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

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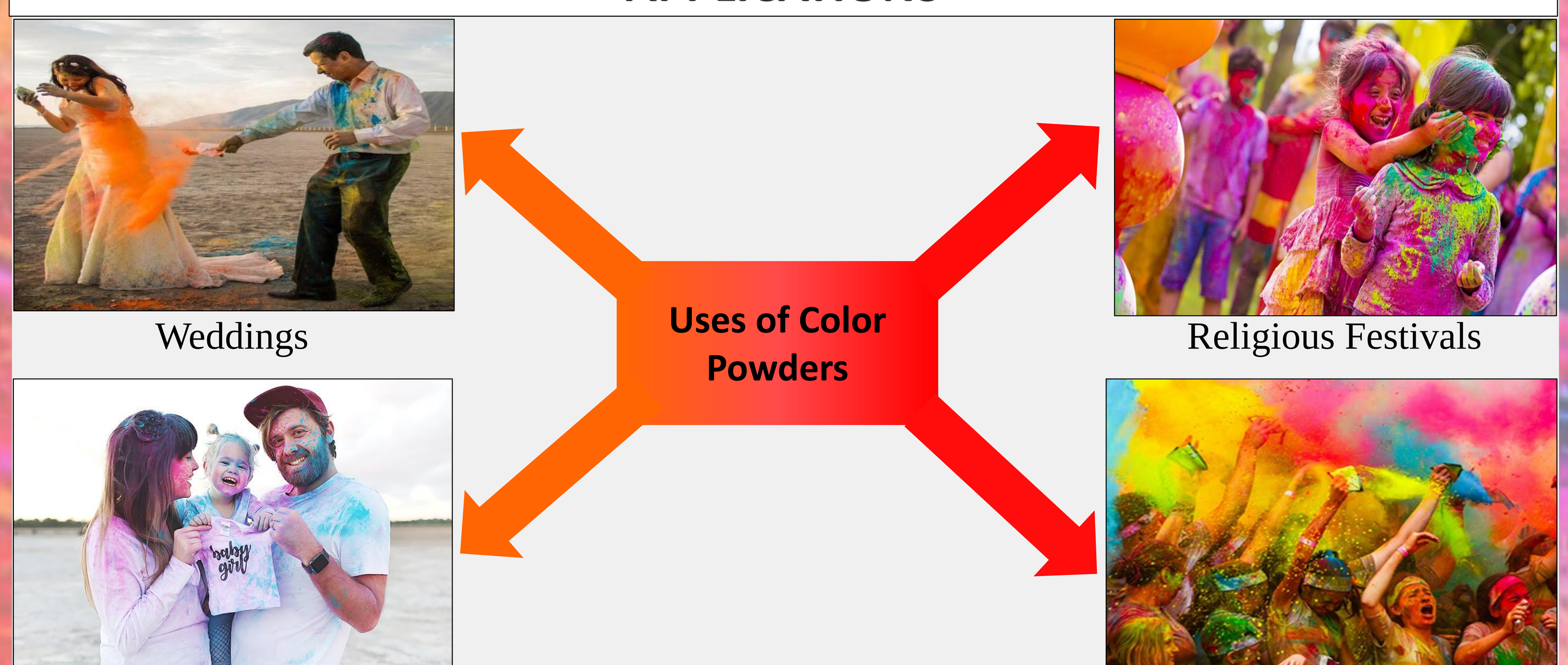
INTRODUCTION

The color dye industry is one of the largest sectors that spans the global market. Synthetic color dyes are multi-faceted 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[1]. 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, specifically 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[2] 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



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 FTWS-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.

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

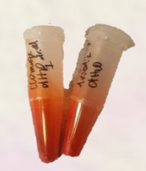
Enwave ProRaman-L spectrometer with 785 nm laser was used to analyze the color extracts.

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

Thermo iS™50 FTIR analysis of CC Red and CC Blue dye extract using an ATR and a DTGS detector.

MATERIALS & METHODS (Continued)

Method (Continued):



Dried color dye standards were also prepped for other instrumentation methods

500 mHz NMR was used to collect H-NMR for CC Red and CC Blue in DMSO-d6.

India Green and Malachite Green were ran on Waters QTOF-MS with Atmospheric Solid Analysis Probe (ASAP).

Thermo HPLC-MS of CC Red, CC Blue, and India Green using a gradient method with a Synergi RP and an Accucore C18 columns.

RESULTS

Analysis of Dye in CC Blue Powder

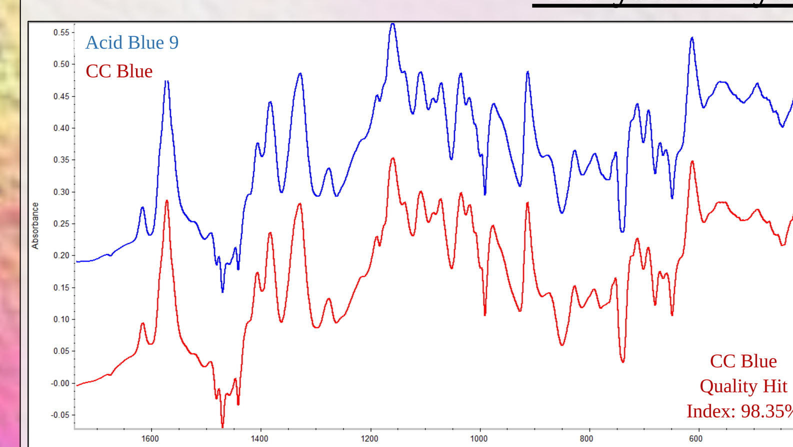


Figure 1: FTIR Spectral Match of Acid Blue 9 and CC Blue.

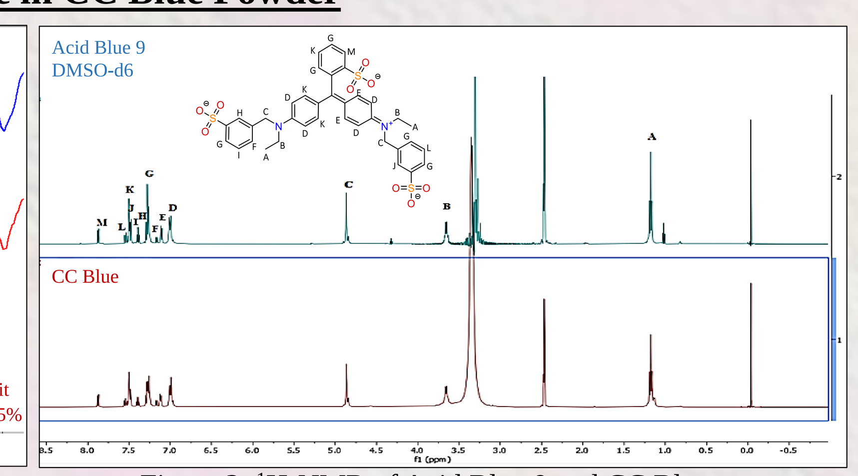


Figure 2: ^{1}H-NMR of Acid Blue 9 and CC Blue.

Key spectral features of Acid Blue 9 that match CC Blue:
- Raman: amine (1620-1627, 1275 cm^{-1}) and sulfonic group (1150-1184 cm^{-1}).
- IR: C=C-H (1630 cm^{-1}), C-N medium stretch (1010 cm^{-1}), and sulfonic group (1300-1350 cm^{-1}).
- ^{1}H –NMR (δ in ppm units): A (C**H**$_3$, 1.17, singlet), B (C**H**$_2$, 3.66, singlet), C (C**H**$_2$, 4.86, singlet), D (Ar**H**, 7.06, doublet), E (Ar**H**, 7.1, doublet), F (C**H**, 7.17, doublet), G (C**H**, 7.28, multiplet), H (C**H**, 7.29, singlet), I (C**H**, 7.37, triplet), J (C**H**, 7.48, singlet), K (Ar**H**, 7.50, multiplet), L (C**H**, 7.55, multiplet), M (C**H**, 7.86, doublet).
- LC-MS: Peak RT (~17.9 mins) for Acid Blue 9 (MW: 792.1222 g/mol); major m/z values of MS: 747.1505 (M-2Na+H), 373.5753 (M-2Na+H)$^{2-}$, 290.0851 (cleavage of single bond of triarylmethane center), and 211.1361 (replacing the loss of SO_3 with H after the single bond cleavage at the triarylmethane center).

Analysis of Dye in CC Red Powder

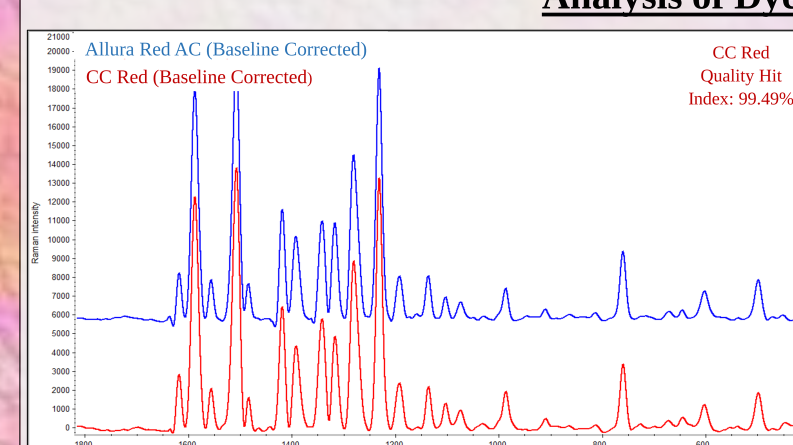
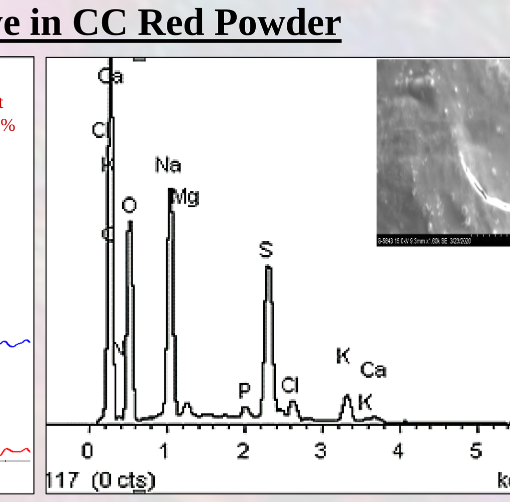


Figure 3: Stacked Raman Spectra of Allura Red AC and CC Red

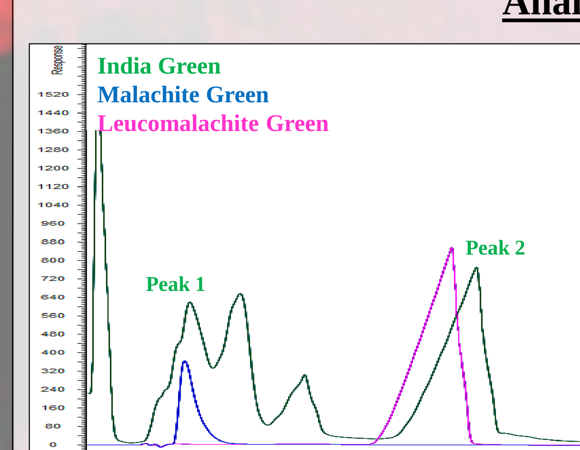


Element	Weight%	Atomic%
C	55.78	66.45
N	0.78	0.79
O	27.33	24.44
Na	7.04	4.38
Mg	0.37	0.22
P	0.37	0.17
S	5.76	2.57
Cl	0.85	0.34
K	1.45	0.53
Ca	0.27	0.10
Totals	100.00	

Figure 4: SEM-EDS Elemental Analysis of CC Red Sample

Key features of Allura Red AC
- Raman: phenyl stretch (1610-1620 cm^{-1}), sulfonic group (1140-1180 cm^{-1}), and aromatic azo peak (1425-1450 cm^{-1}).
- IR: aromatic ring (1650 cm^{-1}), azo bond (1215 cm^{-1}), and sulfonic group (1350 cm^{-1}).
- ^{1}H –NMR (δ in ppm units): A (C**H**$_3$, 2.46, singlet), B (O-C**H**$_3$, 2.55, singlet), C (Ar**H**, 6.77, doublet), D (Ar**H**, 7.52, singlet), E (Ar**H**, 7.8, singlet), F (Ar**H**, 7.94, singlet), G (Ar**H**, 7.95, singlet), H (Ar**H**, 7.75, doublet), I (Ar**H**, 8.45, doublet).
- LC- MS: Peak RT (~14.2 mins) for Allura Red AC (MW: 495.9987 g/mol); major m/z values of MS: 451.0270 (M-2Na+H), 225.5135 (M-2Na+H)$^{2-}$ 334.0623 (loss of ring & sulfonic group).

Analysis of Dye in India Green Powder



Accucore C18 Ramp HPLC-MS Method			
Retention (min)	Flow (mL/min)	%A Aceto-nitrile	%B H_2O with 1% Formic Acid
0.00	0.30	95.0	5.0
25.0	0.30	65.0	35.0

Figure 5: Overlaid Chromatograms of Leucomalachite Green (5.73 min), Malachite Green (1.52 min), and India Green Peak 1 (1.60 min) and Peak 2 (6.12 min).

QTOF-MS of Malachite Green and India Green under APCI Positive Ion Mode using ASAP			
Compound	M+1 Peak (amu)	Mass Peak (amu)	Fragment Peak (loss of $–N(CH_3)_2$ (amu)
Malachite Green	330.1848	329.1943	285.2835
India Green	330.2116	329.2114	285.2835

Table 1: Main Characteristic QTOF-MS Peaks of Malachite and India Green in Atmospheric Pressure Chemical Ionization Positive Mode

RESULTS (Continued)

Unknown Dye in India Green (Continued)

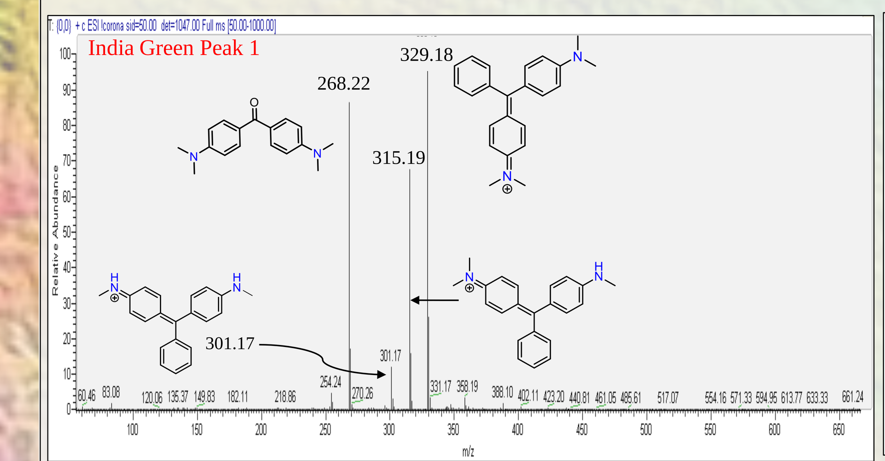


Figure 6: Electrospray Ionization MS of India Green Peak 1 in Positive Ion Mode

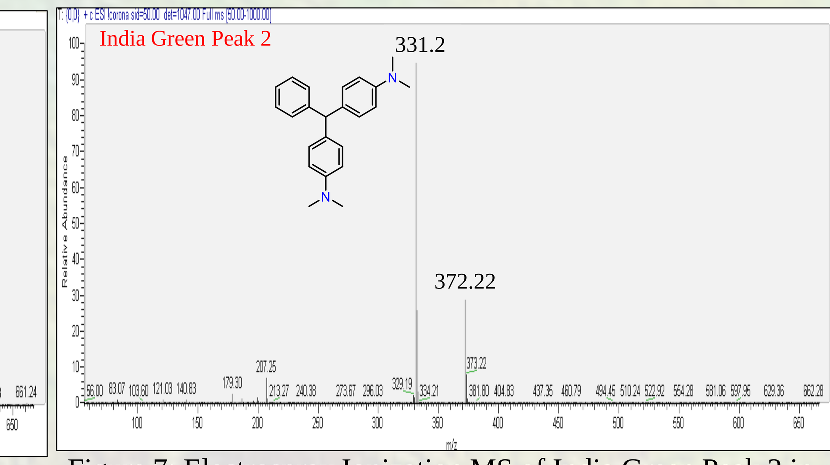


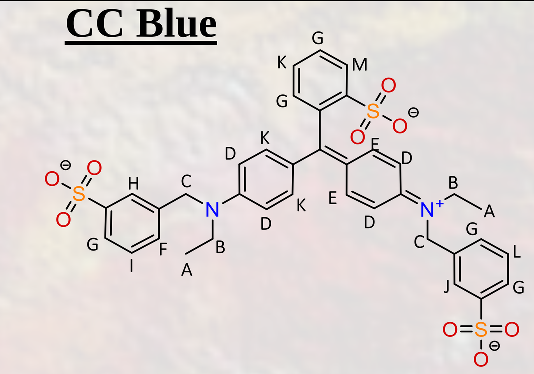
Figure 7: Electrospray Ionization MS of India Green Peak 2 in Positive Ion Mode

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), 329.1943, and 285.2835 (loss of $–N(CH_3)_2$) and India Green (330.2116, 329.2144, and 285.2835 amu).
- LC-MS Data for India Green: RT values of Peak 2 (6.12 min) and LCM (5.73 min) are close; MW [LCM]: 330.2096 g/mol; MS: 372.244 (loss of CH_2 in crystal violet impurity or $C_{25}N_3H_{30}^{+}$) and 331.2174 (LCM+1)
- LC-MS Data for India Green: Peak 1 (RT: 1.60 min) matches the peak of MC (RT: 1.52 min) with MW of 329.2018 g/mol; MS: 315.1861 (MC losing one CH_2), 301.1705 (MC losing two (CH_2) units), and 268.1576 ($C_{17}H_{20}N_2O$ or ketone structure shown in Figure 6)

CONCLUSION

CC Blue

CC Blue Sample

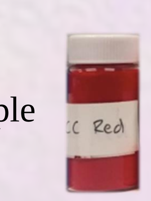


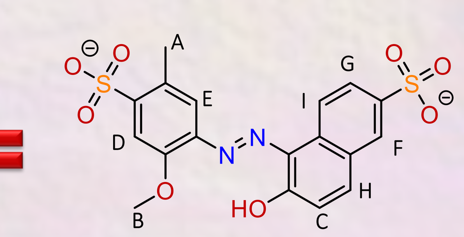
Structure 1: Acid Blue 9

The FTIR spectra of CC Blue dye extract exhibited similar spectral features to the standard dye Acid Blue 9 with hit index of 98%, 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[3].

CC Red

CC Red Sample



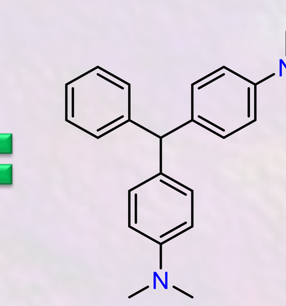


Structure 2: Allura Red AC

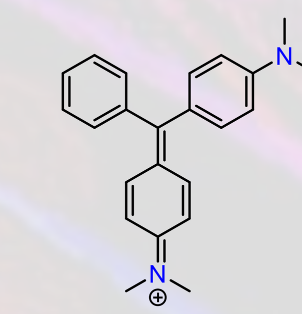
Besides the 99.49% match index of Raman spectra for CC Red and Allura Red AC, the other techniques of H-NMR, LC-MS, and FTIR further confirm the identity of CC Red as Allura Red AC. SEM-EDS analysis of sample also revealed the presence of magnesium, phosphorus, and calcium. Hazardous side effects can be attributed to the degradation of Allura Red AC into carcinogenic compounds[4].

India Green

India Green Sample



Structure 3: Leucomalachite Green



Structure 4: Malachite 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 nontoxic, Malachite Green is a known carcinogen and has environmental toxicity effects on aquatic ecosystems[5].

REFERENCES

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