

Indigo Carmine degradation by hypochlorite in aqueous medium monitored by electrospray ionization mass spectrometry

Ana P. F. M. de Urzedo¹, Clésia C. Nascentes¹, Maria E. R. Diniz¹, Rodrigo R. Catharino², Marcos N. Eberlin² and Rodinei Augusti^{1*}

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

²Thomson Mass Spectrometry Laboratory, Institute of Chemistry, State University of Campinas, 13083-970, Campinas, SP, Brazil

Received 22 February 2007; Revised 20 April 2007; Accepted 24 April 2007

The degradation of the dye Indigo Carmine by hypochlorite in aqueous solution was monitored by electrospray ionization mass spectrometry in the negative ion mode (ESI(-)-MS). Hypochlorite was highly efficient in removing the color of aqueous solutions of the dye. ESI(-)-MS monitoring showed that concomitant with the Indigo Carmine consumption two transient species appeared (detected as doubly charged anions) probably formed via a net insertion of two hydroxyl groups at the exocyclic C=C bond followed by the incorporation of two (mainly) or one oxygen atoms at the indolic rings of the dye. Structures of these products were proposed based on the ESI(-)-MS/MS data and high accuracy mass measurements. These two transient intermediates quickly decomposed, both in the condensed and in the gas phase, to yield mono-charged anions. Based on these results, a route for the Indigo Carmine degradation by hypochlorite in aqueous solution has been proposed. Copyright © 2007 John Wiley & Sons, Ltd.

As international environmental standards are becoming more stringent (ISO 14001, October 1996), technological systems for the removal of organic pollutants, including dyes, from aqueous environments have been recently developed. Indigo Carmine is one of the oldest and most used dyes.¹ Its major industrial application is the dyeing of clothes (blue jeans) and other blue denim. It has also been employed as a food dye and as a redox indicator in analytical chemistry.¹

Several investigations have been conducted to verify the efficiency of processes used to promote Indigo Carmine degradation. Zainal and coworkers,² for instance, studied its degradation in aqueous solution by using TiO₂ immobilized on the inner surfaces of a quartz pipe submitted to continuous exposure to UV light. From total organic carbon (TOC) measurements, the authors found a mineralization rate of 83% after a photodegradation period of 4.5 h. In a related study, Hachem and coworkers³ showed that TiO₂ (Degussa P25) exposed to solar irradiation is an efficient system for color removal from aqueous solutions of Indigo Carmine and proposed that a similar methodology could be used to remediate effluents in regions exposed to constant and abundant sunlight. Gemeay and coworkers¹ investigated the degradation of Indigo Carmine promoted by

hydrogen peroxide catalyzed by heterogeneous systems comprised of several transition metal complexes. The authors observed that, in addition to the color removal, the catalytic system was able to promote almost complete dye mineralization whereas its activity depended on several parameters, mainly the redox potential of the central metal ion and the nature of the ligands. Although a laborious process, these results indicated that this heterogeneous catalytic reaction could be conveniently employed as an alternative method for the remediation of effluents contaminated with dyes and other hazardous organic compounds.

Despite the high efficiency of the advanced oxidative processes (AOP), which do not require solid residues management and show other advantageous aspects such as simplicity and low cost, to promote the degradation of target organic compounds, industrial effluents and related samples have usually been treated via chemical oxidation.^{4–6} The use of hypochlorite (ClO⁻) is one of the most employed oxidative methods and it has been extensively applied to the degradation of many organic molecules.^{7,8} It was shown that the pesticides Isoxazole and Diazinon were rapidly degraded by hypochlorite in aqueous solution.⁹ In a related paper, Pizzolato and coworkers⁴ showed that NaOCl was efficient in removing the color of aqueous solutions of the dyes Rhodamine B, Brilliant Green and Crystal Violet. It was proposed that the color removal mechanism was related to the destruction of the chromophores. The analysis of the

*Correspondence to: R. Augusti, Departamento de Química, Universidade Federal de Minas Gerais, Belo Horizonte/MG, Brazil. E-mail: augusti@ufmg.br

Contract/grant sponsor: Minas Gerais State Science Foundation (FAPEMIG), the São Paulo State Science Foundation (FAPESP), and the Brazilian National Research Council (CNPq).

degradation products by gas chromatography/mass spectrometry (GC/MS) indicated the presence of volatile and semi-volatile organochloro compounds.¹⁰

Owing to its outstanding sensitivity, speed, and selectivity, electrospray ionization mass spectrometry (ESI-MS) has been extensively used to investigate the degradation of organic compounds in the environment, especially polar and non-volatile molecules in aqueous media.^{11–15} ESI-MS is particularly well suited for the analysis of charged species in solution, which can be easily transferred to the gas phase for analysis, with the ESI-MS data giving a snapshot of the various charged species present in the equilibrium mixture.^{15–19} Furthermore, under the most commonly used electrospray operating conditions, only rarely do the electrochemical processes seem to affect the identity and the relative abundances of the ions in an ESI mass spectrum.²⁰ ESI-MS with its unique characteristics is therefore becoming a major technique for the elucidation of reaction mechanisms, especially in solutions of polar solvents, via the detection and identification of reactants, products, and intermediates, most particularly of transient species occurring at very low concentrations.^{10,13,21–29}

In this work, we describe the use of electrospray ionization mass spectrometry in the negative ion mode (ESI(-)-MS) to monitor the degradation of Indigo Carmine by hypochlorite in aqueous solution, aiming to detect intermediates and transient species formed under these reaction conditions.

EXPERIMENTAL

Chemicals

Indigo Carmine was purchased from Sigma-Aldrich (Milwaukee, WI, USA) and used without further purification. Reactions were carried out with commercial grade sodium hypochlorite (6%). Doubly distilled water was used to prepare the solutions in all experiments.

Reaction monitoring and analytical methods

In a typical run, aqueous solutions of NaOCl (1.0 mL at $2.0 \times 10^{-3} \text{ mol.L}^{-1}$) and Indigo Carmine (20 mL; 40 mg.L^{-1}) were mixed and maintained under constant and vigorous stirring until complete color removal. ESI-MS and ESI-MS/MS analyses were conducted in a hybrid quadrupole (Q)

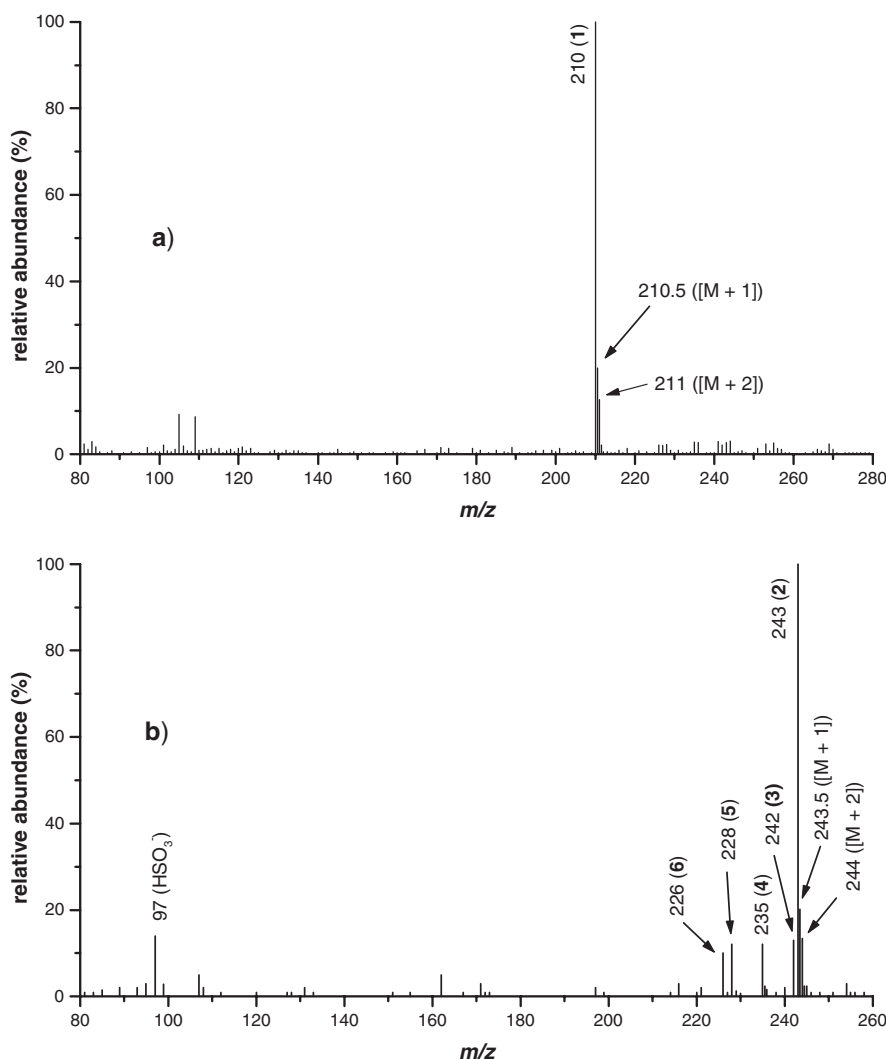
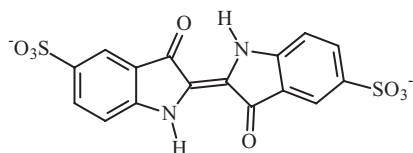


Figure 1. ESI(-) mass spectra of aqueous solutions of: (a) Indigo Carmine at 40 mg.L^{-1} and (b) Indigo Carmine at 40 mg.L^{-1} treated with sodium hypochlorite for 3 min.

1 (m/z 210)

Scheme 1. Structure of the anionic form of Indigo Carmine (1).

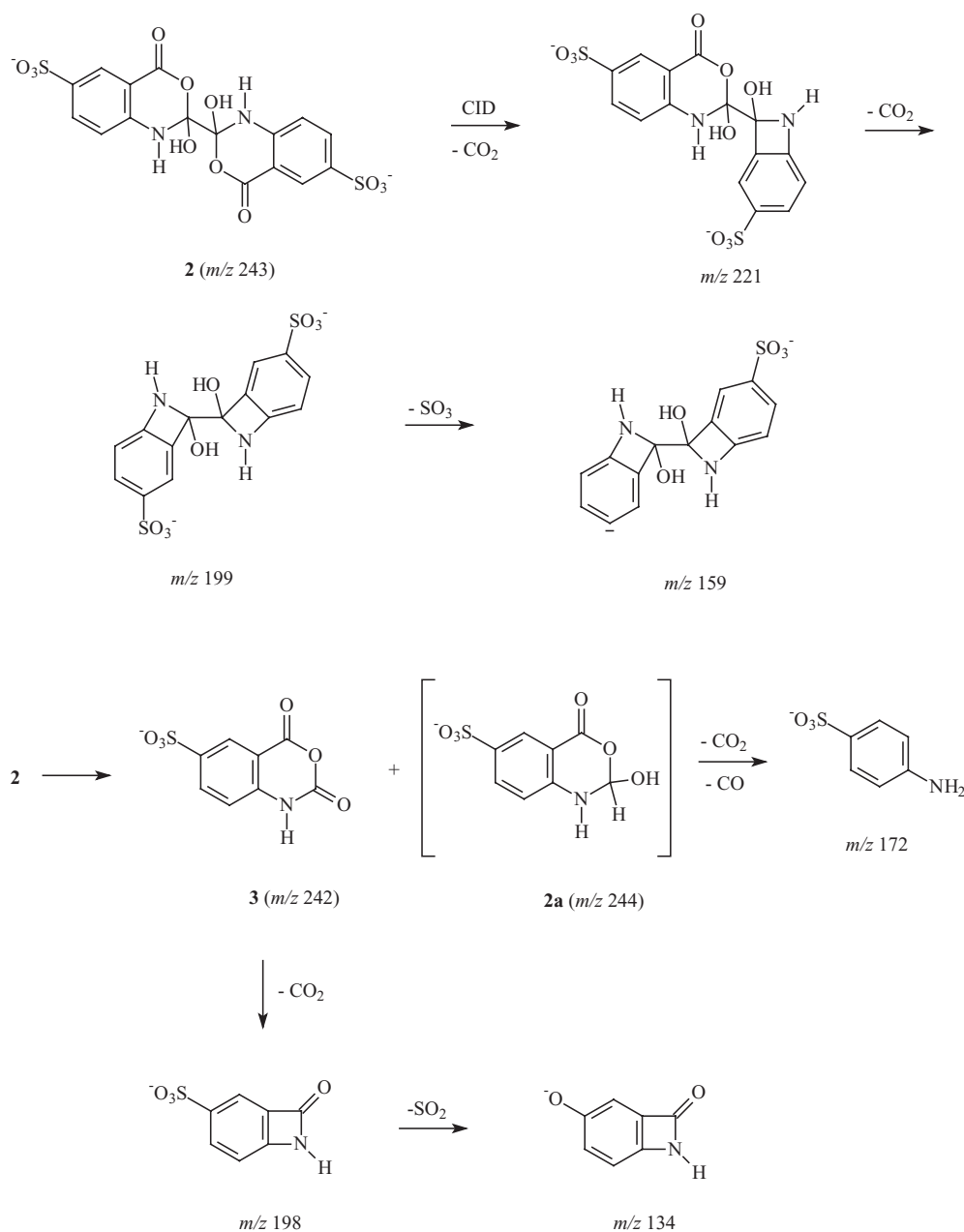
orthogonal time-of-flight (ToF) high-resolution mass spectrometer (Q-ToF, Micromass, Manchester, UK) with a constant nebulizer temperature of 50°C . ESI-MS was carried out in the negative ion mode over a scan range of m/z 100–1000, and the cone and extractor voltages were set to -40

and -10 V, respectively. Samples were directly infused into the ESI source at flow rates of $10 \mu\text{L}\cdot\text{min}^{-1}$ via a microsyringe pump. MS/MS experiments were carried out by selecting a specific ion in Q1, with a window width of 1 m/z unit, and then fragmenting it via collision-induced dissociation (CID) with argon in the collision chamber. Product ion analysis was accomplished with the orthogonal ToF analyzer. Accurate mass data were obtained through calibration of the instrument with a water/methanol solution of caffeine.

RESULTS AND DISCUSSION

Degradation of the dye Indigo Carmine by NaOCl in aqueous solution

Hypochlorite quickly promoted the degradation of Indigo Carmine in aqueous solution, as verified by the almost



Scheme 2. Proposed fragmentation pathways upon mass selection and collision-induced dissociation (CID) of anion 2.

complete color removal after a reaction time as short as 3 min (see Experimental section for the reaction conditions). Figure 1(a) shows the ESI(-) mass spectrum of the Indigo Carmine aqueous solution. Note the major doubly charged anion of m/z 210 (**1**, Scheme 1) and its isotopologues of m/z 210.5 ($M + 1$) and 211 ($M + 2$) with relative signal intensities (100%, 20%, and 13%, respectively) that fit well with those calculated for $C_{16}H_8N_2O_8S_2$. Anion **1** is by far the predominant species in aqueous solution, especially at neutral and basic pH, and it can be gently transferred to the gas phase and detected by ESI-MS. The mass-selection and collision-induced dissociation (CID) of **1** (mass spectrum not shown) yielded mainly a product ion of m/z 80 attributed to the radical anion SO_3^- .

Figure 1(b) displays the ESI(-) mass spectrum of the Indigo Carmine aqueous solution taken 3 min after addition of hypochlorite (NaOCl). Note that whereas the ion of m/z 210 (**1**) is no longer detected, ions of m/z 243 (the major one), 242, 235, 228, 226, and 97 are clearly observed. Note also that none of these anions displays isotopic distributions consistent with the presence of one or more chlorine atoms in their structures. Therefore, it seems that no chlorinated products have been formed despite the ability of hypochlorite to act as a powerful chlorinating agent.³⁰

The anion of m/z 243 (**2**) is doubly charged as evidenced by the presence of the ($M + 1$) and ($M + 2$) isotopologues of m/z 243.5 and 244, respectively (Fig. 1(b)). The $\Delta m/z$ for the doubly charged anions **2** and **1** is 33 units, which indicates that **2** could be formed from **1** via the incorporation of two oxygen atoms (32 Da) and two hydroxyl groups (34 Da). Based on this and some general concepts of the reactivity of organic molecules (as discussed below), the structure shown in Scheme 2 was proposed for **2**. Note also that the experimental relative signal intensities (Fig. 1(b)) observed for the anions of m/z 243, 243.5, and 244 (100%, 20%, and 13%, respectively) are consistent with those calculated for $C_{16}H_{10}N_2O_{12}S_2$.

The mass-selection and CID of **2** yielded mainly the following ions: m/z 242, 221, 199, 198, 172, 159, and 134 (Fig. 2). We suggest that the dissociation pattern fits well with

the proposed structure for this ion (Scheme 2). Based on these results, we propose a plausible route for the degradation of Indigo Carmine by NaOCl in aqueous solution (Scheme 3).

We suggest that the formation of **2** occurred via an initial electrophilic addition of HOCl (hypochlorous acid),³¹ which is in equilibrium with its conjugated base ^-OCl (hypochlorite anion) in aqueous solution,³⁰ towards the exocyclic C=C bond of **1** to yield the chlorohydrin intermediate **1a** of m/z 236/237 (not detected in the ESI(-) mass spectrum shown in Fig. 1(b)). The continuous formation of this intermediate, which has a much less extensive π -electron conjugation system than **1**, could cause the quick and intense solution color removal, as experimentally observed. The next step in this reaction sequence is suggested to involve the replacement of Cl^- by OH^- in **1a**, an easy and quick process that takes place especially in basic aqueous solution,³² to generate the dihydroxylated intermediate **1b** of m/z 227, which was also not detected in the spectrum shown in Fig. 1(b). Species **1a** and **1b** were not detected by ESI(-)-MS probably owing to their high instability and very short-lived character. The mechanism for the **1b** \rightarrow **2** conversion is proposed to involve the chlorination of the indolic nitrogen of **1b**, a previously reported process,^{33,34} to yield the unstable intermediate **1c**. Simultaneous nucleophilic attacks of the hydroxyl groups at both the carbonyl carbons of **1c**, causing rupture of the indolic rings and the consequent formation of expanded six-membered rings, followed by the elimination of two molecules of HCl will lead to the transient species **1d**. Finally, we suggest that anion **2** is formed via the insertion of two molecules of H_2O at the C=N bonds of **1d**. To the best of our knowledge, this is the first time that **2** has been detected as an intermediate in the oxidative decomposition of Indigo Carmine and its formation is a further example of hypochlorite reactivity in aqueous solution. Finally, it is proposed that decomposition of **2** via cleavage of the exocyclic C-C single bond yielded anions **3** of m/z 242 and **2a** of m/z 244. Anion **2a**, which was not detected in the mass spectrum shown in Fig. 1(b), can be easily oxidized to **3**, especially under these strong oxidative conditions (Scheme 3). Note that it is also proposed that anion **3** was

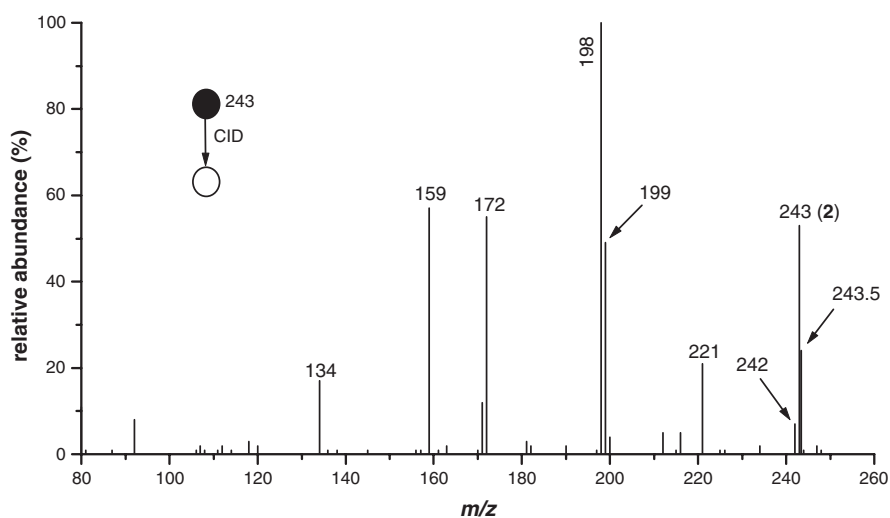
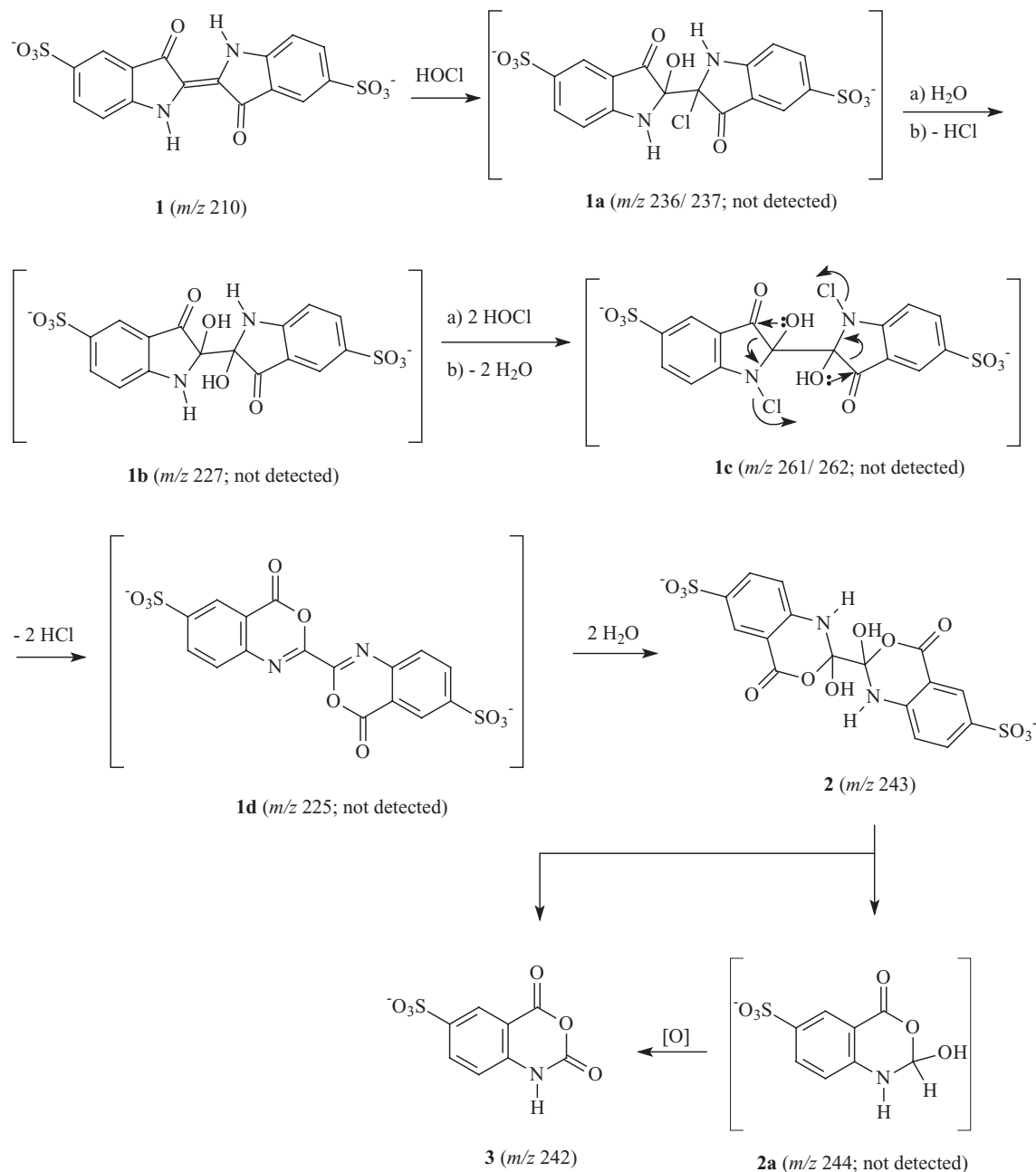


Figure 2. ESI(-)-MS/MS experiment with anion **2** of m/z 243.



Scheme 3. Proposed reaction pathways and mechanisms for the degradation of Indigo Carmine (**1**) by hypochlorite in aqueous solution.

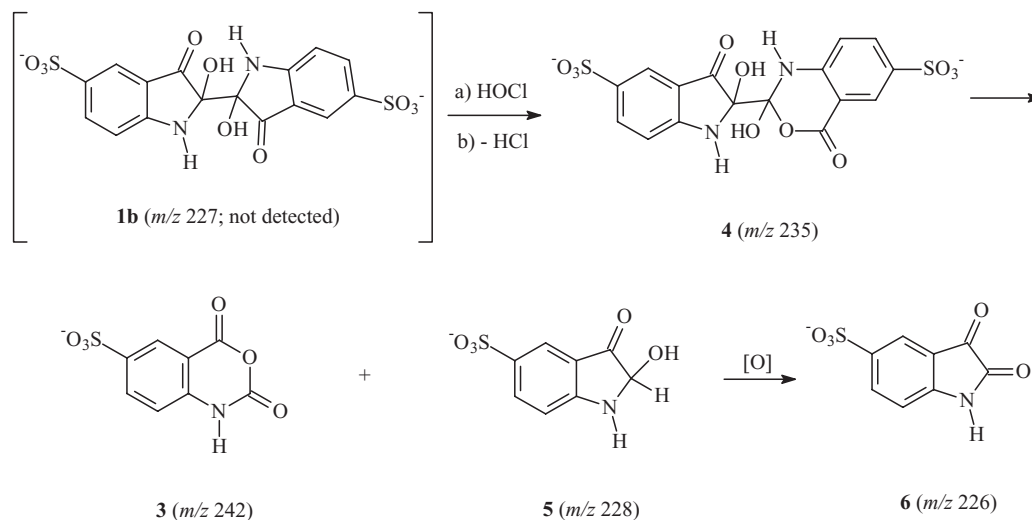
formed in the gas phase as a result of the CID of **2** (Fig. 2 and Scheme 2).

The insertion of one oxygen atom at only one indolic ring of **1b**, via a very similar mechanism to that shown in Scheme 3, was proposed to explain the formation of **4** of m/z 235 (Fig. 1(b) and Scheme 4). Note that the formation of anions **3** of m/z 242 and **5** of m/z 228 can be easily explained by postulating a simple cleavage of the exocyclic C–C bond of **4**. Finally, the formation of anion **6** of m/z 226 can be easily rationalized by supposing an subsequent oxidation of **5** (Scheme 4).

The mass-selection and CID of **4** yielded mainly the product ions of m/z 242 (**3**), 228 (**5**), and 198 (Fig. 3). An

explanation for such a dissociation, which is consistent with the proposed structure for anion **4**, is shown in Scheme 5. Note that anions **3** and **5** are thus formed in both the condensed (Scheme 4) and the gas (Scheme 5) phase as a result of the decomposition of **4**. ESI-MS/MS experiments (spectra not shown) with anions **3**, **5**, and **6** shown mainly the formation of product ions via losses of small molecules such as CO_2 , H_2O , and CO . These findings thus corroborate the proposed structures for such anions.

Finally, high accuracy mass measurements show good agreement between the theoretical and experimental m/z values for the doubly charged anions: **2** (formula: $\text{C}_{16}\text{H}_{10}\text{N}_2\text{O}_{12}\text{S}_2^-$; theoretical: 242.9838; experimental: 242.9831; difference:



Scheme 4. Proposed route for the formation of **4** of m/z 235 and its subsequent decomposition to yield anions **3** (m/z 242), **5** (m/z 228), and **6** (m/z 226).

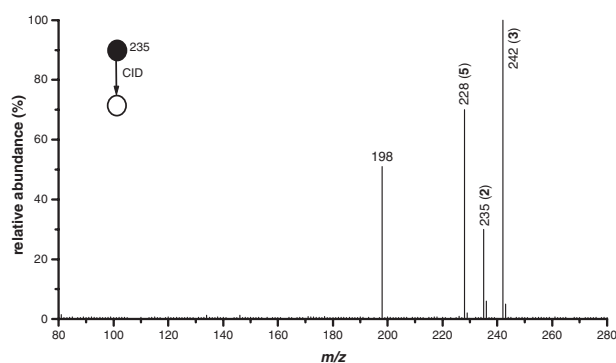


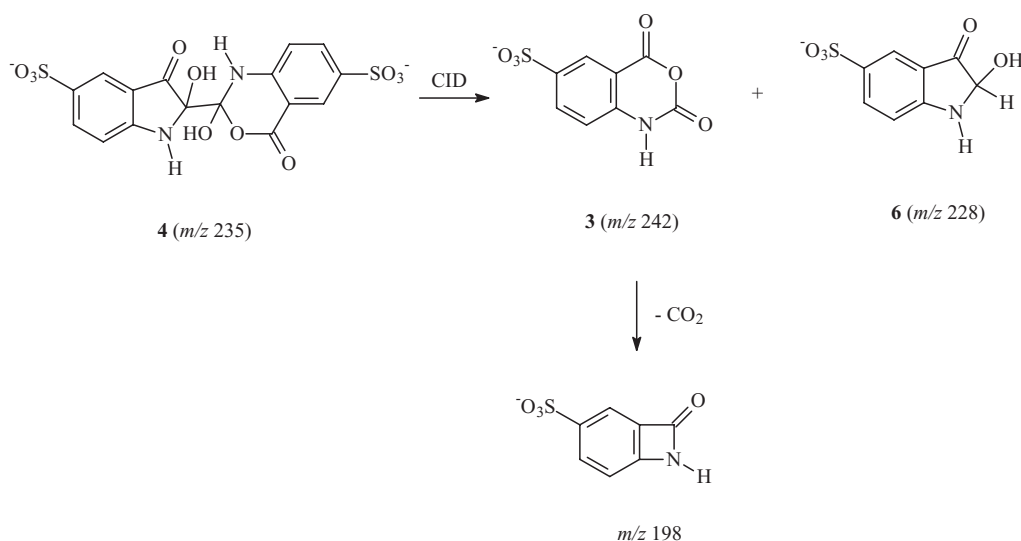
Figure 3. ESI(-)-MS/MS experiment with anion **5** of m/z 235.

3 ppm); **4** (formula: $C_{16}H_{10}N_2O_{11}S_2$; theoretical: 234.9863; experimental: 234.9875; difference: 5 ppm); and the mono-charged anions: **3** (formula: $C_8H_4NO_6S$; theoretical: 241.9759; experimental: 241.9750; difference: 4 ppm); **5** (formula:

$C_8H_6NO_5S$; theoretical: 227.9967; experimental: 227.9955; difference: 5 ppm); **6** (formula: $C_8H_4NO_5S$; theoretical: 225.9810; experimental: 225.9825; difference: 7 ppm).

CONCLUSIONS

The monitoring of the Indigo Carmine degradation by hypochlorite in aqueous solution via direct infusion ESI(-)-MS allowed us to detect unprecedented intermediates, whose structures were proposed based on the ESI(-)-MS/MS data and high accuracy mass measurements. Hence, as well as the double-addition of hydroxyl groups at the exocyclic C=C bond of the dye, hypochlorite was also able to promote the insertion of two oxygen atoms at both indolic rings to yield the oxidized product **2** of m/z 243. Furthermore, a single oxygen atom insertion at just one indolic ring yielded product **4** of m/z 235. Such unstable species would be hardly detected via GC/MS and HPLC/MS, the traditional



Scheme 5. Proposed fragmentation pathways upon mass-selection and collision-induced dissociation (CID) of anion **4** of m/z 235.

techniques used for such analyses. This study further demonstrates the advantageous features of direct infusion ESI-MS for the interception and characterization of transient reaction intermediates. The present results reveal novel routes for Indigo Carmine oxidative degradation and an interesting feature of hypochlorite reactivity.

Acknowledgements

We thank the Minas Gerais State Science Foundation (FAPEMIG), the São Paulo State Science Foundation (FAPESP), and the Brazilian National Research Council (CNPq) for financial support and research fellowships.

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