

Electrospray ionization mass spectrometry monitoring of indigo carmine degradation by advanced oxidative processes

Ilza Dalmázio,¹ Ana P. F. M. de Urzedo,¹ Tania M. A. Alves,² Rodrigo R. Catharino,³ Marcos N. Eberlin,³ Clésia C. Nascentes¹ and Rodinei Augusti^{1*}

¹ Department of Chemistry, Federal University of Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil

² René Rachou Research Center, Oswaldo Cruz Foundation, Belo Horizonte, MG, 30190-002, Brazil

³ ThoMSon Laboratory for Mass Spectrometry, Institute of Chemistry, State University of Campinas, Campinas, SP 13083-970 Brazil

Received 24 August 2006; Accepted 7 November 2006

The degradation of the dye indigo carmine in aqueous solution induced by two oxidative processes ($\text{H}_2\text{O}_2/\text{iodide}$ and O_3) was investigated. The reactions were monitored by electrospray ionization mass spectrometry in the negative ion mode, ESI(-)-MS, and the intermediates and oxidation products characterized by ESI(-)-MS/MS. Both oxidative systems showed to be highly efficient in removing the color of the dye aqueous solutions. In the ESI(-)-MS of the indigo carmine solution treated with H_2O_2 and $\text{H}_2\text{O}_2/\text{iodide}$, the presence of the ions of m/z 210 (indigo carmine in its anionic form, 1), 216, 226, 235, and 244 was noticeable. The anion of m/z 235 was proposed to be the unprecedented hydroperoxide intermediate 2 formed in solution via an electrophilic attack by hydroxyl and hydroperoxyl radicals of the exocyclic C=C bond of 1. This intermediate was suggested to be rapidly converted into the anionic forms of 2,3-dioxo-1H-indole-5-sulfonic acid (3, m/z 226), 2-amino- α -oxo-5-sulfo-benzeneacetic acid (4, m/z 244), and 2-amino-5-sulfo-benzoic acid (5, m/z 216). In the ESI(-)-MS of the indigo carmine solution treated with O_3 , two main anions were detected: m/z 216 (5) and 244 (4). Both products were proposed to be produced via an unstable ozonide intermediate. Other anions in this ESI(-) mass spectrum were attributed to be $[4 - \text{H} + \text{Na}]^-$ of m/z 266, $[4 - \text{H}]^{2-}$ of m/z 121.5, and $[5 - \text{H}]^{2-}$ of m/z 107.5. ESI-MS/MS data were consistent with the proposed structures for the anionic products 2–5. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: advanced oxidation processes; indigo carmine; ESI-MS; reaction monitoring

INTRODUCTION

Dyes in wastewater represent a serious problem, as they can cause considerable environmental damage such as toxicological effects on a number of microorganisms and color pollution.^{1,2} Different methodologies have been proposed to remove these organic pollutants from wastewater, for instance, adsorption, oxidation, reduction, and electrochemical reactions.^{3–5} In recent years, however, advanced oxidation processes (AOPs) have drawn considerable attention.^{6–8} In the AOP, *in situ* generated hydroxyl radicals, extremely reactive and powerful oxidizing species, promote the oxidation of the target organic compounds.⁹ However, the complete mineralization, i.e. the conversion of the organic material to CO_2 , H_2O , and other small molecules, does not always take place. Most of the AOPs comprise combinations of UV light with H_2O_2 , TiO_2 , O_3 , ultrasonic radiation, and Fenton reagent.¹⁰ The use of H_2O_2 and O_3 in degradation processes has been frequently reported.^{9,10} New systems, such as $\text{H}_2\text{O}_2/\text{iodide}$, have also been used because of their higher efficiency in the oxidation of organic compounds.¹¹

The degradation studies of dyes in aqueous solutions have usually focused on the discoloration efficiency promoted by the AOPs, whereas less attention has been given to the investigation of the reaction mechanisms and the characterization of secondary products and intermediates. For instance, GC-MS analysis was recently applied to identify products of the degradation of a number of azo dyes.^{12,13} However, the hydrophilic and unstable intermediates and products, probably formed under these reaction conditions, could not be detected. An alternative technique to be used in these studies is electrospray ionization mass spectrometry (ESI-MS). This technique possesses the remarkable capability to gently transfer ionic species from the condensed to the gas phase without inducing undesirable side reactions.^{14–20} Because of that, the composition of electrosprayed ions usually reflects that in solution.^{21–24} Furthermore, these desirable characteristics have allowed ESI-MS to be successfully employed as an important analytical tool for real-time monitoring of a great variety of chemical reactions.^{25–27}

Indigo carmine has been extensively employed in industrial processes,²⁸ even though it belongs to a highly toxic class of dyes (indigoid).^{29,30} Thus, in this paper we investigate the degradation of indigo carmine in water solutions induced by two oxidative processes: $\text{H}_2\text{O}_2/\text{iodide}$ and O_3 . Aiming to

*Correspondence to: Rodinei Augusti, Department of Chemistry, Federal University of Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil. E-mail: augusti@qui.ufmg.br

detect transient species and unstable oxidation products, we monitored the reactions by real-time ESI-MS in the negative ion mode, ESI(-)-MS. The anionic species were further characterized by ESI(-)-MS/MS, via their mass selection and fragmentation upon collision-induced dissociation (CID).

EXPERIMENTAL

Chemicals

The reagents were purchased from Sigma-Aldrich [Milwaukee, WI, USA; indigo carmine, potassium iodide (KI)], Merck (Whitehouse Station, NJ, USA; H₂O₂ 32%, formic acid, NaOH), and Tedia (Fairfield, OH, USA; chromatographic grade methanol). Ozone was produced at a flow rate of 1.15×10^{-6} mol min⁻¹ by passing synthetic air through an ozone generator (Eletro-Triozone, Brazil). The flux of ozone was determined by bubbling the gaseous stream through an aqueous solution of KI followed by the titration of the I₂ formed with standardized Na₂S₂O₃ solution. Doubly distilled water was used to prepare the solutions in all experiments.

Degradation reactions

H₂O₂

H₂O₂ (1 ml; 0.7 mol l⁻¹) was added to an aqueous solution of indigo carmine (100 ml; 153 mmol l⁻¹; pH = 8 adjusted with 1 mol l⁻¹ NaOH).

H₂O₂/iodide

H₂O₂ (1 ml; 0.7 mol l⁻¹) and KI (1 ml; 2 mmol l⁻¹) were added to an aqueous solution of indigo carmine (100 ml; 153 mmol l⁻¹; pH = 8 adjusted with 1 mol l⁻¹ NaOH).

Ozonation

An aqueous solution of indigo carmine (20 ml; 86 mmol l⁻¹) was continuously bubbled with a constant flux of ozone until complete discoloration.

Aliquots of all systems were collected and analyzed by ESI(-) MS and ESI(-) MS/MS.

Analytical methods

Total organic carbon (TOC)

The total organic content (TOC) experiments were carried out in a TOC 5000A (Shimadzu, Minnesota, USA) instrument at 680 °C using a platinum catalyst.

Mass spectrometry

ESI-MS and ESI-MS/MS analyses were conducted in two mass spectrometers both operating in the negative ion mode: (1) LCQ Advantage (ion trap analyzer, ThermoElectron, CA, USA), and (2) time-of-flight analyzer Q-ToF (Micromass, UK). In the first one, the mass spectra were obtained as an average of 50 scans, each requiring 0.2 s. The aliquots were directly injected into the ESI ion source at a flow rate of 5 µl min⁻¹ using a micro syringe. Typical ESI conditions were as follows: heated capillary temperature 200 °C; sheath gas (N₂) flow rate 20 units (*ca.* 0.30 l min⁻¹); spray voltage 4.5 kV; capillary voltage 25 V; tube lens offset voltage 25 V. For ESI-MS/MS, the precursor ions were first isolated by applying

an appropriate waveform across the end cap electrodes of the ion trap to resonantly eject all trapped ions except those ions of the *m/z* ratio of interest. The isolated ions were then subjected to a supplementary a.c. signal to resonantly excite them and so cause CID. The relative collision energy was set to a value at which product ions were produced in measurable abundance (it was varied from 20 to 32%). The isolation width used in the MS/MS experiments was 2 *m/z* units.

In the Q-ToF mass spectrometer, the capillary and cone voltages were set to 3 kV and 40 V, respectively, with a desolvation temperature of 100 °C. To each sample (0.1 ml), 1 ml of a solution of methanol/deionized water (7:3 v/v) and 0.1 ml of an aqueous solution of ammonia (0.1% v/v) were added. This diluted solution was then directly infused into the ESI source at a flow rate of 10 µl min⁻¹ via a micro syringe pump. The mass analyzer was set to operate at a scan range of *m/z* 50–1000. MS/MS experiments were carried out by mass selection of a specific ion in Q1 and then submitted to CID with argon in the collision chamber. Collision energy was optimized for each component, varying from 15 to 50 V. The product ion MS analysis was accomplished with the high-resolution orthogonal TOF analyzer.

RESULTS AND DISCUSSION

The experiments involving the H₂O₂ and H₂O₂/iodide systems were monitored by using exclusively the LCQ Advantage mass spectrometer (with an ion trap analyzer). On the other hand, the ozonation process was monitored by using both mass spectrometers described in the experimental section. In the last case, both instruments produced very similar results with the detection of a similar set of ions. Therefore, the ions identified by ESI(-)-MS, and characterized by ESI(-)-MS/MS (as will be discussed later in this paper) are really products formed from reactions taking place in aqueous solution rather than from in-source processes. In aqueous solution, indigo carmine and its oxidation products are predominantly in their anionic forms, with the sulphonyl substituents bearing the negative charges. These anions are thus gently transferred to the gas phase under the ESI conditions and subsequently analyzed by the mass spectrometer.

H₂O₂ and H₂O₂/iodide system

At first, the degradation of indigo carmine in aqueous solution by H₂O₂ was evaluated. A slight discoloration of the solution was observed after a reaction time of 15 min. In Fig. 1(a), which displays the ESI(-)-MS of the dye aqueous solution, an intense anion of *m/z* 210, corresponding to indigo carmine in its anionic form (doubly charged) (1, Scheme 1), was observed. Its mass selection and fragmentation (spectrum not shown) upon CID yielded the following fragments: *m/z* 356 (1 - SO₂^{-•}), 340 (1 - SO₃^{-•}), 276 (1 - SO₂ - SO₃^{-•}), and 260 (1 - SO₃ - SO₃^{-•}). After a reaction time of 15 min, an intense anion of *m/z* 235 was detected in the ESI(-)-MS (Fig. 1(b)). The ESI(-)-MS/MS of this anion is shown in Fig. 2. The presence of a significant fragment ion of *m/z* 242 (as well as the fragments of *m/z* 228

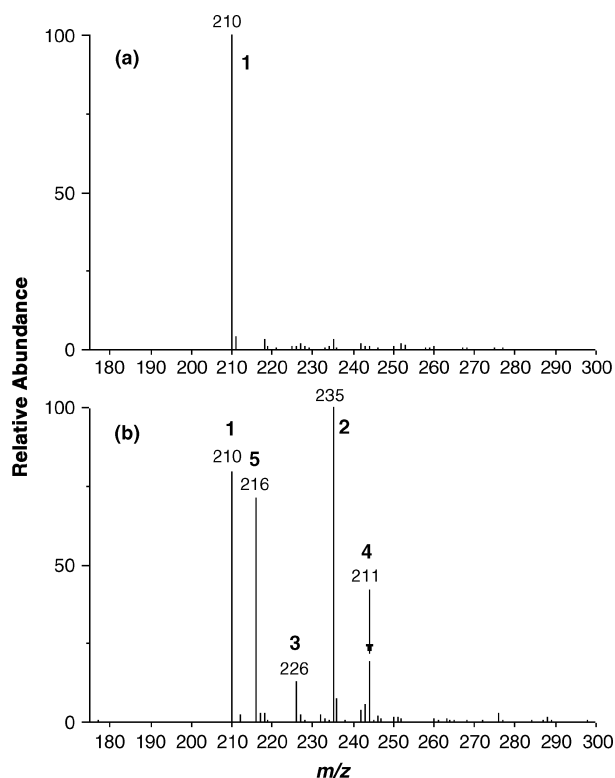
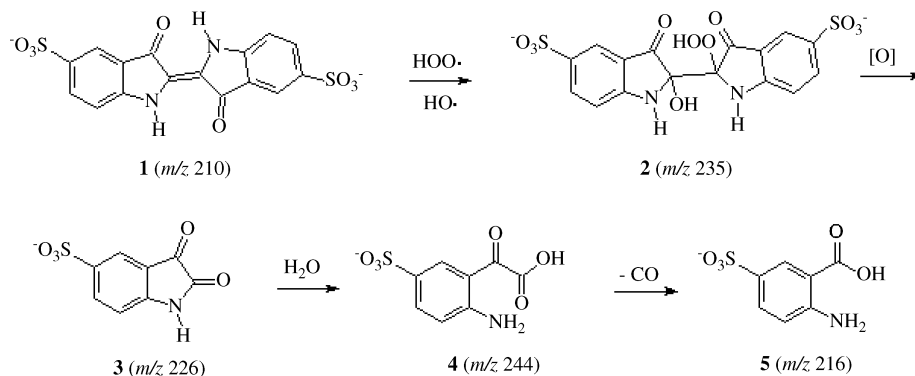


Figure 1. Real-time monitoring of the reaction between indigo carmine (**1**) and H₂O₂ in an aqueous medium: (a) ESI(-) mass spectrum of the initial solution; (b) ESI(-) mass spectrum after a reaction time of 15 min.

and 226) in this mass spectrum indicates that the anion of *m/z* 235 is doubly charged with a mass difference of 50 Da in relation to the dianion **1** (of *m/z* 210). On the basis of these data, the structure **2** was proposed for this anion (Scheme 1), which is consistent with the fragment ions observed in its ESI(-)-MS/MS (Fig. 2), as outlined in Scheme 2.

The formation of **2**, a hydroperoxide intermediate, is supposed to involve a simultaneous electrophilic attack by hydroxyl and hydroperoxyl radicals, reactive species commonly produced upon the decomposition of H₂O₂,³¹ of the exocyclic C=C bond of **1** (Scheme 1). Kettle and coworkers proposed, but did not confirm, the formation of a similar hydroperoxide intermediate during the oxidation of indigo carmine by superoxide.³² The intermediate **2** can be easily oxidized to yield anion **3** (of *m/z* 226), which is then



Scheme 1. Proposed route for the degradation of indigo carmine in aqueous solution by H₂O₂ and H₂O₂/iodide systems. Note the unprecedented hydroperoxide intermediate (**2**) suggested to be formed under these reaction conditions.

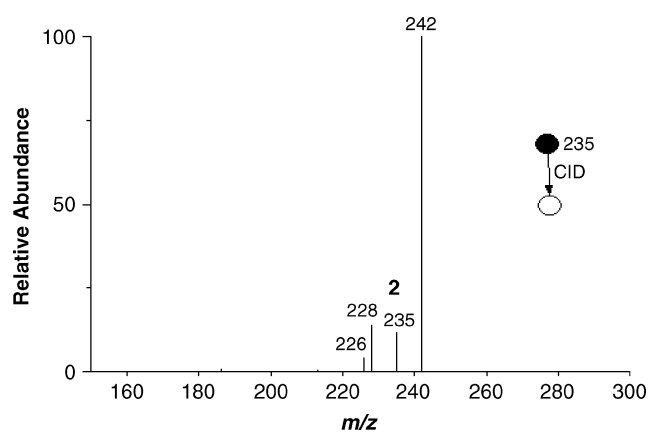


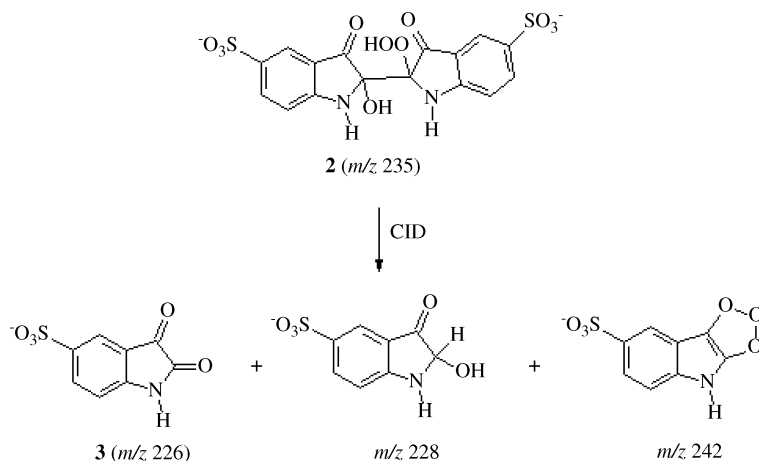
Figure 2. ESI(-)-MS/MS of the precursor ion of *m/z* 235 (**2**).

quickly converted to anion **4** (of *m/z* 244) by the addition of H₂O. Anion **4** can subsequently be converted to **5** (of *m/z* 216) by the release of a molecule of CO (Scheme 1 and Fig. 1(b)).

The mass selection and fragmentation of **3** (*m/z* 226) yielded mainly a fragment ion of *m/z* 198 via the loss of a CO molecule. The CID of **4** produced characteristically the fragment ion of *m/z* 200 by the release of a CO₂ molecule. The mass selection and fragmentation of **5** (*m/z* 216) yielded a series of major and structurally diagnostic fragment ions arising from the losses of H₂O (*m/z* 198), CO₂ (*m/z* 172), and SO₃ (*m/z* 136) (mass spectra not shown). Again, these fragmentation patterns seem to be consistent with the proposed structures for these anions.

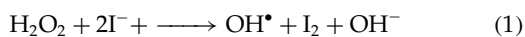
Remarkably, the product anions of *m/z* 227 or 243, which could be formed via an electrophilic attack of two hydroxyl radicals or two hydroperoxyl radicals on the exocyclic C=C bond of **1**, respectively, were detected in much smaller intensity than anion **2** (*m/z* 235) in the ESI(-)-MS (Fig. 1(b)). The reasons for that are not fully understood. Longer reaction times (for instance, 40 min) did not produce substantial solution discoloration or a significantly different ESI(-)-MS (not shown), thus indicating that the reaction did not proceed further.

In a second set of experiments, the indigo carmine degradation by the H₂O₂/iodide system was evaluated. The addition of iodide caused a noticeable increase in the dye degradation rate, although the TOC content remained practically constant during this reaction process, with



Scheme 2. Fragments formed upon mass selection and dissociation of the precursor anion **2**.

extensive solution discoloration after a reaction time as short as 5 min. This was probably caused by the well-known^{11,33} ability of iodide to induce the decomposition of H₂O₂ yielding hydroxyl radicals, as shown in Eqn (1):



After approximately 60 min, the solution was almost completely discolored. Figure 3 displays representative ESI(−) mass spectra acquired after reaction times of 20 (Fig. 3(a)) and 60 (Fig. 3(b)) min. In Fig. 3(a), the anion **5** (of *m/z* 216) is by far the most intense, whereas a much less

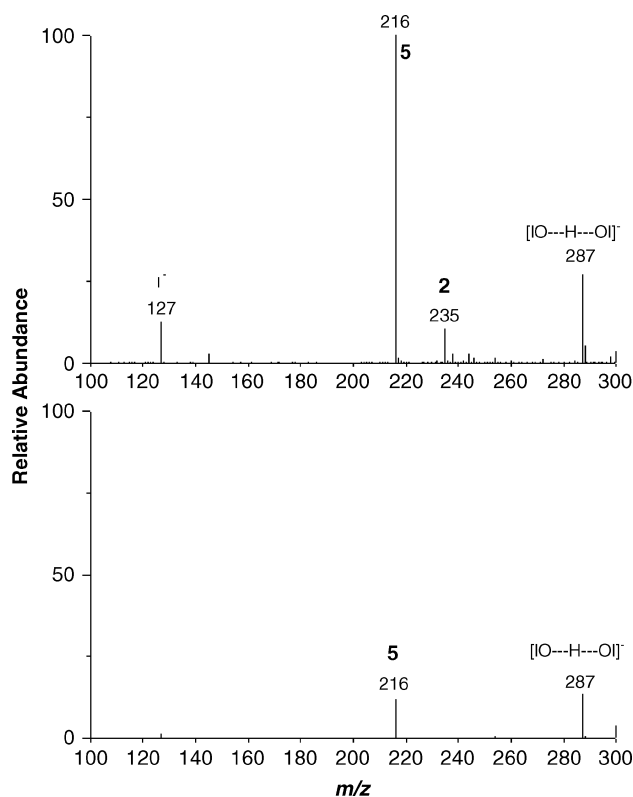


Figure 3. ESI(−)-MS monitoring of the degradation of indigo carmine (**1**) by the H₂O₂/iodide system in an aqueous medium after reaction times of (a) 20 min and (b) 60 min. To allow a more consistent comparison, the absolute abundance scales in both spectra in arbitrary units (abscissa) are the same.

intense anion of *m/z* 235 (the hydroperoxide intermediate **2**) is also noticeable. The absence of the anion of *m/z* 210 (indigo carmine in its deprotonated form, **1**) in this spectrum confirms that the dye had already been completely consumed after this reaction time. These results indicate that even under these stronger oxidizing conditions the reaction goes through identical pathways, i.e. indigo carmine is first oxidized to the unstable hydroperoxide intermediate **2** which is then quickly converted to the products **4** and subsequently **5** (Scheme 1). After longer reaction times (60 min, for instance), the analysis of the ESI(−)-MS (Fig. 3(b)) revealed that the intensity of the anion of *m/z* 216 (**5**) has markedly decreased, thereby indicating its later and continuous consumption and probable conversion to lighter compounds (which were not detected). The anions of *m/z* 127, detected in the ESI(−)-MS of Fig. 3(a), and 287, detected in both ESI(−)-MS of Fig. 3(a) and (b), were attributed to be the iodide anion (I[−]) and maybe the proton-bound dimer of the hypoiodite anion, i. e. [IO−H−OI][−], respectively.

Ozonation

When an aqueous solution of indigo carmine was continuously bubbled with ozone, a complete discoloration was observed after 10 min. In addition, the TOC content stayed the same as the initial dye solution, thereby indicating that

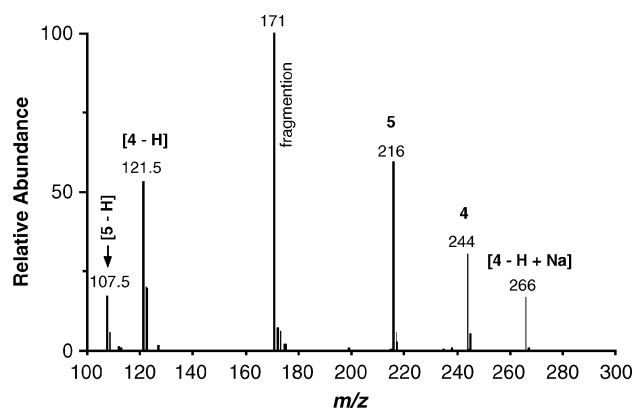


Figure 4. ESI(−)-MS monitoring of the degradation of indigo carmine (**1**) by O₃ in an aqueous medium after a reaction time of 10 min.

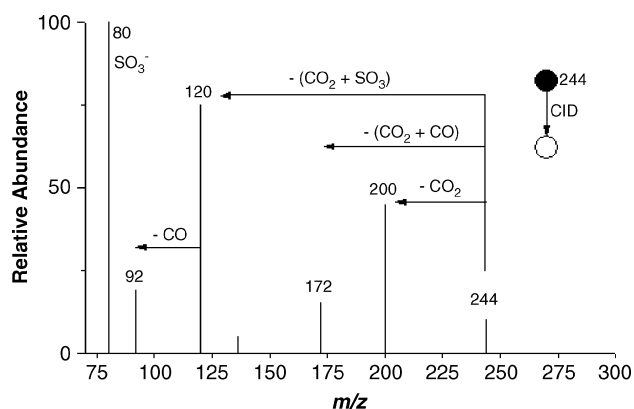


Figure 5. ESI(-)-MS/MS of the precursor ion of m/z 244 (**4**).

indigo carmine was not being mineralized, but rather converted into other products. In the ESI(-)-MS (Fig. 4), two intense anions of m/z 216 and 244, which were not detected in the initial dye solution, could be clearly observed. On the basis of their fragmentation profiles (as previously mentioned), the structures **5** and **4** (as displayed in Schemes 1 and 3) were proposed for the anions of m/z 216 and 244, respectively. For instance, the ESI(-)-MS/MS of anion **4** (Fig. 5) shows losses of CO_2 (m/z 200), CO_2 plus CO (m/z 172), and CO_2 plus SO_3 (m/z 120), which corroborate its proposed structure (Schemes 1 and 3). Furthermore, high-accuracy m/z measurements show good agreement between the experimental and theoretical m/z values for **4** (chemical composition: $\text{C}_8\text{H}_6\text{NO}_6\text{S}$; experimental: 243.9926; theoretical: 243.9916; difference: 4 ppm) and **5** (chemical composition: $\text{C}_7\text{H}_6\text{NO}_5\text{S}$; experimental: 215.9960; theoretical: 215.9967; difference: 3 ppm).

Other anions in the ESI(-) mass spectrum (Fig. 4) were attributed to $[\mathbf{4} - \text{H} + \text{Na}]^-$ (m/z 266), $[\mathbf{4} - \text{H}]^{2-}$ (m/z 121.5), and $[\mathbf{5} - \text{H}]^{2-}$ (m/z 107.5). The ion of m/z 171 results from the dissociation of the anions $[\mathbf{4} - \text{H} + \text{Na}]^-$, $[\mathbf{4} - \text{H}]^{2-}$, and $[\mathbf{5} - \text{H}]^{2-}$, as confirmed by the analysis of their ESI(-)-MS/MS (spectra not shown).

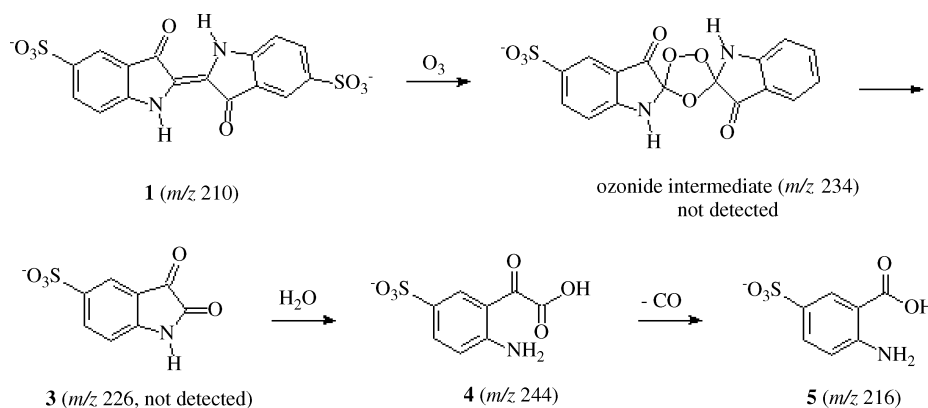
The reaction route that was proposed on the basis of the mass spectrometry results, as well as on the fundamental and well-established concepts on the reactivity of organic molecules, for the degradation of indigo carmine by ozone is displayed in Scheme 3. The initial pathway involves a 1,3

dipolar cycloaddition of O_3 toward the exocyclic $\text{C}=\text{C}$ bond of **1** to generate an unstable intermediate called *ozonide*³¹ (of m/z 234), which was not detected in the ESI(-)-MS (Fig. 4). It is well known that O_3 , a very powerful oxidizing agent ($E^\circ = 2.07$ V), usually reacts via 1,3 dipolar cycloaddition with most species containing multiple bonds (such as $\text{C}=\text{C}$, $\text{C}=\text{N}$, $\text{N}=\text{N}$).³⁴ In sequence, this ozonide intermediate is proposed to undergo a rearrangement to yield the dicarbonyl anion **3** (of m/z 226), which was also not detected in the ESI(-) mass spectrum (Fig. 4). The carboxylic acid **4** (of m/z 244) was proposed to be formed via a nucleophilic attack of a water molecule on the carbon of the $-\text{HNCO}-$ moiety of **3** followed by the lactam ring opening. Finally, the anion **5** (of m/z 216) was suggested to be formed via the release of CO from **4** (or, alternatively, via the oxidation of the terminal carbonyl of **5**). Note that product **3** (m/z 226) was exclusively detected (but in a relatively small intensity) in the ESI(-)-MS of the dye solution treated with H_2O_2 (Fig. 1(a)), which was the mildest oxidative system investigated here. Probably, under stronger oxidative conditions (such as H_2O_2 /iodide and O_3), such a product is quickly degraded to yield anions **4** (m/z 244) and subsequently **5** (m/z 216).

Product **3** was previously described as arising from photochemical and photocatalytic degradation of indigo carmine.³⁵ In this example, the products were detected by techniques other than ESI-MS, such as NMR, UV-vis and FTIR spectroscopy. GC/MS and HPLC/UV were also used to identify other products, i.e. small carboxylic acids arising from the dye benzene ring breakdown.³⁶

CONCLUSIONS

Both oxidative systems (H_2O_2 /iodide and O_3) showed to be highly efficient in removing the color of indigo carmine aqueous solutions. The monitoring by ESI(-)-MS allowed the *in situ* identification of a series of oxidized products and transient species, which were further characterized by ESI(-)-MS/MS. For instance, an unstable hydroperoxide intermediate was for the first time detected under such reaction conditions. These findings were conveniently used to suggest routes for the degradation of indigo carmine induced by both oxidative systems. Finally, ESI-MS (and MS/MS) demonstrated to be suitable techniques for real-time



Scheme 3. Suggested pathways for the degradation of indigo carmine in an aqueous solution by O_3 . Note that the ozonide intermediate was not detected in the corresponding ESI(-)-MS.

monitoring of AOPs of indigo carmine. It can be envisaged, therefore, that it can be applicable to the degradation studies of other classes of environmentally relevant compounds.

Acknowledgements

Authors would like to thank FAPESP, FAPEMIG and CNPq for financial support.

REFERENCES

- Oetzuerk A, Abdullah MI. Toxicological effect of indole and its azo dye derivatives on some microorganisms under aerobic conditions. *Sci. Total Environ.* 2006; **358**: 137.
- Tünay O, Kabdasli I, Eremektar G, Orhon D. Color removal from textile wastewaters. *Water Sci. Technol.* 1996; **34**: 9.
- Crini G. Non-conventional low-cost adsorbents for dye removal: a review. *Bioresour. Technol.* 2006; **97**: 1061.
- Forgacs E, Cserháti T, Oros G. Removal of synthetic dyes from wastewaters: a review. *Environ. Int.* 2004; **30**: 953.
- Awad HS, Galwa NA. Electrochemical degradation of acid blue and basic brown dyes on Pb/PbO₂ electrode in the presence of different conductive electrolyte and effect of various operating factors. *Chemosphere* 2005; **61**: 1327.
- Lachheb H, Puzenat E, Houas A, Ksibi M, Elaloui E, Guillard C, Herrmann JM. Photocatalytic degradation of various types of dyes (Alizarin S, Crocein Orange G, Methyl Red, Congo Red, Methylene Blue) in water by UV-irradiated titania. *Appl. Catal. B: Environ.* 2002; **39**: 75.
- Marco S, Peres LA, Peres JA. Decolorization of the azo dye Reactive Black 5 by Fenton and photo-Fenton oxidation. *Dyes Pigm.* 2006; **71**: 236.
- Alaton IA, Balcioglu IA, Bahnemann DW. Advanced oxidation of a reactive dyebath effluent: comparison of O₃, H₂O₂/UV-C and TiO₂/UV-A processes. *Water Res.* 2002; **36**: 1143.
- Huang CP, Dong C, Tang Z. Advanced chemical oxidation: its present role and potential future in hazardous waste treatment. *Waste Manag.* 1993; **13**: 361.
- Parag R, Pandit GB, Pandit AB. A review of imperative technologies for wastewater treatment I: oxidation technologies at ambient conditions. *Adv. Environ. Res.* 2004; **8**: 501.
- Mohamed AA, Iwatsuki M, Elshahat MF, Fukasawa T. Catalytic determination of iodide using the promethazine-hydrogen peroxide redox reaction. *The Analyst* 1995; **120**: 1201.
- Feng W, Nansheng D, Helin H. Degradation mechanism of azo dye C. I. reactive red 2 by iron powder reduction and photooxidation in aqueous solutions. *Chemosphere* 2000; **41**: 1233.
- Herrera F, Lopez A, Mascolo G, Albers P, Kiwi J. Catalytic combustion of Orange II on hematite. Surface species responsible for the dye degradation. *Appl. Catal. B: Environ.* 2001; **29**: 147.
- Domingos JB, Longhinotti E, Brandao TAS, Bunton CA, Santos LS, Eberlin MN, Nome F. Mechanisms of nucleophilic substitution reactions of methylated hydroxylamines with bis(2,4-dinitrophenyl)phosphate. Mass spectrometric identification of key intermediates. *J. Org. Chem.* 2004; **69**: 6024.
- Koch KJ, Gozzo FC, Nanita SC, Takats Z, Eberlin MN, Cooks RG. Chiral transmission between amino acids: chirally selective amino acid substitution in the serine octamer as a possible step in homochirogenesis. *Angew. Chem. Int. Ed. Engl.* 2002; **41**: 1721.
- Meurer EC, Sabino AA, Eberlin MN. Ionic transacetalization with acylium ions: a class-selective and structurally diagnostic reaction for cyclic acetals performed under unique electrospray and atmospheric pressure chemical ionization in-source ion molecule reaction conditions. *Anal. Chem.* 2003; **75**: 4701.
- Griep-Raming J, Meyer S, Bruhn T, Metzger JO. Investigation of reactive intermediates of chemical reactions in solution by electrospray ionization mass spectrometry: radical chain reactions. *Angew. Chem. Int. Ed. Engl.* 2002; **41**: 2738.
- Sabino AA, Machado AHL, Correia CRD, Eberlin MN. Probing the mechanism of the Heck reaction with arene diazonium salts by electrospray mass and tandem mass spectrometry. *Angew. Chem. Int. Ed. Engl.* 2004; **43**: 2514.
- Meyer S, Metzger JO. Use of electrospray ionization mass spectrometry for the investigation of radical cation chain reactions in solution: detection of transient radical cations. *Anal. Bioanal. Chem.* 2003; **377**: 1108.
- Augusti DV, Carazza F, Okuma AA, Augusti R. Reactivity of some novel multifunctional benzoyl esters towards nucleophiles investigated by electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 2003; **17**: 1084.
- Pereira RMS, Paula VI, Buffon R, Tomazela DM, Eberlin MN. New iridium(I) complexes with labile ligands: reactivity and structural characterization by atmospheric pressure mass and tandem mass spectrometry. *Inorganica Chim. Acta* 2004; **357**: 2100.
- Gozzo FC, Santos LS, Augusti R, Consorti CS, Dupont J, Eberlin MN. Gaseous supramolecules of imidazolium ionic liquids: magic numbers and intrinsic strengths of hydrogen bonds. *Chem. Eur. J.* 2004; **10**: 6187.
- Toma SH, Nikolaou S, Tomazela DM, Eberlin MN, Toma HE. Synthesis, spectroscopy, tandem mass spectrometry, and electrochemistry of the linearly bridged μ -(trans-1,4-bis[2-(4-pyridyl)ethenyl]-benzene)-{Ru₃O(CH₃COO)₆(py)₂}_2 cluster. *Inorganica Chim. Acta* 2004; **357**: 2253.
- Hinderling C, Adlhart C, Chen P. Olefin metathesis of a ruthenium carbene complex by electrospray ionization in the gas phase. *Angew. Chem. Int. Ed. Engl.* 1998; **37**: 2685.
- Dalmázio I, Santos L, Lopes RP, Eberlin MN, Rodinei A. Advanced oxidation of caffeine in water: on-line and real-time monitoring by electrospray ionization mass spectrometry. *Environ. Sci. Technol.* 2005; **39**: 5982.
- Bier M, Lavery K, Conlin L. Monitoring biomolecule reaction rates in real-time using a micro-reactor and an electrospray mass spectrometer. *Adv. Mass Spectrom.* 2001; **15**: 523.
- Milagre CDF, Milagre HMS, Rodrigues JAR, Rocha LL, Santos LS, Eberlin MN. On-line monitoring of bioreductions via membrane introduction mass spectrometry. *Biotechnol. Bioeng.* 2005; **90**: 888.
- Green FJ (ed.). *The Sigma Aldrich Handbook of Stains, Dyes and Indicators Aldrich Chemical*. Aldrich Chemical: Milwaukee, 1990; 403.
- Butterworth KR, Hoosen J, Gaunt IF, Kiss IS, Grasso P. Long-term toxicity of indigo carmine in mice. *Food Cosmet. Toxicol.* 1975; **13**: 167.
- Gaunt IF, Kiss IS, Grasso P, Gangolli SD. Short-term toxicity study on indigo carmine in the pig. *Food Cosmet. Toxicol.* 1969; **71**: 17.
- March J. *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*. John Wiley and Sons: New York, 1992.
- Kettle AJ, Clark BM, Winterbourn CC. Superoxide converts indigo carmine to isatin sulfonic acid: implications for the hypothesis that neutrophils produce ozone. *J. Biol. Chem.* 2004; **279**: 18521.
- Mitic SS, Miletic GZ, Kostic DA. Kinetic determination of traces of iodide by its catalytic effect on oxidation of sodium pyrogallol-5-sulfonate by hydrogen peroxide. *Anal. Sci.* 2003; **19**: 913.
- Hoigne J, Bader H. The role of hydroxyl radical reactions in ozonation processes in aqueous solutions. *Water Res.* 1976; **10**: 377.
- Galindo C, Jacquesb P, Kalta A. Photochemical and photocatalytic degradation of an indigoid dye: a case study of acid blue 74 (AB74). *J. Photochem. Photobiol. A* 2001; **141**: 47.
- Vautier M, Guillard C, Herrmann J. Photocatalytic degradation of dyes in water: case study of indigo and of indigo carmine. *J. Catal.* 2001; **201**: 46.