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Speciation of chromium dyes by high-performance liquid chromatography with inductively coupled plasma mass spectrometric detection

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Abstract

High-performance liquid chromatography (HPLC) coupled to inductively coupled plasma mass spectrometry (ICP-MS) was employed for the separation and detection of chromium species in azo dyes, Acid Blue 158 and Acid Blue 193; mainly Cr(III) and Cr(VI). The dyes were first analyzed for total metal content using ICP-MS and their stability in solution was studied by measuring their absorbance through a range of pH values. Then an isocratic chromatographic method employing reversed-phase liquid chromatography with mass spectrometric detection was developed. Applying this method to the separation of these dyes, the absolute detection limits of Acid Blue 158 and 193 were 1 and 5 ng respectively. Additionally, Acid Blue 158 did not contain any unbound chromium species. On the other hand, Acid Blue 193 contained uncomplexed and potentially bioavailable Cr(III). Acid Blue 193 did not have any toxic Cr(VI) present in the samples.

Keywords: Chromium; Azo dyes; Dyes

1. Introduction

Azo dyes are used in diverse applications including textile dyes, paint pigments, printing inks, food coloring, plastics and cosmetics [1]. The textile industry is the largest consumer of these products and accounts for two thirds of the dyestuff market [2]. The high number of azo dyes is attributed to the wide variety of structures that yield large numbers of chromophores in the visible region. The typical structure of azo dyes is Ar–N=N–Ar[1] where Ar and Ar[1] are substituted phenyl or naphthyl groups. The substituents may also include additional azo groups, chelated metal ions and charged organic groups.

The manufacturing and use of azo dyes creates wastes that are discarded in waste water and solid residues. These dyes can be reduced in the environment [3] and in vivo [4] to produce carcinogens such as naphthylamines [4,5], substituted phenylamines, or benzidine analogs [6,7]. In addition, the presence of unchelated metal in the environment, even in trace amounts, can pose serious health risks to many organisms. Acid Blue 193 and Acid Blue 158 are two commonly used chromium azo dyes and their structures are shown in Fig. 1.

Speciation (separation and quantitation of different 'forms' of an element) of chromium metal is of

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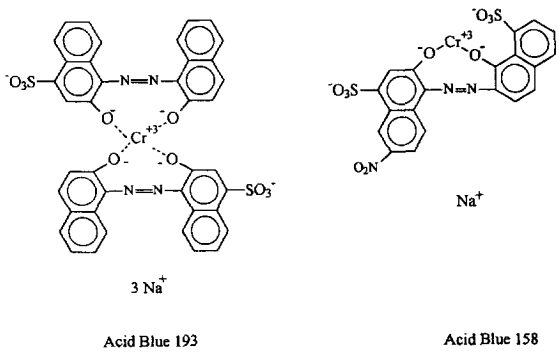

Fig. 1. Structures of Acid Blue 193 and 158.

toxicological interest because it makes its way into the environment via industrial wastes. The two most common forms of chromium are Cr(III) and Cr(VI). Cr(III) is an essential nutrient found in many foods while Cr(VI) is the toxic form of the metal. Hexavalent chromium is known to cause lung cancer and produce DNA mutations, while Cr(III) is present in most soils and is less mobile in the environment than Cr(VI) [8].

Separation methods for azo dyes are frequently based on high-performance liquid chromatography (HPLC) with ultraviolet–visible (UV–Vis) detection [9,10]. Structure elucidation or identification of dyes by mass spectrometry has been reported by using electron ionization for non-polar petroleum azo dyes [11] and field desorption [12] or fast atom bombardment [13] for very polar colorants such as sulfonated and phosphonated azo dyes. Liquid chromatography with electrospray mass spectrometric detection for azo dyes was reported by Straub et al. [14]. Bruins et al. published the separation of azo dyes by liquid chromatography with atmospheric pressure ionization mass spectrometry [15]. In addition, gas chromatography–mass spectrometry [16] and capillary zone electrophoresis [17] have been employed for the separation of azo dyes.

Inductively coupled plasma mass spectrometry (ICP-MS) is a robust technique for elemental analysis. Solution sample introduction typically involves the nebulization of the sample into the plasma followed by evaporation of the solvent, atomization and ionization of the metal of interest. Detection is then accomplished by the mass spectrometer at very low levels. When coupled to HPLC, the separation

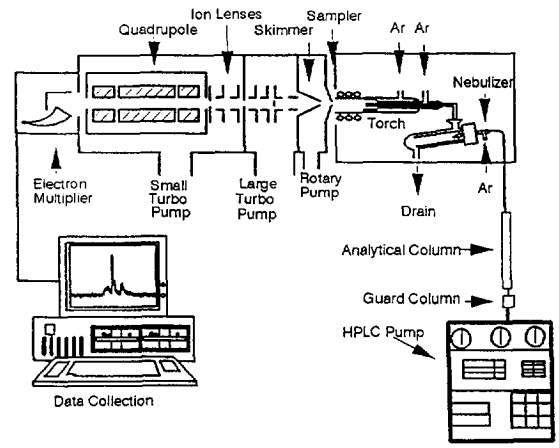

Fig. 2. Schematic diagram of HPLC–ICP-MS.

power of chromatography allows the various chemical forms of different metals to be isolated and detected. A schematic diagram of HPLC–ICP-MS is shown in Fig. 2.

In a joint effort with the University of Cincinnati, Department of Environmental Health, we have attempted to establish whether the chromium-complexed dyes, Acid Blue 158 and 193, are stable in the environment as currently used, or whether they enter into physical, chemical or biological processes that release the metal from the organic complex. In this paper, we undertook the first step in this project which involves the development of a chromatographic method which can separate and detect Cr(III), Cr(VI) and other inorganic chromium species of Acid Blue 158 and 193 at sub-ng levels.

2. Instrumentation

The ICP-MS instrument used in this experiment was a PlasmaQuad II (VG Elemental, Winsford, UK). The ICP-MS conditions were typical: forward power (1500 W), reflected power (<5 W), coolant gas (15.5 l min^{-1}), auxiliary gas (1.3 l min^{-1}), nebulizer gas (0.7 l min^{-1}) and oxygen gas (0.07 l min^{-1}). The conditions were optimized daily to get the best chromium signal. The sample introduction system was equipped with concentric nebulizer, Type C-1 (Precision Glass Blowing of Colorado,

Parker, CO, USA) and a double-pass Scott-type spray chamber cooled to −2°C with a Neslab Endocal refrigerated chiller (Neslab Instrument, Portsmouth, NH, USA). A nickel sampler and skimmer, each with a 1.0-mm diameter orifice, were used. Optimum signals were obtained by nebulizing a solution of 20 ng g^{-1} of chromium in the mobile phase and then adjusting the sampling position and ion-lens voltages for the best signal. Chromium was monitored at *m/z* 53 (abundance 9.55%) to avoid interferences from ArC^+ at *m/z* 52. A Hewlett–Packard HP 8452A diode array spectrophotometer (Boise, ID, USA) was also employed. The data collected were converted to a Lotus 123 format for data manipulation. All calculations, unless otherwise stated, were based on peak areas and three trials.

3. Reagents

Chromium(III) chloride hexahydrate ($CrCl_3 \cdot 6H_2O$), potassium dichromate ($K_2Cr_2O_7$) and ammonium sulfate [$(NH_4)_2SO_4$] were purchased from Aldrich (Milwaukee, WI, USA) and used without further purification. HPLC-grade methanol and ammonia solution were purchased from Fischer (Fairlawn, NJ, USA). Doubly distilled concentrated nitric acid was purchased from GFS (Columbus, OH, USA). The dye samples, Acid Blue 158 and Acid Blue 193, were received from the Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers, US Dye Manufacturers Operating Committee of ETAD (Washington, DC, USA) as press cakes and were used without further purification. All solutions and mobile phases were prepared with distilled, de-ionized water (18 MΩ, Barnstead, Newtown, MA, USA). Samples were filtered through 0.45-μm disposable syringe filters made of nylon 66 (Alltech, Deerfield, IL, USA) prior to their injection on the analytical column.

4. Chromatographic conditions

The HPLC system was a Dionex Model DX-300 metal free system 300 (Sunnyvale, CA, USA), consisting of an eluent de-gas module EDM2, a gradient pump AGP, a Dionex AI-450, RELEASE 3.10 chromatography software program and a Dionex UV–Vis monochromator. A Rheodyne Model 9125 metal free injector (Cotati, CA, USA) with a 100-μl polyether ether ketone (PEEK) sample loop was employed. Connections between the injector, guard column and separation column were made using PEEK tubing (Alltech). A piece of PEEK tubing connected the outlet of the column to the nebulizer of the ICP-MS system. The analytical column was a Dionex AS 7 (250 mm×4 mm) anion-exchange column (10-μm particle diameter, 100 μequiv. alkyl quaternary ammonium functional group) with a NG 1 (100 mm×4 mm) guard column. For the separation on Dionex AS 7, the mobile phase consisted of 50 m*M* ammonium sulphate in doubly deionized water (18 MΩ). The pH of the solution was adjusted to 9 using ammonium hydroxide. The standard used for uncomplexed chromium(III) was prepared from $CrCl_3 \cdot 6H_2O$ in doubly deionized water. It was diluted to the desired concentrations using the mobile phase. The samples were also prepared in mobile phase. The flow-rate was 1 ml min^{-1}.

The separation of both Acid Blue 193 and Acid Blue 158 was performed on a Baxter B and J OD5 Octadecyl column (250 mm×4.6 mm with 5-μm particle diameter). The mobile phase consisted of water–methanol (80:20, v/v). The standard used for uncomplexed chromium(III) was prepared from $CrCl_3 \cdot 6H_2O$ in doubly deionized water. It was diluted to the desired concentration using the mobile phase. The dye samples were prepared in mobile phase. The flow-rate used was 1 ml min^{-1}.

5. Results and discussion

One method for the separation and detection of trace metal species at sub-ng levels is by using liquid chromatography coupled to ICP-MS. Liquid chromatography provides adequate and reliable separation while ICP-MS gives quantitation and low detection limits. In some instances, UV–Vis detection is used to investigate the presence of a conjugated system in the samples.

The dye samples were analyzed first for metal content using solution nebulization with ICP-MS detection. Direct solution nebulization was utilized with no chromatographic column and elemental

chromium was found in the dye samples. Since chromatographic separation was not used, the various ionic forms of chromium were detected as elemental chromium. A calibration curve was then generated for chromium using chromium standards in a concentration range between 1 and 200 ng g^{-1}. The dyes were found to contain a total chromium content of 2.9% and 5.8% for Acid Blue 198 and 153, respectively. These values include not only the metal incorporated in the dye but also account for any uncomplexed metal present in the sample. All solutions prepared for the rest of the study were based on these values.

Preliminary studies were then performed to determine the stability of Acid Blue 193 in the mobile phase by monitoring its absorbance spectra at various pH values over a period of time. The wavelengths monitored were from 400–700 nm with a maximum at 578 nm. The results show that the absorbance of the dye changes after the first 24 h indicating a change in its concentration. Fig. 3 shows the absorbance of 1 μg g^{-1} samples of Acid Blue 193 monitored over a period of two days. The absorbance changes dramatically with time and serves to illustrate how difficult it is to preserve the species in its original form when doing speciation studies. Similar results were obtained for Acid Blue 158. Therefore, fresh dye samples were prepared and used daily.

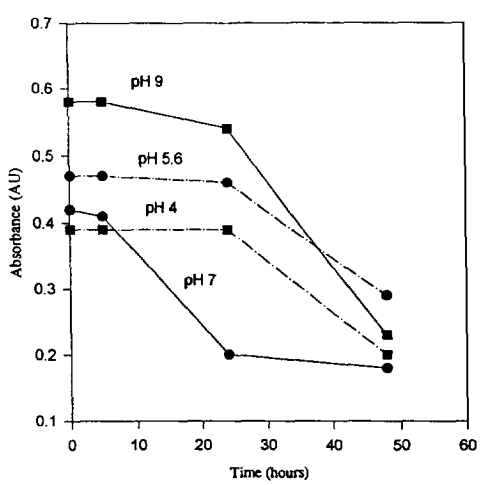

Fig. 3. Absorbance monitored over a period of time for Acid Blue 193 at different pH values.

5.1. Anion-exchange chromatography

The separation of standards of Cr(III) and Cr(VI) on an anion exchange column was developed previously in our laboratories [18]. Since chromatographic separation had not been applied to 'real' samples that contain chromium in various ionic forms, we attempted to apply it to the dye samples to expand the applications of the separation method. The chromatogram for 50 ng of Acid Blue 158 is shown in Fig. 4. The retention time for the single peak of Acid blue 158 did not correspond to the retention times of uncomplexed Cr(III) and Cr(VI) indicating that the sample did not contain any unbound Cr(III) or Cr(VI). However, when 100 ng of Acid Blue 193 were injected, a peak that corresponded to uncomplexed Cr(III), the hexaaquo-chromium(III) complex, appeared as shown in Fig. 5. The sample did not contain any Cr(VI). All attempts to resolve the peaks of Acid Blue 193 by changing the ionic strength, pH and flow rate of the eluent failed. Since the separation could not be achieved by anion-exchange chromatography, reversed-phase liquid chromatography was explored to resolve the peaks of Acid Blue 193. A new separation method was developed for that purpose.

5.2. Reversed-phase liquid chromatography

5.2.1. Acid Blue 193

Several mobile phases with various organic concentrations ranging from 5–50% methanol in water

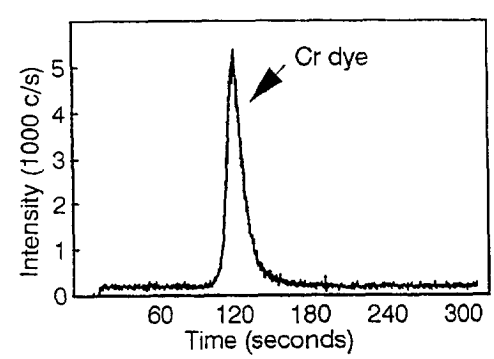

Fig. 4. Chromatogram of 50 ng of Acid Blue 158 by anion-exchange chromatography. The mobile phase consisted of 50 m*M* ammonium sulfate (pH 9). The flow-rate was 1 ml min^{-1}.

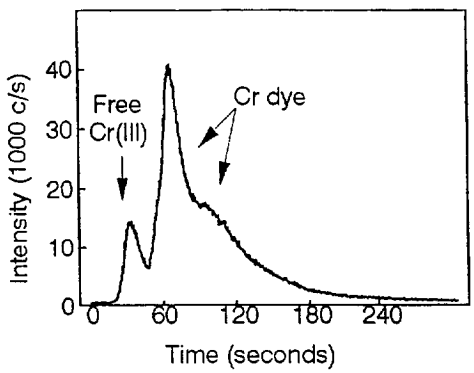

Fig. 5. Chromatogram of 100 ng of Acid Blue 193 by anion-exchange chromatography. Chromatographic conditions same as Fig. 4.

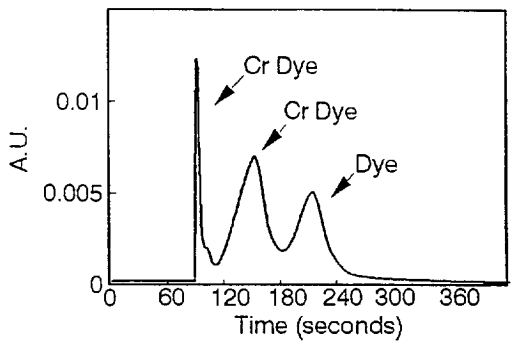

Fig. 7. Chromatogram of 50 ng of Acid Blue 193 by reversed-phase liquid chromatography with UV–Vis detection. The wavelength monitored is 582 nm. Chromatographic conditions same as Fig. 6.

were investigated for the reversed-phase liquid chromatographic method with mass spectrometric detection. The optimized separation of a standard of Cr(III) and Cr(VI) was with water–methanol (80:20, v/v). The flow-rate was 1 ml min^{-1}. Fig. 6 shows the separation of 50 ng of Acid Blue 193 using these same conditions. The second peak in the chromatogram has the same retention time as that of a Cr(III) standard and is therefore assigned as uncomplexed Cr(III) i.e. the hexaaquo form. The first and third peaks contain chromium and were detected by mass spectrometry at m/z 53. In an attempt to gain more information about these species, UV–Vis detection (wavelength 572 nm) was used instead of ICP-MS detection. The chromatogram of 50 ng of Acid Blue 193 is shown in Fig. 7. The first and second peaks had identical retention times as the first and third peaks of the chromatogram with ICP-MS detection, indicating that these peaks contained chromium metal and a conjugated organic species. Also, a third peak appeared in the UV–Vis chromatogram and was not seen by ICP-MS which indicates an organic species with no chromium metal.

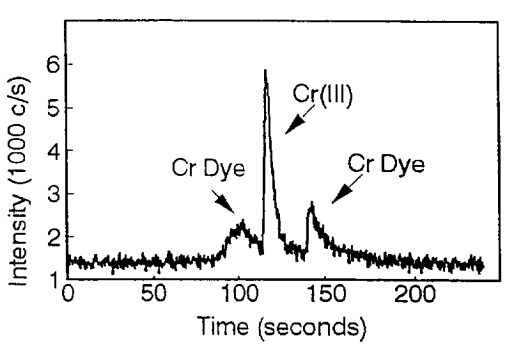

Fig. 6. Chromatogram of 50 ng of Acid Blue 193 by reversed-phase liquid chromatography. The mobile phase composition was water–methanol (80:20, v/v). The flow-rate was 1 ml min^{-1}.

Figures of merit for Acid Blue 193 were calculated based on Cr(III). The calibration curve was linear with an r^2 value of 0.9952. The slope of the log–log plot was 0.9937. Relative standard deviation for five 100 ng g^{-1} Acid Blue 193 injections was 3%. The absolute detection limit for Cr(III) in the sample was 5 ng. The linear dynamic range was 3 ranging from 10 ng g^{-1} to 10 μg g^{-1}. The recovery of Acid Blue 193 was 93%.

To rule out the possibility that Acid Blue 193 was decomposing on the column, the flow-rate of the eluent was varied between 0.8, 1.0 and 1.2 ml min^{-1} and all peak areas were calculated. Each chromatogram showed three peaks. At a flow-rate of 0.8 ml min^{-1}, the ratio of peak areas 1:2, 2:3 and 1:3 was 0.9, 1.0 and 0.9 respectively. At 1.0 ml min^{-1}, the ratio of peak areas 1:2, 2:3 and 1:3 was 0.9, 1.0 and 0.9. Also at 1.2 ml min^{-1}, the ratios were 0.9, 1.1, and 0.9. Since the ratio of peak areas of the sample remained constant at different flow-rates, it was then assumed that the dye did not decompose on the column. However, the changes in the flow-rate used above result in less than 50% residence time on

the column and that small window of time may be insufficient to detect any degradation.

5.2.2. Acid Blue 158

Using the same mobile phase, 100 ng of Acid Blue 158 was injected and the chromatogram is shown in Fig. 4. Again, only one peak appeared in the chromatogram denoting that the dye sample did not have any unbound metal and all the chromium metal was complexed to the organic dye. Attempts at changing the chromatographic conditions (mobile phase composition, pH, flow-rate) did not produce any other peaks, and so the sample was considered to contain one species only.

Since this sample did not contain any uncomplexed chromium and it was standardized previously (total metal content), it was then treated as a standard and a calibration curve of dye samples with concentrations ranging from 10 ng g^{-1} to 10 $\mu g\ g^{-1}$ was generated. The figures of merit were depicted as follows: The calibration curve was linear with $r^2 = 0.9991$ and the slope of the log–log plot=0.9978. The linear dynamic range of five 100 ng g^{-1} injections of Acid Blue 158 was 3%. The detection limit was 3 ng g^{-1} and the absolute detection limit was 1 ng. The recovery was 98%.

The presence of unbound Cr(III) in Acid Blue 193 but not in Acid Blue 158 may be attributed to impurities and failure to get complete purification after the synthesis of the dyes. Additionally, although fresh dye samples were prepared daily to preserve the chromium species in their original form, the dyes may well have differing stability constants in the particular environment in which they were prepared.

In conclusion, a new method for the separation and detection of chromium in Acid Blue 193 and Acid Blue 158 was developed. The isocratic separation was performed by using reversed-phase liquid chromatography with ICP-MS detection. Acid Blue 158 did not contain any uncomplexed Cr(III) or Cr(VI) and all the chromium metal was incorporated in the dye. The absolute detection limit was 1 ng. Although Acid Blue 193 did not contain any Cr(VI), unbound Cr(III) was found in the sample. The absolute detection limit for Acid Blue 193 was 5 ng.

Acknowledgments

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