytic degradation of Methylene Blue (a thiazin class of dye) and the products were identified using the LC–MS/MS technique (Rauf et al., 2010). The intermediate structures were confirmed and a suitable mechanism was suggested for dye degradation. Sakalis et al. (2007) have used LC–diode array detection–MS/MS to study the degradation of electrochemically treated acid yellow azo dye. They proposed simple degradation mechanisms based on the tandem mass spectrometric identification of several degradation products. In all of the above studies the mode of degradation involved the initial attack of the ·OH radicals on the dye molecule followed by further reactions resulting in the formation of various intermediates. However, limited information is available in terms of elucidating the intermediate products of a diazo dye with various functional groups.

In this paper an attempt was made to investigate the intermediates produced during the degradation of Amido Black dye (a diazo dye) in the presence of H₂O₂ and UV light. This particular dye was chosen because it has a variety of functional groups such as the sulfonate, nitro, aryl, amine, hydroxyl and azo groups. These groups will influence the cleavage pathway of the parent molecule and their intermediates. The overall emphasis was on finding out the various intermediates (using LC–MS/MS data) formed during the course of the reaction and proposing a suitable mechanistic pathway for the dye degradation.

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2. Materials and methods

2.1. Preparation of samples and decoloration studies

Amido Black (C.I name Acid Black 1, C.I number 20 470, MW = 616.5 g mol⁻¹, purity >95%) was obtained from Fluka and used as such. Stock solution of 1 mM for the dye was prepared in 100 mL of deionized water in a 250 mL flask. Necessary dilutions of this stock were performed with deionized water to obtain a series of dye solutions with varying concentrations. Hydrogen peroxide (35% w/w) was obtained from Merck and was freshly diluted in water before use. A known amount of the diluted solution was mixed with a given amount of H₂O₂ and the mixture was irradiated with UV light. After a given interval of time, the absorbance of the solution decreased (indicative of degradation of the dye molecule). Photolytic oxidation studies were carried out at 25 ± 2 °C.

Changes in absorbance value of the dye were monitored on a CARY 50 UV/Vis spectrophotometer, using a 1 cm cell. For photolytic experiments, the samples were irradiated with a UV lamp (UVGL-58, J-129, Upland). The instrument operates at 0.12 A with a UV power output of 6 W at 365 and 254 nm; however, the lamp was selectively used in the 254 nm output mode (by using appropriate cut-off filters) for these studies. The light intensity was calculated to be 300 W m⁻². The change in absorbance value of Amido Black at its λ_{max} value (618 nm) reflected the degradation of the dye molecule. A 50 mL dye solution taken in a beaker was exposed to a stand mounted UV lamp at a distance of 4 cm and after appropriate time intervals 3 mL of the solution was removed from the beaker and scanned in the spectrophotometer for absorption values.

2.2. Product Analysis and identification using LC–UV/Vis–MS/MS studies

The chromatographic experiments using HPLC–UV/Vis system were carried out on a HP1100 LC (Agilent, USA) coupled with a binary solvent gradient pump and an automatic injector. The dye degradation products were separated using Agilent Zorbax SB-C₁₈ column 150 mm × 4.6 mm packed with 5 μ m particle size. The detection system was a diode array detector (Agilent, USA) with detection range between 200 and 780 nm. The signal acquired from the detector was recorded by HP Chemstation software. The mobile phase consisted of two solutions namely A and B. Solution A was made from 0.1 M ammonium formate (pH 6.7), whereas solution B was made from a mixture of acetonitrile + methanol (1:1). The gradient elution was from 0% to 80% in 40 min; the flow rate was 1 mL min⁻¹ and the injection volume was 100 μ L.

The gradient HPLC separation was coupled with LC/MSD trap 6310, ion trap mass spectrometer (Agilent technologies). The mass spectrometer was equipped with an electrospray ionization (ESI) source and operated in positive polarity. The ESI conditions were as follows: capillary voltage: 3.5 kV, endplate offset was fixed at 500 V; skimmer at 40 V; trap drive at 53 V; the nebulizer pressure was 483 kPa; drying gas flow was 12 L min⁻¹ and drying temperature was 350 °C. The mass range was from 50 to 700 Da. Tandem MS experiment was done using the Auto MSⁿ mode wherein Helium gas was used as a collision gas.

3. Results and discussion

3.1. Degradation of dye

The molecular structure of the dye is shown in Fig. 1, along with its absorption spectra in aqueous solution. Initial experiments with either UV light or H₂O₂ alone did not cause any significant changes

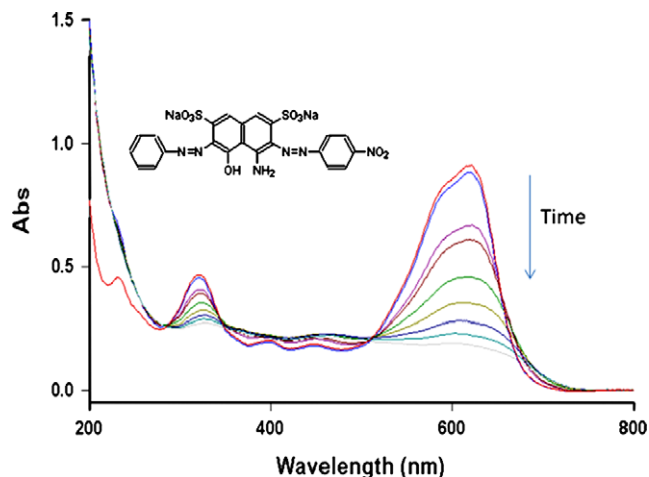


Fig. 1. UV/Vis spectral changes of Amido Black during UV/H₂O₂ treatment ([dye] = 20 μ M; [H₂O₂] = 3.3 mM, pH = 7).

in the degradation of this dye. However, the dye started degrading immediately in the presence of H₂O₂ and UV radiation together. Changes in the absorption spectra of the dye solution were monitored at regular time intervals.

Hydroxyl radicals have a very short lifetime, so that they can only react where they are formed. The lifetime of \cdot OH radicals is approximately 70 ns in the presence of 1 mM phenol, and its diffusion coefficient is estimated to be 2.3×10^{-5} cm² s⁻¹ (Land and Ebert, 1967). The radical can diffuse through an average distance of 180 Å and thus oxidation reactions can be successfully performed in homogeneous media. Since the hydroxyl radicals generated in solution remain in the vicinity of the dye molecule during their short lifetime, it would be safe to say that they are probably the main source of initiating the degradation reaction of the dye molecule.

Various parameters, such as dye concentration, H₂O₂ concentration, and solution pH, which could affect the degradation of the dye, were also investigated. We found that the dye degradation (the apparent rate as well as the % decoloration) was very much dependent on the initial dye concentration, concentration of H₂O₂ used, as well as the pH of the solution (data available in Supplementary material (SM), Table SM 1–3). Moreover, it was found that the kinetics of the Amido Black dye degradation followed the pseudo first order kinetics as shown in Fig. SM-1. In order to ensure that reasonable amounts of breakdown products would be obtained for analyses, the dye degradation conditions chosen for the degradation mechanism studies were as follows: 20 μ M Amido Black, 3.3 mM H₂O₂, and pH 7.

3.2. LC–UV/Vis–MS studies for product analysis

The absorption spectra of the dye solution + H₂O₂ were recorded before and after various irradiation times and are shown in Fig. 1. The long wavelength band at 618 nm could be attributed to the highly conjugated system of azo functionality linked to aromatic substituents, whereas, the two short wavelength bands at 226 and 318 nm could be attributed to the naphthalene and *p*-nitrobenzene parts, respectively.

Fig. 2 shows the HPLC spectra of the dye at different time intervals of irradiation. It is evident that the H₂O₂/UV process is not involved only in the decoloration of the dye, but also in its degradation at a slower rate. A 3D plot (Fig. SM-2) of the dye solution at 0, 10, and 30 min of irradiation provides a clear comparison of the changing composition of various species which are being

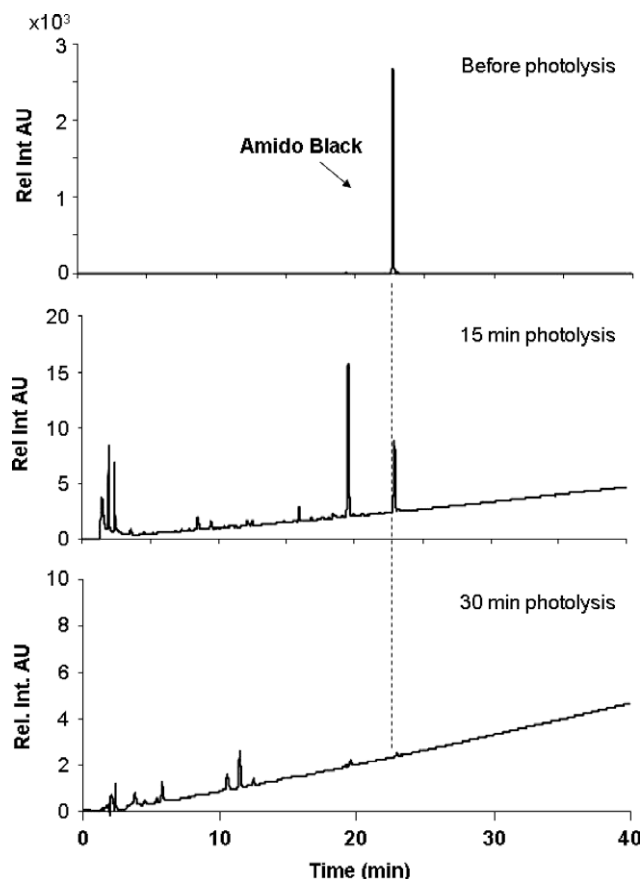


Fig. 2. HPLC chromatograms of Amido Black dye before and after photolysis for 10 and 30 min., using UV/Vis detection at wavelength 618 nm.

formed at different time intervals. As expected, an intermediate that was observed at 10 min was further degraded by the reactive $\cdot\text{OH}$ radicals to give much simpler compounds by the end of the experiment.

To gain further understanding of the various types of product formation in the photolytic degradation of Amido Black dye, mass spectrometric (MS) analysis was carried out and the results are shown in Fig. 3. Many peaks of different intensities were observed in the LC–MS chromatogram of the dye after 30 min of H_2O_2 and UV treatment (see Fig. 3a). As an example the extracted ion chromatogram and MS scan of an intermediate with mass of 521 Da are shown in Fig. 3b and c, the MS/MS spectrum of which was used to predict its structure (see Fig. 3d). Fig. 4 shows the proposed structures of other degradation products based on the MS/MS studies. The MS/MS data are provided in Fig. SM-3a–3j. These structures show that out of the many different functional groups present in the dye, only the amino and hydroxyl functionalities were intact after 30 min of irradiation. This may be attributed to the selective attack of $\cdot\text{OH}$ radical onto the dye molecule.

3.3. Mechanism of intermediate formation

Dye degradation resulting in various intermediates were distinguished by using the LC/MS technique, however a plausible mechanism for the formation of these intermediates is an important aspect towards the overall understanding of diazo dye degradation. On the basis of the information collected from our MS/MS studies three major degradative routes based on radical mechanisms can be distinguished. These are listed below and are depicted in Schemes 1–3.

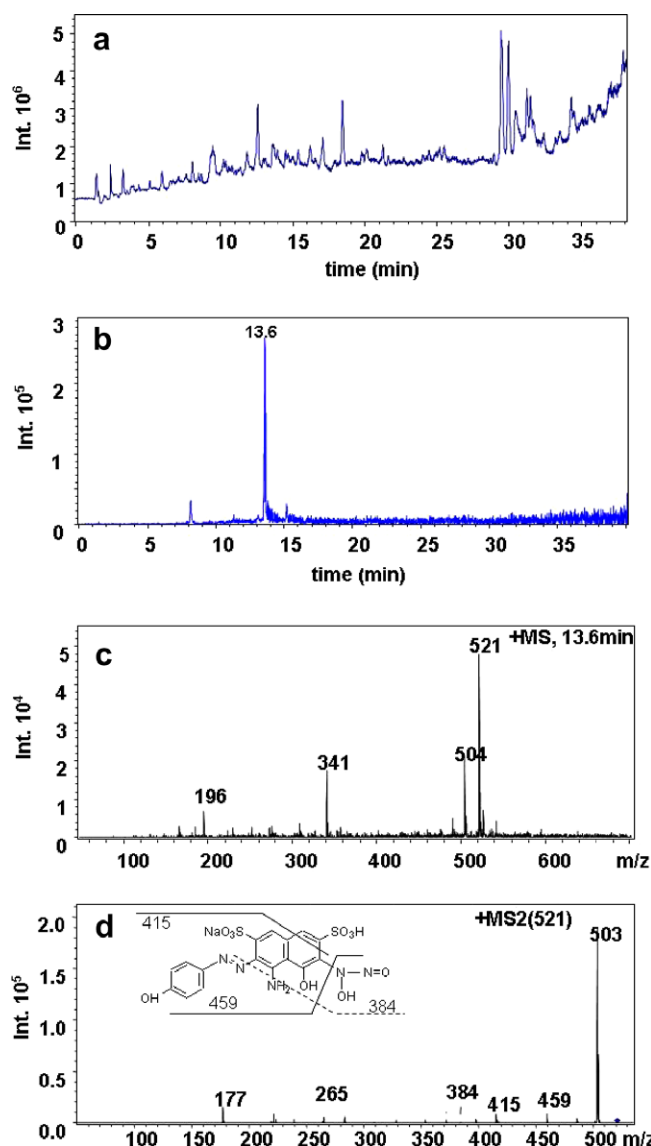


Fig. 3. (a) LC–MS profile of Amido Black sample after 30 min of degradation. (b) Extracted ion chromatogram of 521 intermediate, (c) the mass spectrum of intermediate 521, (d) MS/MS spectrum of 521.

3.3.1. Radical cleavage of the azo functionality

The presence of π -bonds in the azo groups makes them the preferred site of $\cdot\text{OH}$ radical attack (Karkmaz et al., 2004; Ozen et al., 2004; Chen et al., 2008; Erdemoglu et al., 2008). Attack can occur at the proximal or distal nitrogen atom of the azo group leading to a variety of cleavage pathways resulting in a number of identifiable products (Fig. 4) such as hydroxylamines (structures 1, 11), hydroxyl hydrazines (structures 8, 10, 11), nitroso compounds (structures 1, 4), and diazo compounds (structures 6, 9). The cleavage of $\text{N}=\text{N}$ group can also lead to amines (structure 3) and ketamines (structure 2). Interestingly, a closer look at the intermediates (Fig. 4) shows that the azo group attached to the unsubstituted phenyl is more susceptible to $\cdot\text{OH}$ attack. This can be interpreted on the basis that the azo group attached to the nitrophenyl group is relatively electron deficient compared to that attached to the phenyl. Loss of $\text{N}=\text{N}-\text{Ar}$ is also another possibility that can arise to produce phenols (Fig. 4, structure 7) and arenes (Fig. 4, structure 5).

3.3.2. Radical denitration (Scheme 1)

The $\cdot\text{OH}$ attack onto the nitro group results in the loss of nitric acid (HNO_3) and the formation of an aromatic radical (Scheme 1)

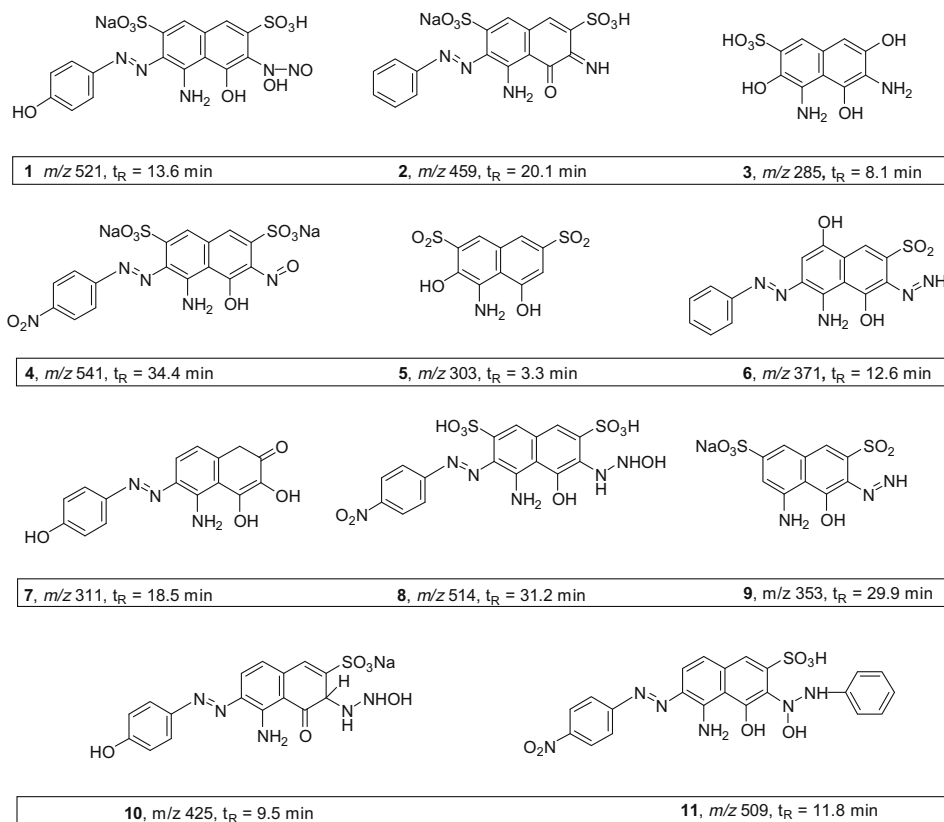
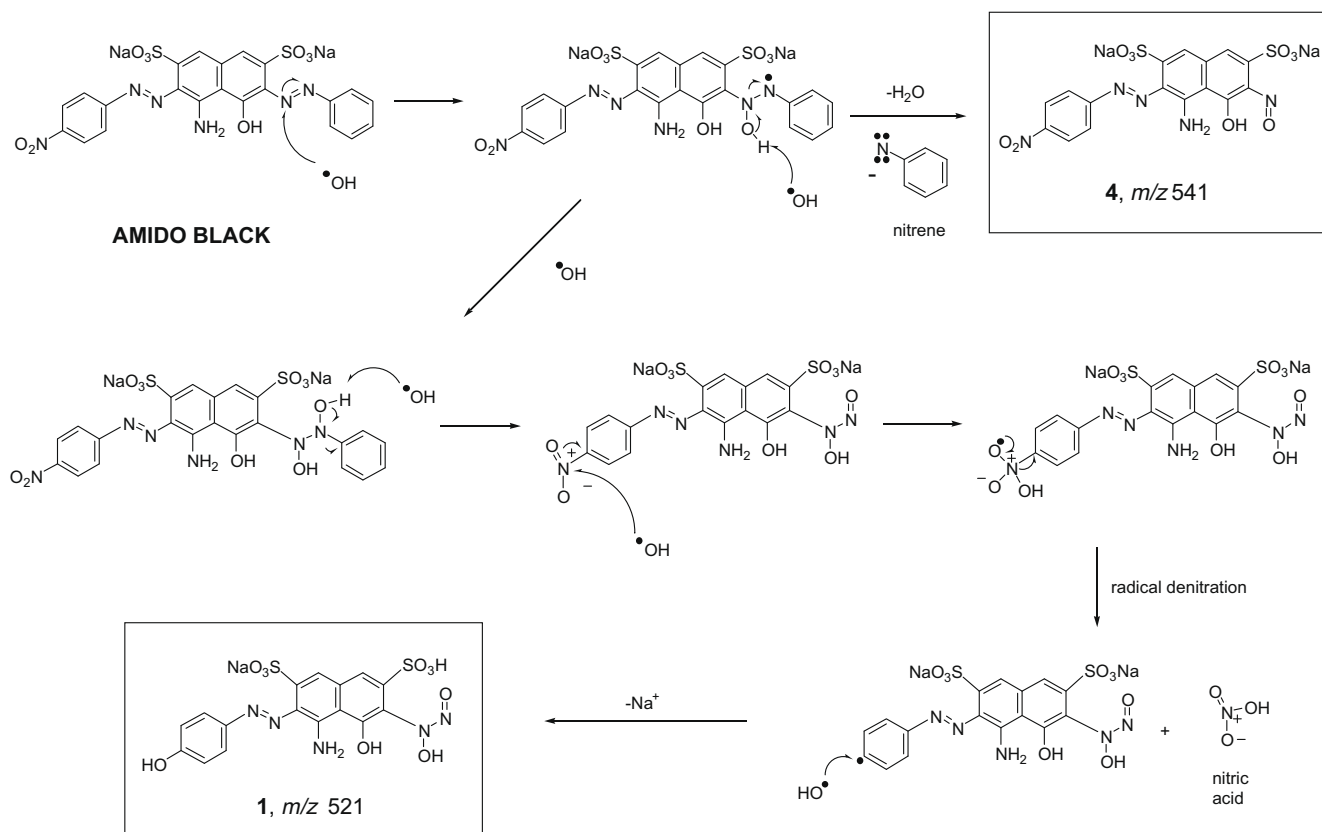
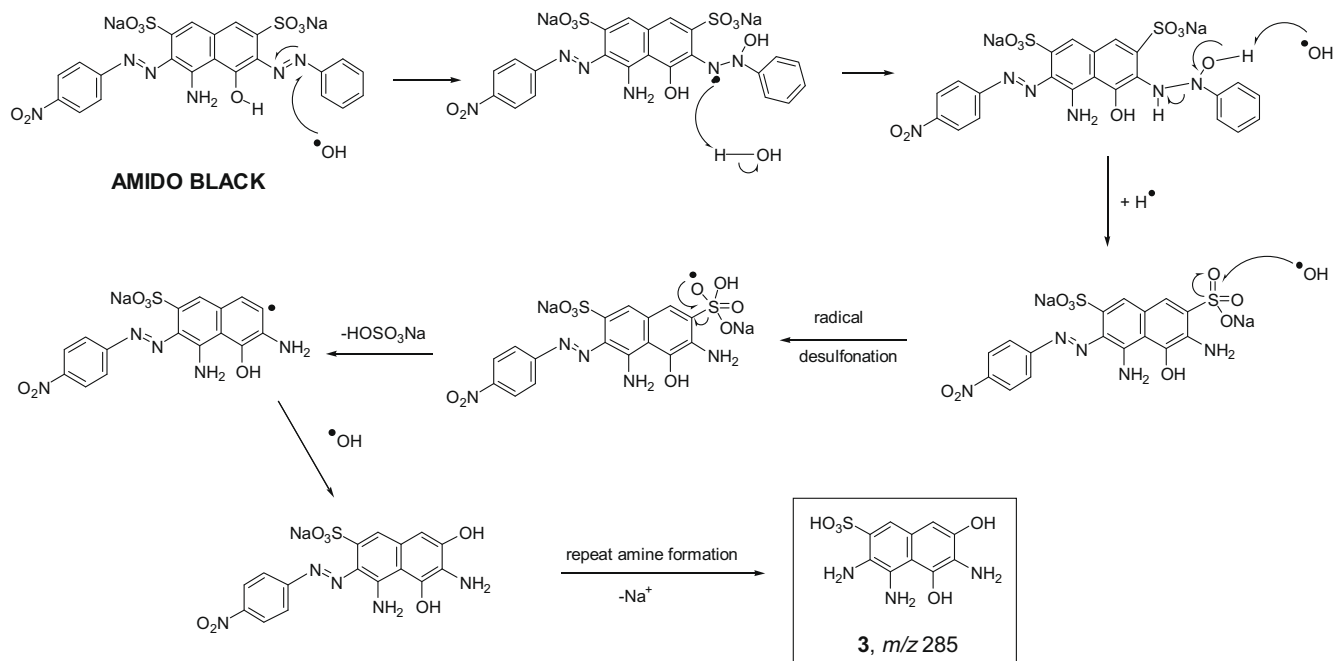


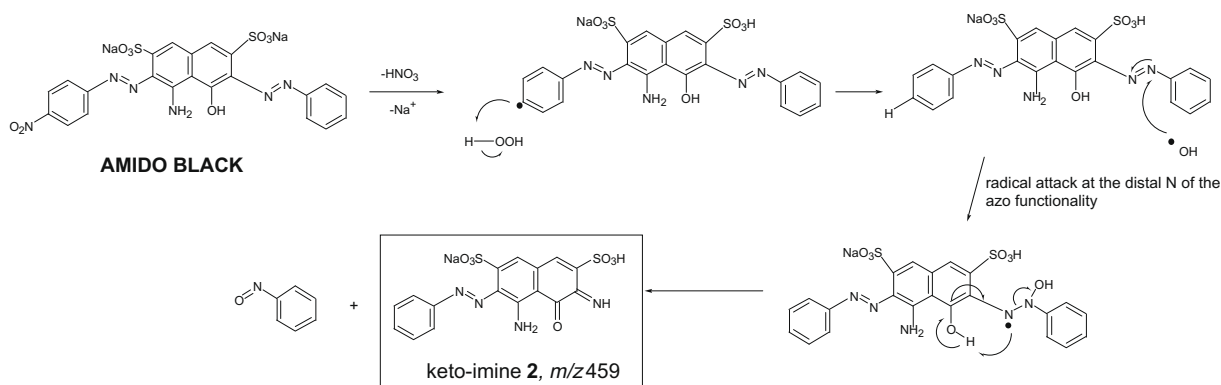
Fig. 4. Proposed structures for degradation products of Amido Black based on MS-MS data.



Scheme 1. Radical denitration and cleavage of the azo functionality: formation of hydroxylamine and nitroso functionalities.



Scheme 2. Radical desulfonation and formation of an amine from an azo functionality.



Scheme 3. Formation of keto-imine.

which can be quenched by either $\cdot\text{OH}$ to form a phenol or by H atom to form an arene (Holčapek et al., 2007). Examples of denitrated products are proposed in Fig. 4 (structures 2, 6, 7, 10).

3.3.3. Radical desulfonation and formation of sulfones (Scheme 2)

The loss of H_2SO_4 or NaHSO_4 was also observed (Fig. 4, structures 3, 6, 7, 10, 11). This is rationalized by $\cdot\text{OH}$ attack onto sulfur followed by its elimination. The resulting aromatic radical was quenched (Scheme 2) to produce phenols or arenes (Karkmaz et al., 2004; Ozen et al., 2004; Chen et al., 2008; Erdemoglu et al., 2008). The removal of $\cdot\text{ONa}$ from a sulfonate is also possible leading to a sulfone as shown in Fig. 4 (structures 5, 6, and 9).

Based on these mechanisms it was noted that the degradation was initiated by selective attack of the $\cdot\text{OH}$ radicals on preferential sites of the molecules, namely the nitro, sulfonate and azo groups.

In summary, the present findings showed that Amido Black degraded by radical initiated reactions and that the degradation pathway can be complex and can lead to generation of multitude of intermediates. This is supported by the variety of intermediates formed during the course of reaction. We interpret this to be a direct consequence of different mechanisms operating simultaneously on the dye and its primary degraded products.

Additionally, we observed that the relative population of the various intermediates was changing over time, thus pointing to another level of complexity and dynamism in such degradation studies.

4. Conclusions

Amido Black, a diazo dye was subjected to UV light in the presence of H_2O_2 and the degradation products/intermediates were analyzed by LC/MS. We propose that the overall photolytic oxidation of the dye was most likely initiated by $\cdot\text{OH}$ radicals. LC–MS/MS studies suggested possible pathways of dye degradation and revealed a variety of intermediate compounds which were formed during the course of the reaction. Three different mechanistic pathways were proposed for the degradation of the dye (radical denitration, radical desulfonation, and radical diazotization). Interestingly, we showed that the dye degradation starts by the cleavage of $-\text{N}=\text{N}-$ group bearing the unsubstituted phenyl ring. Based on our observations it was found that given the choice of two azo groups, $\cdot\text{OH}$ radicals preferentially attack the more electron rich diazo functionality of the molecule.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.chemosphere.2010.04.065](https://doi.org/10.1016/j.chemosphere.2010.04.065).

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