B47 Differentiation of Henna-Based Hair Dyes Using Attenuated Total Reflectance/ Fourier Transform Infrared (ATR/FTIR) Spectroscopy

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After attending this presentation, attendees will better understand how ATR/FTIR spectroscopy and a valid statistical classification method can be used to analyze spectra of henna-based hair powders of varying colors and from several manufacturers.

This presentation will impact the forensic science community by describing a new application of ATR/FTIR, a rapid, non-destructive spectroscopic technique, to discriminate henna-based hair dye powders in the analysis of forensic evidence.

Hair is frequently encountered at crime scenes, especially those in which a struggle has taken place between the victim and the perpetrator, but also from daily hair shedding. Microscopy is used to evaluate hair evidence. Henna (*Lawsonia inermis* L.) is a plant that has been used for centuries for hair dyeing and applying body decoration, such as body painting and palm coloring, primarily in Asia/the Middle East. Henna is increasing in popularity in the United States because of the desire of people to use a more natural method for cosmetics. Henna-based hair dyes are available in a variety of colors that are different due to the varying combinations of plant powders that include henna, cassia, and indigo. Henna is pulverized into a powder for purchase, which is then made into a paste for application. To make the paste, the powder is mixed with water. When the henna dye is applied, the henna only coats the outside of the strand instead of permeating it, making this a safe and natural alternative to synthetic dyes. As the interaction of the molecules on the diamond surface are probed in ATR/FTIR spectroscopy, the chemical features of the henna and other dyes in henna-dyed hair are recorded when it is applied to the hair. The data results were gathered from the spectra and Principle Component Analysis (PCA) was performed to create classification models, which statistically classified the powders into distinct groups based on color. ATR/FTIR analysis is a powerful tool for trace examiners because it is a quick and non-destructive technique and requires a minimal amount of sample with little-to-no sample preparation. Although the trace analyst could strip the hair of the chemical, the henna can serve as an additional tool to differentiate hair evidence.

This study evaluated the ATR/FTIR absorbance spectra for 49 different henna-based hair dye powders from 4,000-500cm^{-1} with a spectral resolution of 1.929cm^{-1} using a Thermo Fisher Scientific Nicolet™ iS™10 spectrometer running the Omnic™ software and equipped with the Smart iTR™ ATR attachment. Background spectra of air were recorded at ambient temperature. The samples were purchased from eleven different suppliers. Thirteen colors were chosen due to their availability from the individual suppliers. Thirty-two scans were recorded for each replicate; each henna sample was recorded multiple times and was used to build an FTIR henna spectral library. The average ATR/FTIR absorbance was averaged over all scans by saving the data as .CSV text files that were imported into Microsoft® Excel® for spectral analysis. Predominantly variations were observed in the fingerprint region. Absorbance ratios were analyzed at selected frequencies to produce a table of ATR/FTIR results that emphasized the variations and could be clustered using PCA statistical analysis. Statistics were used to differentiate the henna samples from one another. The Unscrambler Multivariate Analysis Software PCA will be employed for statistical analysis. ATR/FTIR spectroscopy combined with chemometrics increases the selectivity of the method.

To compare to the ATR/FTIR results, the powders and pastes were also analyzed using a smartphone application called ColorAssist. ColorAssist uses the iPhone® camera with or without the flash to capture RGB values of colored materials. The dry powders and wet paste could not be differentiated by color or manufacturer using an Excel® scatter plot of RGB data results. This research supports the use of ATR/FTIR spectroscopy to differentiate henna-containing evidence. The ATR/FTIR database will provide another tool to help solve forensics cases and can be applied in most laboratories as they are widely equipped with this instrument.

Henna, ATR/FTIR Spectroscopy, Hair

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