A102 Rapid IR and Gel Electrophoresis "Fingerprinting" Methods for Characterizing Body Samples Including Blood, Saliva, and Hair Evidence

Kelly M. Elkins, PhD, Metropolitan State College of Denver, Chemistry Department, PO Box 17362, CB 52, Denver, CO 80217*

After attending this presentation, attendees will gain an appreciation for methods that differentiate between body samples that may contain blood, saliva, and hair using attenuated total reflectance infrared spectroscopy and agarose gel electrophoresis. In particular, each one of these three evidence samples and their components exhibit different absorption frequencies and intensities and electrophoretic mobility. Attendees will also learn how the use of appropriate reference standards can assist in spectral and gel interpretation, especially in the case of complex mixtures.

This presentation will impact the forensic science community by establishing the scientific basis for direct spectroscopic and electrophoretic methods of qualitatively identifying evidence at a crime scene. Both methods allow an investigator to determine the presence of biological samples at a crime scene in an expeditious manner using non- destructive techniques. The determination of having saliva or blood or both present may help the investigator to decide how to collect and package the sample for further DNA processing. Rapid tools for differentiating body samples and non-biological samples such as paint might also help investigators determine the relevance of an item of trace evidence. Electrophoresis has found utility in species determination.

Methods to replace the currently used presumptive test reagents for blood (luminol, phenolphthalein, and leucomalachite green) and saliva (Phadebas tablets) are continuously being pursued. These tests often render a sample unusable for genetic testing. FT-IR has been employed to examine the hemoglobin component of blood (Dong and Caughey, 1994, Gregoriou et al., 1995) and ATR FT-IR was recently employed to analyze hair morphology (Lyman and Schofield, 2008). Although FT-IR spectroscopy has not been used directly to analyze saliva or amylase, it has been used in characterizing amylase activity in digesting starch, amylose and amylopectin by analyzing the degradation of the original substrate molecule (Marcazzan et al., 1999). IR has also been used to measure the adsorption of salivary proteins onto soft denture lining materials (Imai and Tamaki, 1999). Protein electrophoresis is used to evaluate, diagnose, and monitor a variety of diseases and conditions. It is used clinically to separate and evaluate the albumin, α1, α2, β, and γ proteins and to evaluate blood disease by differentiating between the different types of hemoglobin in the blood including A, F, S, C, E, D. H. Kelly et al. (1991) used gel electrophoresis to perform studies of human apolipoprotein components of blood from whole blood and blood stains. Gel electrophoresis is a simple, rapid, reliable, and economical technique that allows for the simultaneous analysis ("fingerprinting") of a variety of polymorphic enzymes and proteins and allows for the estimation of nano- and picogram quantities of enzymes and proteins. This technique has been used to differentiate blood samples and to attribute them to either brown or black bear species. It can also date the time elapsed since the sample was deposited (Wolfe, 1983). Electrophoresis and isoelectric focusing have been used to separate and to identify proteins from blood (Grunbaum, 1977), hair (Lee et al., 1978; Folin and Cau, 1990; Khawar et al., 1995) and saliva (Tenjo et al., 1993; Yasuda et al., 1996). SDS- PAGE and immunoblotting was used to detect ABH blood group antigens in saliva and their stability according to storage of saliva samples (Kim, 2003). Human hair keratin was analyzed by electrophoresis to obtain specific-species patterns (Folin and Contiero, 1996) and the human hair proteome was recently analyzed by LC-MS and LC-MS/MS (Lee et al., 2006). Tie et al. (1993) used capillary electrophoresis to characterize human seminal plasma.

The research described in this presentation utilizes the entire IR spectrum including the amide banding regions and the fingerprint region of the spectrum. Although the FT-IR spectra of blood dried at three temperatures (21ºC, 40ºC, 120ºC) to simulate conditions of arson or fire do not differ in the frequencies of the exhibited peaks, these spectra can reveal a complex mixture relative to a hemoglobin standard. All spectra obtained were of solid samples which demonstrate a major advantage to the use of ATR FT-IR spectroscopy: it is fast, reliable, and requires little or no sample preparation. FT-IR absorbance spectra (32 scans, 4000 to 400 cm^{-1} spectral range, 1.929 cm^{-1} spectral resolution) using the ATR diamond crystal in ambient temperature as a background, IR spectral data sets were recorded for simulated forensic samples (dried blood at varying temperatures, dried saliva, and shed hair) and comparison reference samples of known components (hemoglobin, albumin, amylase, free amino acids). Finally, the spectral regions most relevant to the differentiation of the biological samples were identified. Predictive accuracy appears to be independent of temperature. In the electrophoresis experiments, the components of the complex mixtures of blood and saliva separated and provide an informative fingerprint for these substances as the components vary in size and charge and migrate at different rates. Agarose gel electrophoresis was used because it is non-toxic and sets up and runs more quickly than polyacrylamide gels. Both gels can be commercially purchased pre-made. 0.8% gels were employed to reduce the cost of the analysis. Coomassie blue stain was used to visualize the bands. The use of higher percentage gels reduces discriminating power in the short time frame (30 minutes

 ** Presenting Author*

maximum) used. Electrophoresis is also non-destructive, requires only nanogram or even pictogram quantities of sample, and allows the sample to be recovered from the gel material. Hemoglobin, albumin, amylase reference standards were used to verify the protein composition of the samples. This research could be extended to include qualitative examinations of urine, feces and semen using these methods.

ATR FT-IR, Agarose Gel Electrophoresis, Biological Evidence

 ** Presenting Author*