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Analysis of Sulfonated Anthraquinone Dyes by Electrospray Ionization Quadrupole Time-of-flight Tandem Mass Spectrometry

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Abstract

A tandem mass spectrometric method using a commercial quadrupole–time-of-flight (QTOF) mass spectrometer is described for the identification of sulfonated anthraquinone type dyes, having a 1-amino anthraquinone-2-sulfonate backbone. A total of 9 anthraquinone dye model compounds were evaporated and ionized via negative-ion electrospray ionization (ESI). Ionization of the sulfonated anthraquinone compounds primarily results in the formation of deprotonated molecules, [M-H]-. Once ionized, the ions were subjected to collision-activated dissociation (CAD). The type of neutral molecules or ions cleaved during CAD facilitates identification of the original compound. In most cases, a loss of 64 amu was observed for all dyes and was confirmed to be SO_2 by high resolution mass spectrometry analysis. A unimolecular rearrangement of the sulfonate (SO_3) group was triggered by CAD that allowed loss of SO_2. Also, it was found that different group functionalities attached to the anthraquinone backbone (e.g., secondary aromatic amines and secondary alkyl amines) have specific fragmentation pathways that can be used to distinguish them under similar CAD conditions. For example, an anthraquinone having a secondary amine with an aromatic group attached to it (e.g., Acid Blue 25) can be differentiated from an anthraquinone having a secondary alkyl amine (e.g., Acid Blue 62) based on the product ions. The resultant fragmentation patterns could contribute to the identification of unknown dyes with similar chemical structures. The method was also successfully used in concert with targeted CAD for quantification purposes. The methodology presented here is the first stage in building a high resolution mass spectrometry dye database from the extensive uncatalogued Max Weaver Dye Library at North Carolina State University.

Introduction

The ability to identify unknown dyes in complex mixtures is of great importance in the areas of food, environment, human health, and forensics [1-5]. For example, acid dye manufacturing constitutes one of the highest production worldwide, and is normally used on nylon, synthetic polyamides, wool, silk, paper, inks and leather [6]. The continuous development of new products requiring improved dyeing properties (i.e., leveling characteristics, washfastness, and lightfastness) requires the use of suitable analytical tools for structural identification of new dyes, their byproducts and degradation products, as well as synthetic impurities. However, the chemical structures of dyes are often protected by patents, making their characterization a challenge. In addition, the same dye type may have a different structure depending on the colorant manufacturer. For this reason, we set about to develop a suitable methodology for the analysis of these compounds, both qualitatively and quantitatively.

Mass spectrometry (MS) has evolved into an essential and powerful tool for mixture analysis [7,8]. The high sensitivity, specificity, and speed of MS provides rapid and useful molecular-level information regarding complex mixtures. The development of atmospheric pressure ionization techniques-such as electrospray ionization (ESI)-smoothed the coupling of high-performance liquid chromatography (HPLC)-an invaluable tool in mixture analysis-with MS [9,10]. This approach allows for the determination of the molecular weights and elemental composition of known and unknown analytes. In order to obtain detailed information on the molecular structures of these analytes, tandem mass spectrometry (MS/MS) is required [11-17]. MS/MS elucidates the structures of ionized compounds by their fragmentation reactions through collision-activated dissociation (CAD) [18-20].

The analysis of anionic dyes, such as sulfonated and sulfated dyes, by MS has commonly used negative-ion ESI as an ionization source due to the polar character of the dyes [21-23]. The use of this ionization technique produces a series of deprotonated molecules, which can be very useful in the determination of the molecular weight (MW) of the dye [21]. The total number of acid groups can also be determined with negative-ion ESI based on the number of protons replaceable by sodium ions [24].
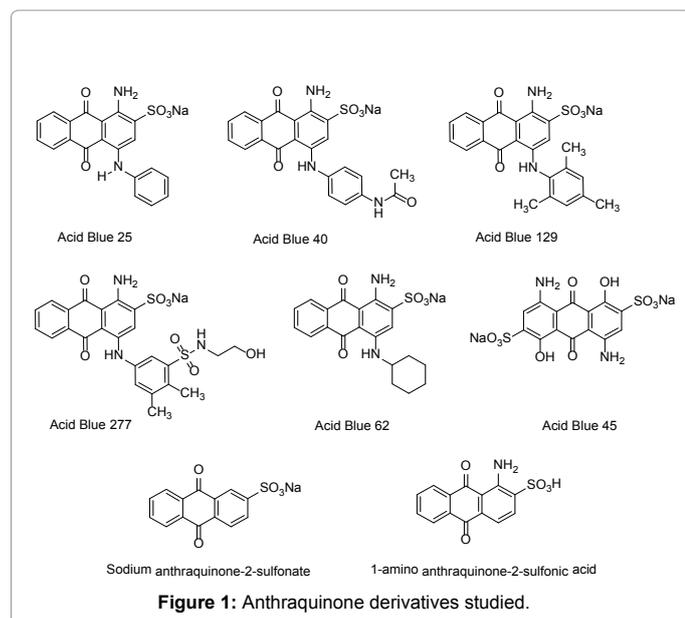
We report here the use of ESI in combination with a quadrupole-time-of-flight (QTOF) MS/MS as a way to determine specific fragmentation pathways of commercial sulfonated anthraquinone dyes for identification purposes. In this study, a series of sulfonated acid dyes containing the structure of 1-amino anthraquinone-2-sulfonate (Figure 1) were analyzed by HPLC coupled to ESI-MS and ESI-MS/MS with the purpose to investigate the featured fragment loss of sulfonated anthraquinone dyes, as well as the fragmentation mechanism by which these molecules disassociate. This will be part of the first phase of building a high resolution mass spectrometry dye database from the extensive uncatalogued Max Weaver Dye Library donated from Eastman Chemicals, with approximately 100,000 dyes, to North Carolina State University.

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Figure 1: Anthraquinone derivatives studied.

No.	Name	Manufacturer	Molecular formula	MW[a]
1	Acid Blue 25	M. Dohmen	$C_{20}H_{14}N_2O_5S$ Na	416.38
2	Acid Blue 45	Ciba Geigy	$C_{14}H_{10}N_2O_{10}S_2Na_2$	474.33
3	Acid Blue 40	M. Dohmen	$C_{22}H_{16}N_3O_6S$ Na	473.43
4	Acid Blue 62	Classic Dyestuffs	$C_{20}H_{19}N_2O_5SNa$	422.43
5	Acid Blue 129	Sigma-Aldrich	$C_{23}H_{21}N_2O_5SNa$	460.48
6	Acid Blue 277	Ciba Geigy	$C_{24}H_{23}N_3O_8S_2Na$	567.57
7	1-amino anthraquinone-2-sulfonic acid	Sigma-Aldrich	$C_{14}H_9NO_5S$	303.29
9	Sodium anthraquinone-2-sulfonate	Sigma-Aldrich	$C_{14}H_7O_5S$ Na	310.26

[a] Molecular Weight (MW)

Table 1: Sulfonated anthraquinone acid dyes and sulfonated anthraquinone model compounds.

Materials and Methods

Chemicals

Methanol and acetonitrile (LC-MS grade) were purchased from Honeywell & Burdick Jackson (Muskegon, MI, USA). The solvents were filtered through 0.22 μm Millipore filters (Whatman, GE Healthcare, UK). Ammonium formate (>99%, HPLC grade, Fluka, Switzerland), formic acid (~98%, MS grade), Sodium anthraquinone-2-sulfonate (**A1**), 1-Amino anthraquinone-2-sulfonic acid (**A2**) and Acid blue 129 were purchased from Sigma Aldrich (St Louis, MO, USA). The acid dyes (Table 1 and Figure 1) were supplied by Ciba Specialty Chemicals, M. Dohmen, and Classic Dyestuffs.

Sample preparation

Standard dye solutions were prepared at 1 mg/mL concentrations in HPLC grade 70:30 (v/v) methanol (MeOH)/ acetonitrile (CH_3CN). All solutions for analysis were prepared by adding 20 μL of standard dye solution to 980 μL of water (Milli-Q).

Instrumentation

Liquid chromatography/mass spectrometry (LC/MS): The experiments were carried out using an Agilent (Santa Clara, CA)

Accurate Mass 6520 Q-TOF mass spectrometer equipped with an ESI source operating in negative-ion mode ((-)ESI) and coupled with an Aglient 1260 SL HPLC system. The chromatography runs were performed using an Agilent Zorbax Eclipse Plus C_{18} column (2.1 × 50 mm, 3.5 μm) with a Zorbax Eclipse Plus C_{18} narrow bore guard column (2.1×12.5 μm, 5 μm). The mobile phase used for separation consisted of a mixture of 20 mM ammonium formate and 0.01% formic acid in H_2O (A) and 70:30 MeOH/CH_3CN (B). The flow rate was 0.5 mL/min with an injection volume of 3 μL. Negative ESI source parameters were as follows: nebulizer pressure, 35 psi; capillary voltage, 4000 V; drying gas flow, 12 L/min at 350 °C; and fragmentor voltage, 110 V. The instrument was operated in 4 GHz high resoltuion mode. Instrument control, data aquisistion, and analysis were performed using Agilent MassHunter Workstation Acquisition and Agilent MassHunter Qualitative Analysis B.06.00.

Collision activated dissociation (CAD): MS/MS experiments were performed on the collision cell (hexapole) of the QTOF by selecting the ion of interest with an isolation width of 1.3 Da (narrow) and colliding the ion with nitrogen gas (99.9995%) with collision energy of 40 V. All fragment ions were guided to the TOF mass analyser for detection.

Targeted MS/MS QTOF quantitation for acid blue 25: Quantitation of Acid Blue 25 (AB25) was achieved by using the fragment ion (m/z 329.0926) abundance generated from the corresponding deprotonated dye molecule. Five calibration solutions of AB25 were prepared (20.0, 40.0, 60.0, 80, and 100.0 μg/mL in 70:30 MeOH/CH_3CN) for calibration. A solution containing 50.0 μg/mL was analyzed by this MS/MS method and validated with a traditional liquid chromatographic method. All experiments were run at least three times for reproducibility purposes.

Results

A total of nine anthraquinone compounds were examined via (-) ESI/MS/MS. Most analytes primarily formed stable deprotonated molecules upon negative-ion ESI when using the 70:30 MeOH/CH_3CN as the solvent. The deprotonated molecules were subjected to CAD to obtain structural information. High-resolution measurements were carried out to verify the identities of the neutral molecules lost upon fragmentation. A detailed discussion on each of the anthraquinone derivatives studied is provided below.

Anthraquinones model compounds

A1, was the simplest anthraquinone derivative studied; it contained a sulfonate group at the 2 position with no other substituents on the aromatic system. Under negative-ion ESI conditions **A1** forms an abundant deprotonated molecule [M-H], which fragments upon CAD by loss of SO_2-confirmed by exact mass measurements (Table 2) to generate a unique fragment ion with a mass-to-charge ratio (m/z) of 223.0429. The loss of this neutral SO_2 molecule suggested a rearrangement of the sulfonate group (SO_3), which is in agreement with previous theoretical [25] and experimental [26] studies on aromatic systems. The fragment ion after the SO_3 rearrangement is considered a phenoxide anion and is useful for identification of similar structures.
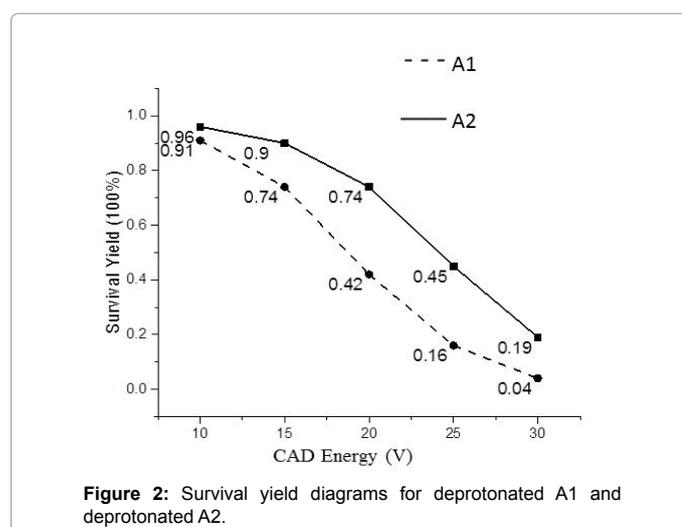
A2, having an amino group at the ortho-positon to the sulfonate group, was found to undergo loss of SO_2 upon CAD of the deprotonated ion, giving a single fragment ion with m/z 238.0512 (Table 2). This fragment ion also generates a phenoxide anion, which was confirmed by high resolution MS.

In order to understand the fragmentation efficiency of these two

Analyte (MW)	MS (m/z)[a]	MS/MS CAD fragment ions (m/z)
A1 (310.26)	[M-H]$^{-b}$ (287.0022)	**287.0022 – SO_2 (223.0409)**
A2 (303.28)	[M-H]$^-$ (302.0129)	**302.0129 – SO_2 (238.0512)**
Acid blue 25 (416.38)	[M-H]$^-$ (393.0529)	**393.0529 – SO_2 (329.0926)** 393.0529 – NHC_6H_5 (301.0944) 393.0529 – SO_2 - NHC_6H_5 (329.0926)
Acid blue 40 (474.33)	[M-H]$^-$ (450.0763)	**450.0763 – SO_2 (386.1151)** 450.0763 – SO_2 – C_2H_3O (386.1151) 450.0763 – SO_2 – $C_8H_5N_2O$ (237.0429) 450.0763 – SO_2 – C_8H_8NO (252.0540)
Acid blue 45 (473.43)	[M-H]$^-$ (428.9781)	**428.9781– SO_2 (365.0052)** 428.9781– $C_6H_{12}N$ (289.0043) 428.9781– SO_2 – SO_2 (285.0527)
Acid blue 62 (422.43)	[M-H]$^-$ (399.1009)	**399.1009 –C_6H_{11} (316.0155)** 399.1009 – SO_2 (289.0060) 399.1009 – SO_2 – C_6H_{11} (252.0533)
Acid Blue 129 (460.48)	[M-H]$^-$ (435.1020)	**435.1020– SO_2 (371.1412)** 435.1020– SO_2 – C_6H_{11} (252.0548) 435.1020– SO_2 – $C_7H_{12}N$ (225.0464)
Acid blue 277 (567.57)	[M-H]$^-$ (544.0846)	544.0846 – SO_2 (480.1213) **544.0846 – $C_2H_6NO_3S$ (420.0780)** 544.0846 – SO_2 – $C_2H_6NO_3S$ (356.1165) 544.0846 –$C_{10}H_{15}N_2O_3S$ (301.0045) 544.0846 – SO_2 –$C_{10}H_{15}N_2O_3S$ (237.0427)

[a]The m/z value is the monoisotopic mass. [b]Deprotonated molecule, the sodium (Na+) cation under negative-ion ESI conditions will not be added to the m/z value. The m/z value of deprotonated molecule will differ by one or two sodium ions and/or one hydrogen atom from the total molecular weight of the dye.

Table 2: Ions formed upon ESI as well as the product ions formed upon CAD experiments for sulfonated dyes.


Figure 2: Survival yield diagrams for deprotonated A1 and deprotonated A2.

Scheme 1: Proposed mechanism of the loss of SO_2 from A2 (Rationalization of SO_3 rearragment and the generation of the phenoxide anion).

molecules, the two compounds were fragmented at the same CAD energies (ranging from 10 to 35V in 5V intervals). The Survival Yield (SY) methodology [27-29] was used as defined in Eqn. 1:

$$SY = \frac{I_p}{I_p + \sum I_f} \qquad (1)$$

where I_p is the intensity of the precursor ion (deprotonated molecule) and $\sum I_f$ is the sum of fragment intensities. A higher SY suggests the compound requires higher energy to be fragmented. Comparison of the SY between the compounds **A1** and **A2** is shown in Figure 2. The results suggest that the **A2** requires higher energy to fragment.

A possible explanation of the extra energy needed for fragmentation of **A2** can be related to hydrogen bonding between the hydrogen atom of the amino group and the oxygen atom of the sulfonate group. This can form a six-membered ring that stabilizes the deprotonated
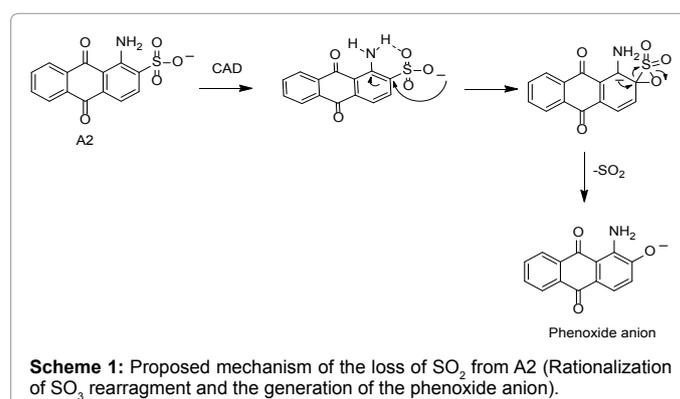
molecule. A proposed mechanism of the sulfonate rearrangement is depicted in Scheme 1. The formation of an epoxide that leads to the cleavage of the SO_2 molecule has been proven as a favorable pathway by theoretical studies [25].

Acid dyes containing a secondary aromatic amine: Acid blue dyes 25, 40, 129 and 277 form abundant deprotonated molecules [M-H]$^-$ upon ionization via negative-ion ESI. All deprotonated acid dyes fragment upon CAD with a main fragment ion loss of SO_2 and a minor fragment ion loss of the secondary aromatic amine (NHC_6H_5, Table 2). These results suggest that the lowest energy pathway of fragmentation is the rearrangement of the sulfonate group to lose SO_2 as compared to the loss of the secondary aromatic amine. This characteristic fragmentation pathway is useful in determining possible substituents present on the anthraquinone backbone (Figure 3).
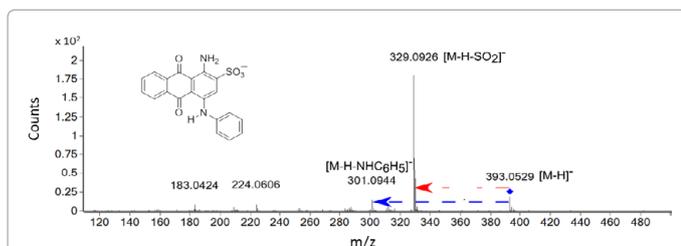
Acid Blue 62 (AB62) generates an abundant deprotonated molecule upon ionization via negative-ion ESI. CAD experiments showed that deprotonated AB62 dissociated via loss of alkyl group C_6H_{11} as the main fragment ion (m/z 316.0155) followed by loss of SO_2 (yielding an anion with a m/z of 289.0060, Table 2). These results suggested that

the lowest fragmentation energy pathway is the loss of the cyclohexyl group from the deprotonated acid blue as compared to the loss of SO_2 (Figure 4). Furthermore, this dissociation pathway is different from the ones observed for acid dyes containing secondary aromatic amines as substituents on the anthraquinone backbone.
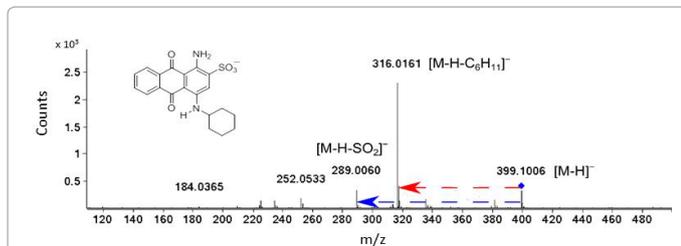
Acid Blue 277 forms an abundant deprotonated molecule upon ionization via negative-ion ESI. This ion fragments via loss of $C_2H_6NO_3S$ to generate the main fragment ion with a m/z 420.0780 followed by the loss of SO_2 (m/z 480.1213, Table 2). This result suggests that the lowest energy path of fragmentation is loss of alkyl groups attached to aromatic ring of the secondary aromatic amine.

The CAD studies of the nine anthraquinone derivatives showed an interesting loss of SO_2, which can generate a very stable phenoxide anion useful for quantification by MS/MS. AB25 was selected to test the efficiency of quantitation by this method. The fragment ion with m/z 329.0926, generated upon CAD of the deprotonated ion (m/z 393.0529), was selected for calibration and sample quantification. The calibration curve is showed in Figure 5.
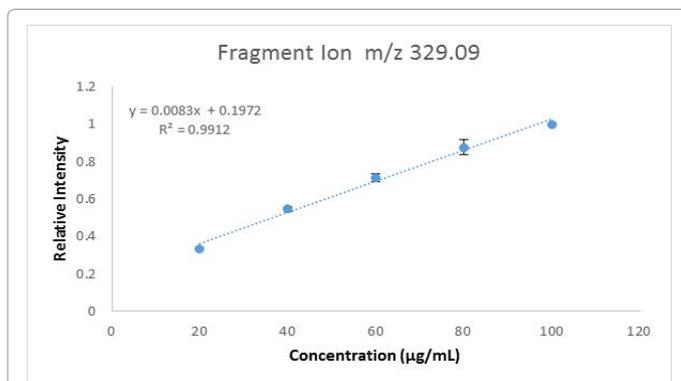
A solution of AB25 with a concentration of 50.0 µg/mL was quantified by HPLC and by targeted MS/MS. The results of the AB25 solution were for HPLC 50.77 µg/mL and for Targeted MS/MS 54.64 µg/mL. The percent error for the MS/MS method was 7.62% compared to the HPLC quantification value. The overall results strongly suggest that the phenoxide anion generated by loss of SO_2 by CAD is an exceptional ion for performing MS/MS quantification.

Conclusions

The deprotonated molecules formed upon negative-ion ESI were subjected to CAD events revealing fragmentation patterns that facilitated the identification of the anthraquinone derivatives. A loss of SO_2 was observed for all the sulfonated anthraquinone dyes, which indicated a characteristic rearrangement of -SO_3 functional group to form a stable phenoxide fragment ion. It became clear that this was the dominant fragment ion when the dye contained a secondary aromatic amine substituent (e.g., Acid Blue 25). In the presence of alkyl substituent groups on the anthraquinone backbone, the SO_3 rearrangement was not the main fragmentation pathway. Quantification by QTOF MS/MS of a sulfonated anthraquinone was feasible when the stable phenoxide anion was selected as observed in the AB25 experiments.

Overall, the fragmentation of acid dyes with similar group functionalities was analyzed. This provided tools for fingerprinting anthraquinone dyes and generating a database of dyes containing sulfonated anthraquinone structures. The results of this study will be applied on the Max Weaver Dye Library for structure characterization.

Figure 3: MS/MS spectrum obtained after CAD of the deprotonated molecule of acid blue 25 (m/z 393.0529).

Figure 4: MS/MS spectrum obtained after CAD of the deprotonated molecule of acid blue 62 (m/z 399.1006).

Figure 5: Quantitative analysis of AB 25 (20.0 µg/mL-100.0 µg/mL) Bars represent standard deviation analysis for three replicates. The intercept is 0.1972 and slope 0.0083.

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