The Investigation into Spectral Differences in Aged Semen Stains to Determine a Biological Time Clock

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Introduction

- Bodily fluids are one of the more common types of evidence found at crime scenes, and they contain valuable DNA information[1].
- Many techniques used on bodily fluids can be destructive or require a great amount in order for detection[1].
- Attenuated total reflectance fourier-transform infrared spectroscopy (ATR FTIR) is one of the many few instruments that is non-destructive and requires miniscule amounts for analysis[2].
- It has been previously proven that ATR FTIR can in fact be used in the identification of bodily fluids, including semen[2].
- As semen ages, proteins degrade over time[1].
- The degradation of proteins in human semen could be used to establish a biological clock to determine the age since deposition of a semen stain.
- The effect of the environment on both the positive identification of semen using ATR FTIR and the biological clock of protein degradation was investigated.

Overview:
The protein degradation overtime in aged semen stains was investigated to establish a biological clock that could be used for age determination.

- Various semen stains were left to age up to 30 days.
- The ratios of chemical shifts of common proteins was graphed against the age of the sample to determine a relationship. There was a decrease in some ratios after 1 day.
- Week old stains were left to age in sunlight and darkness to determine if the environment affects the identification and/or biological clock of the stain. Sunlight accelerated the degradation.

Methods

Aged Study:

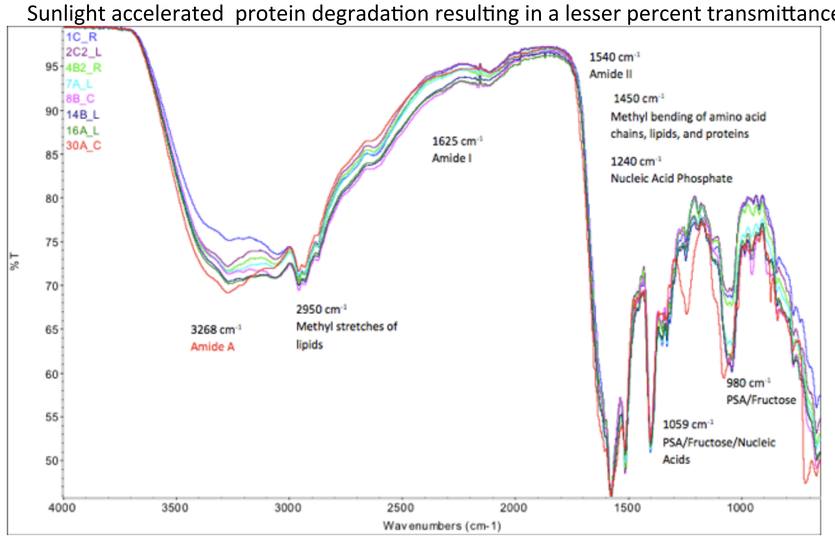
- Three 10 µL samples of semen were placed on the three plastic surfaces for each age.
- Samples were left to age in the lab between 1 to 30 days. Once the samples aged, the stains were analyzed utilizing ATR FTIR. Each sample was ran three times.
- Utilizing the human semen signature, the spectra were observed for the percent transmittance values for the referenced chemical shifts.
- Ratios of each chemical shift were observed in comparison to the age of the sample.

Environmental Study:

- Six 10 µL samples of semen were placed on the six plastic surfaces. Three stains were left to dry on a window sill and three were left to dry in a closed file cabinet draw for 1 week.
- Each sample was ran three times on the ATR FTIR.
- Utilizing the human semen signature, the spectra were observed for the percent transmittance values for the referenced chemical shifts.
- The relationship between the average percent transmittance for each chemical shift and the environments in which the stain aged in were shown graphically.

Results

- When determining if protein degradation attributes to spectral differences, the shifts at 3268 and 2950 cm^{-1} demonstrated the greatest change over time.
- In all ratio relationships there is a decrease after day 1 and slight changes after 2 days .
- Sunlight accelerated protein degradation resulting in a lesser percent transmittance.



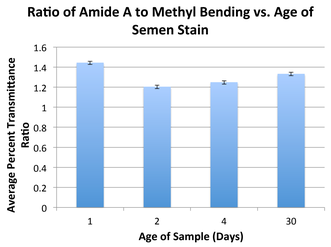


Figure 2. Comparison of ratio of amide A to methyl bending of amino acids, lipids and proteins of the aged samples.

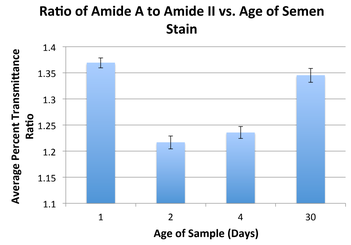


Figure 3. Comparison of ratio of amide A to amide II of aged samples.

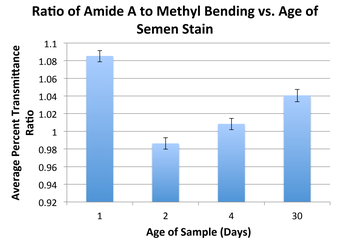


Figure 4. Comparison of ratio of amide A to methyl bending of amino acids, lipids and proteins of the aged samples.

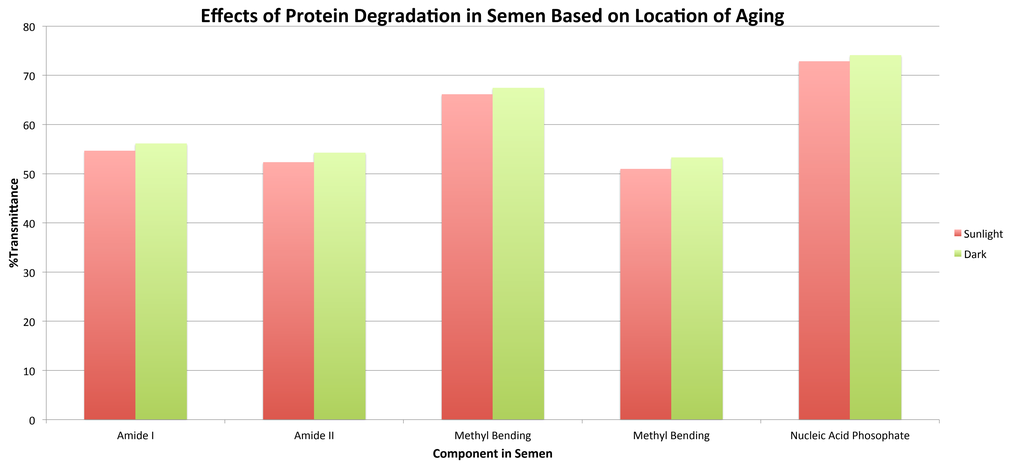


Figure 5. The average percent transmittance of each chemical shift in 7 day old semen stains aged in sunlight and darkness.

Conclusions

- When comparing the ratios of each chemical shift, Amide A ratios showed more evidence of protein degradation.
- Protein degradation was evident from days 1 to 2.
- Sunlight accelerated the protein degradation in the stains resulting in a lesser average percent transmittance.
- Principle Component Analysis (PCA) was done identifying that the Amide A shift contained two components. The first component was present in days 1-16 while the second was present in the 30 day samples.
- The second component observed could have potentially been a suppressed signal or a newly formed signal.
- The difference in these components could attribute the no change in the ratios between days 1 and 30.

Future Directions

- Investigate the differences seen after 2 days.
- Continue this method for greater aged samples.
- Determine if various substrates can effect the identification and the biological time clock.
- Apply this method to Raman spectroscopy for a comparison.

References

1. Virkler, Kelly; Lednev, Igor K. *Analysis of Body Fluids for Forensic Purposes: From Laboratory Testing to Non-destructive Rapid Confirmatory Identification at a Crime Scene*. Forensic Science International 188 (2009) 1-17.
2. Elkins, Kelly M. *Rapid Presumptive "Fingerprinting" of Body Fluids and Materials by ATR FT-IR Spectroscopy*. Journal of Forensic Sciences (2011) 1580-87.

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