Infrared Insights: Using FTIR Spectroscopy to Predict DNA Viability in Burnt Bone Samples

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INTRODUCTION

The identification of fragmentary burnt human remains in forensic investigations is primarily accomplished through DNA analysis. While this method is effective, it is time-consuming, costly, and particularly challenging when dealing with burned remains. In cases involving charred or calcined bone, there may be little to no recoverable DNA, but extraction and analysis procedures are often still performed before this determination is made. Developing a reliable pre-screening method using spectroscopic techniques could significantly streamline this process. An early assessment of organic content could help save time and resources while ensuring efforts focus on samples more likely to yield viable DNA profiles, enhancing overall efficiency of forensic identification.

This study therefore evaluates the feasibility of using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy to assess the organic content in burnt bone samples and predict the success of DNA extraction.

MATERIALS & METHODS

Sample Preparation: 33 bone samples were collected from the experimental burning of complete human cadavers[1]. 9 exhibited thermal alteration (**Figure 1A**), 10 exhibited carbonization (**Figure 1B**), 10 exhibited calcination (**Figure 1C**), and 4 reference samples exhibited minimal thermal alteration (**Figure 1D**). Samples were powdered using a freezer mill for one minute with no subsequent sieving performed.

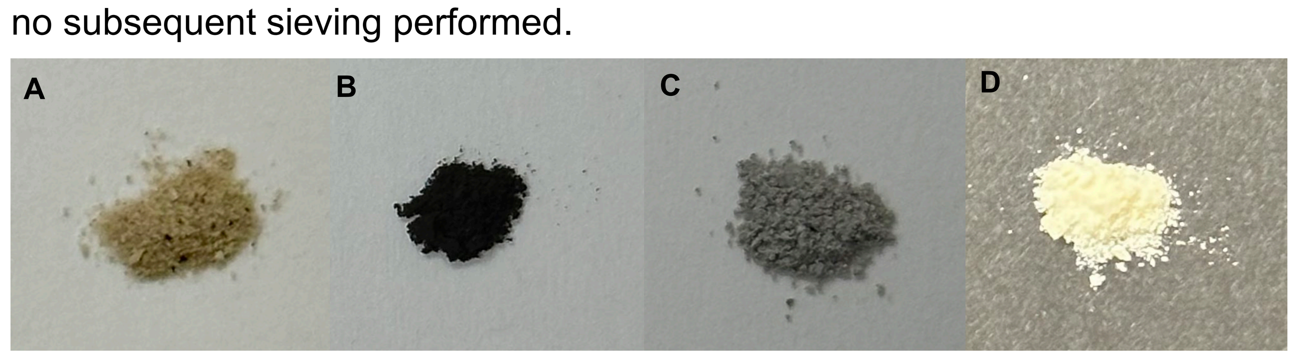

Figure 1: Color variation between samples exhibiting thermal alteration (A: sample 11), carbonization (B: sample 20), calcination (C: sample 29), and minimal thermal alteration (D: sample 1).

ATR-FTIR: All spectra were collected using a Thermo Scientific Nicolet 6700 FTIR with Smart iTR ATR Sampling Accessory. Five replicates for each sample were collected on a zinc selenide crystal using 64 scans, a spectral range of 650-4000 cm^{-1}, and a resolution of 4 cm^{-1}. Spectra were baseline corrected, smoothed, and normalized to the PO_4 band.

Amide I/PO_4 and Carbonate/PO_4 ratios: The Amide I/PO_4 ratio was calculated using the area of the amide I band between 1710 cm^{-1} and 1590 cm^{-1} using a horizontal baseline and the PO_4 band area between 1110 cm^{-1} and 940 cm^{-1} after a baseline correction between 1160 cm^{-1} and 890 cm^{-1} [2]. The carbonate/PO_4 ratio was calculated using the peak heights of PO_4 at 1035 cm^{-1} and CO_3 at 1415 cm^{-1} [3].

DNA Analysis: DNA extracts from all samples were quantified using the Quantifiler Trio DNA Quantification Kit (ThermoFisher Scientific), and autosomal STR profiles were generated using the Globalfiler PCR Amplification Kit (ThermoFisher Scientific) on Applied Biosystems 3500 Genetic Analyzer.

Data Processing: ATR-FTIR data was analyzed using Python version 3.12, and STR profile interpretation was performed with GeneMapper ID-X Software v1.6.

RESULTS & DISCUSSION

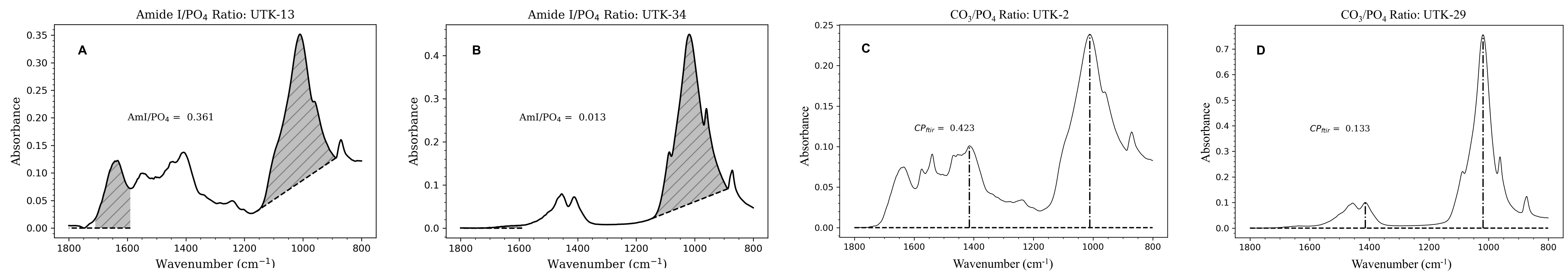

Figure 2: ATR-FTIR spectra displaying the difference between the Amide I/PO_4 ratio and the CO_3/PO_4 ratio for samples with successful allele recovery (**A and C**) compared to unsuccessful allele recovery (**B and D**).

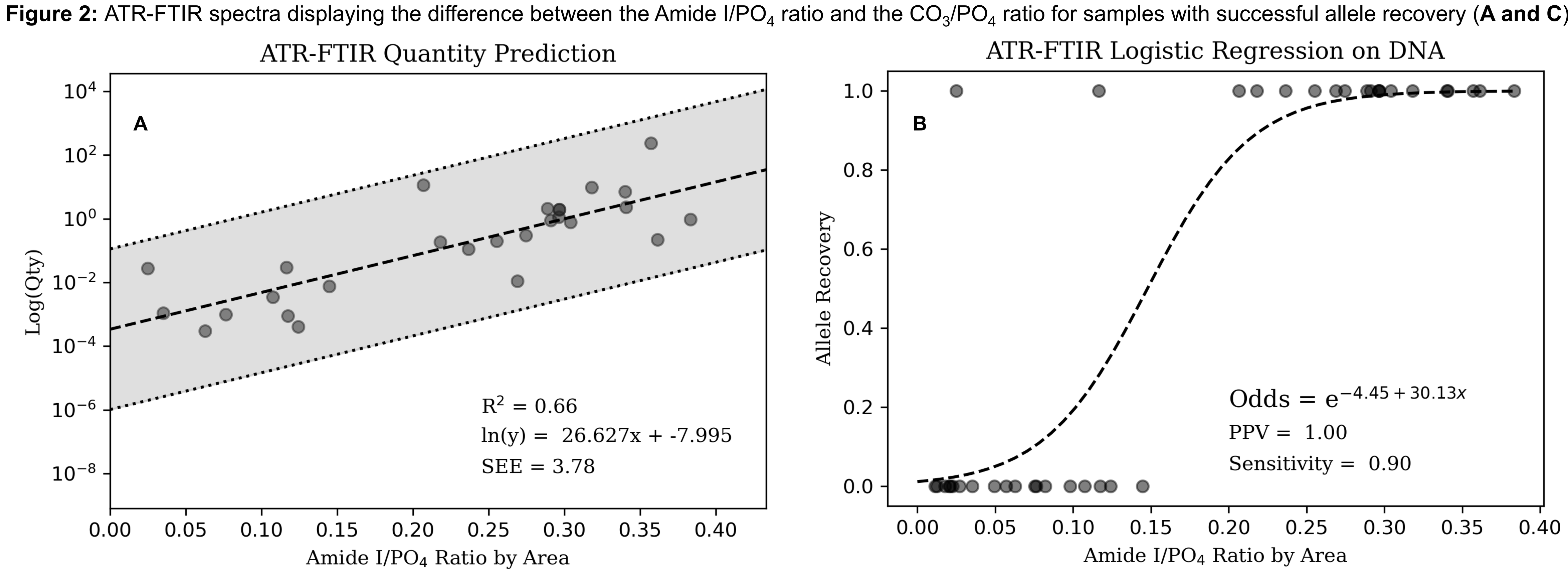

Figure 3: Linear regression correlating the Amide I/PO_4 ratio to the log of the DNA quantification data **(A)**, and a logistic regression on the correlation of the Amide I/PO_4 ratio and percent STR allele recovery **(B)**.

- The ATR-FTIR spectra obtained for all 33 samples were consistent and reproducible, with no spectral features indicative of the presence of contaminants.
- Clearly resolved spectra exhibiting characteristic absorption bands corresponding to the phosphate functional group were consistently produced **(Figure 2)**.
- However, prominent absorption bands associated with the amide I and carbonate functional groups were not produced with the same level of consistency and showed variability in peak intensity and resolution **(Figure 2 B and D)**.
- The CO_3/PO_4 ratios **(Figure 2 C and D)** did not provide reliable estimates of the quantity of DNA across the sample set.

Sample Type	Average Amide I/PO_4 Ratio	% STR Allele Recovery
4 References	0.165 – 0.361	60 – 100%
9 Thermally Altered		
1 Carbonized		
8 Carbonized	0 – 0.164	0 – 59%
9 Calcined		
1 Carbonized	0.124	95.45%
1 Calcined	0.029	82.22%

Table 1: Correlation of the average Amide I/PO_4 ratio to the percent (%) allele recovery for each sample. Green highlight indicates greater than 60% allele recovery as predicted by an Amide I/PO_4 ratio greater than 0.165. Red indicates no or minimal allele recovery, less than 60%, with an Amide I/PO_4 ratio less than 0.165. Blue indicates greater than 60% allele recovery despite an Amide I/PO_4 ratio less than 0.165.

- The Amide I/PO_4 index exhibited a logarithmic correlation with the DNA quantity present in a sample **(Figure 3)**.
- A linear regression on the log of the DNA quantification data achieved an R^2 of 0.66 and a Standard Error of the Estimate of 3.46 **(Figure 3A)**.
- Fitting a logistic regression on the allele recovery percentage yielded a Positive Predictive Value of 1.00 and a sensitivity of 0.90 based on the Amide I/PO_4 ratio **(Figure 3B)**.
- As shown in **Table 1**, 60% allele recovery was observed with a 90% likelihood above a 0.165 Amide I/PO_4 ratio threshold.

CONCLUSIONS

- ATR-FTIR spectroscopy produced well-defined spectra consistently exhibiting characteristic absorption bands corresponding to the amide I and phosphate functional groups.
- The Amide I/PO_4 ratio exhibited a strong association with the organic content of bones.
- Thus, the Amide I/PO_4 index can serve as a predictive indicator of DNA yield and STR typing success.
- ATR-FTIR can serve as a minimally destructive method for estimating the potential success of genetic profiling from bone material.
- Such predictive capability can meaningfully inform decisions regarding sample prioritization for DNA extraction and contribute to more effective identification efforts in forensic casework involving compromised skeletal remains.
- As all 33 powdered samples used in this study were unsieved, future research using samples sieved to 45 – 75 microns will evaluate the effect of bone powder particle size on the correlation between the FTIR data and DNA yield.

REFERENCES

[1] G. Vidol & J. Devlin. (2019). U.S. Dept. of Justice, National Institute of Justice "Identification of Blunt Force Traumatic Fractures in Burned Bone" 2019-75-CX-0019.

[2] Lebon, M., Reiche, I., Gallet, X., Bellot-Gurlet, L., & Zazzo, A. (2016). Rapid Quantification of Bone Collagen Content by ATR-FTIR Spectroscopy. *Radiocarbon, 58*(1), 131-45. doi.org/10.1017/RDC.2015.11.

[3] Wright, L. E., & Schwarcz, H. P. (1996). Infrared and isotopic evidence for diagenesis of bone apatite at Dos Pilas Guatemala: palaeodietary implications. *Journal of Archaeological Science, 23*(6), 933-944. doi.org/10.1006/jasc.1996.0087.

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