Evaluating the Safety of Color Powders via Chromatographic, Spectroscopic, and Microscopic Analysis of Their Dye Constituents

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INTRODUCTION

The color dye industry is one of the largest sectors that spans the global market. Synthetic color dyes are multifaceted and have various roles in the textile, food, medical, forensic, and beauty industry. Industrially, 10,000 different dyes produce over 500,000 tons of synthetic dye a year. Industrial companies use unique proprietary dyeing processes to create unique color goods. Dyes are heavily regulated by the U.S. Food and Drug Administration due to the harmful side effects, ranging from allergic reactions to carcinogenic effects. European and Asian countries differ from America when it comes to which dyes are acceptable for use. A surge in recreational activities have caused an increase in the use of color powders for events, especially color run races. Races typically have at least 5 types of color powders and use the recommended amount of half a pound to one pound of powder per runner. Due to Internet commerce, it is extremely fast and easy to obtain powders from overseas that may contain toxic dyes with harmful side effects. In addition to this, organizers of color run races do not readily disclose information about the dyes that may cause adverse health effects to runners or users with sensitivity to dyes.

APPLICATIONS

- Weddings
- Religious Festivals
- Color Run Races

OBJECTIVES

- To identify dye components in unknown color powders: CC Blue, CC Red, and India Green
- Develop analytical methods for color dye extraction, identification, and quantitation.

MATERIALS & METHODS

Materials: 0.5 grams of color powder, namely CC Blue, CC Red, and India Green provided by FTW-ABC News Channel, was transferred to 15-mL centrifuge tubes for dye extraction.

Method:

Solid-liquid extraction was done using UHP water for CC Red and CC Blue and acetonitrile for India Green.

Samples were vortexed and sonicated with a Branson sonicator for one hour.

Samples were centrifuged for 15 minutes at 4000 rpm at 4°C.

Ensure ProRaman-L spectrometer with 785 nm laser was used to analyze the color extracts.

Microscopic analysis of dye extracts and standards using Hitachi X-3400N SEM and Oxford Aztec EDS.

Thermo (S9910) FTIR analysis of CC Red and CC Blue dye extract using an ATR and a DTGS detector.

Supernatant samples were filtered with 0.22-micron Nylon filters prior to analysis using chromatographic and spectroscopic techniques.

RESULTS

Analysis of Dye in CC Blue Powder

Figure 1: FTIR Spectrum of Acid Blue 9 and CC Blue

Figure 2: H-NMR of Acid Blue 9 and CC Blue

Figure 3: Stacked Raman Spectra of Allura Red AC and CC Red

Key spectral features of Acid Blue 9 that match CC Blue:

- Amine (1620-1627 cm⁻¹) and sulfonic group (1350-1184 cm⁻¹).
- C-Cl (460 cm⁻¹) in CC medium stretch (1010 cm⁻¹), and sulfonic group (1360-1150 cm⁻¹).
- LC-MS: Peak RT (17.9 min) for Acid Blue 9 (MW: 792.222 g/mol), major m/z values of MS: 747.1505 (M–2NaH)+, 373.5753 (M–NaH)+, 290.0851 (cleavage of single bond of triphenylmethane center), and 211.3841 (replacing the loss of SO₃ with hydroxylic bond cleavage at the triphenylmethane center).

Analysis of Dye in CC Red Powder

Figure 4: H-NMR of Acid Red AC and CC Red

Figure 5: Overlaid Chromatograms of Leucomalachite Green (5.73 min), CC Blue (Baseline Corrected), and CC Red (Baseline Corrected). Structure 3: Leucomalachite Green

Key features of Allura Red AC:

- Aromatic ring stretch (1610-1620 cm⁻¹), sulfonic group (1140-1180 cm⁻¹), and aromatic azo peak (1425-1430 cm⁻¹).
- C-H aromatic ring (1650 cm⁻¹), and bond (1215 cm⁻¹), and sulfonic group (1350 cm⁻¹).
- LC-MS: Peak RT (14.4 min) for Allura Red AC (MW: 495.9987 g/mol), major m/z values of MS: 451.0270 (M–2NaH)+, 225.5135 (M–NaH)+, 334.0623 (loss of ring & sulfonic group).

Analysis of Dye in India Green Powder

Figure 6: H-NMR of Leucomalachite Green and India Green

Figure 7: EDS Analysis of Leucomalachite Green

Table 1: Main Characteristic QTOF MS Peaks of Malachite Green in Leucomalachite Green

Analysis of Unknown Dye in India Green

Figure 8: ES Liquid Chromatogram of Leucomalachite Green

Figure 9: Mass Spectra of Leucomalachite Green (Thin Film) and India Green Peak 1 (1.8 Amu) and Peak 2 (5.1 Amu).

REFERENCES

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RESULTS (Continued)

Key features of Malachite green and Leucomalachite green:

- Raman spectra for India Green, Leucomalachite green (LCM), and Malachite green (MC): phenyl stretch (1610-1650 cm⁻¹) and amine (1225-1250 cm⁻¹).
- QTOF-MS Data for Malachite Green: 330.1848 (M+1), 329.493, and 285.2835 (loss of -CH(CH₃)), and Indian Green (330.2096 g/mol, 329.2116, 259.2144, 258.235 g/mol).
- LC-MS Data for India Green: RT values of Peak 2 (144.0220 mg) and LCM (5.71 min) are close; M/LC (330.0206 g/mol). MS: 372.244 (loss of CH₃ in crystal violet impurity or C₅N₇H₉O₃) and 331.2174 (LCM+1).
- LC-MS Data for India Green: Peak 1 (RT 1.06 min) matches the peak of MC (RT 1.52 min) with MW of 329.2188 g/mol, MS: 315.1681 (MC losing one CH₂), 301.1705 (MC losing two (CH₂) units), and 268.1578 (C₅H₉N₃O or kerite structure shown in Figure 6)

CONCLUSION

The FTIR spectra of CC Blue dye extract exhibited similar spectral features to the standard dye Acid Blue 9 with an index of 99%, along with H-NMR, LC-MS, and Raman analysis confirmation. Potentially hazardous side effects of Acid Blue 9 include extreme eye irritation and hypersensitivity in children.

CC Red

Besides the 99.49% match index of Raman spectra for CC Red and Allura Red AC, the other techniques of H-NMR, LC-MS, and FTIR further confirm the identity of CC Red as Allura Red AC. SEM-EDS analysis of sample also revealed the presence of magnesium, phosphorus, and calcium. Hazardous side effects can be attributed to the degradation of "Allura Red AC into carcinogenic compounds.

India Green

The Raman, LC-MS, and QTOF-MS data of India Green dyes exhibited spectral characteristics similar to those of Leucomalachite Green and Malachite green standards. While Leucomalachite Green is non-toxic, Malachite Green is a known carcinogen and has environmental toxicity effects on aquatic ecosystems.