Monitoring the solid-state UV-VIS profiles of bloodstains

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1. Introduction

- Determining **time since deposition (TSD)** of **bloodstains** is important because it can provide timing and context to a criminal investigation[1,2]
- **Oxidative changes** to the **hemoglobin (Hb)** structure and conformation are used for some TSD estimation methods, and rates are influenced by **temperature** [1-5].

Hb → OxyHb
Central Fe^{2+} ion in Hb is saturated with oxygen, forming oxyHb.

OxyHb → metHb
Central Fe^{2+} ion is oxidised to Fe^{3+}, and the oxygen ligand is reduced to H_2O.

metHb → HC
Central ion consists of both Fe^{2+} & Fe^{3+}, and cell degradation results in a substitution from H_2O to histidine.

- TSD has been probed using **UV-VIS spectroscopy** by resuspending desiccated bloodstains prior to analysis. [1,2]
- We ask, can solid-state UV vis (non-destructive) be used to probe additional information for bloodstain TSD?

2. Methods

- 8 biological replicates of 45.0 µL of **bovine blood** with **acid citrate dextrose anticoagulant solution (ACD-A**) in a 12.5 % v/v ratio.
- **Droplet setup (per replicate)**
  - 45.0 µL stains made on glass slides, and placed in **four temperature conditions**: -20°C, 4°C, 22°C, 45°C
- **UV-VIS spectral acquisition (in solid-state)**
  - Spectra acquired using a Perkin Elmer UV-VIS spectrometer, measuring absorbance from a **wavelength range of 250.00-800.00 nm** at the following timepoints: 3hrs, 4hrs, 24hrs, 48hrs, 96hrs.
- **Data analysis**
  - Graphical plots made using Origin Lab software, and **statistical analyses** (Principal Component Analysis [PCA] and linear mixed-effect modelling) using R Studio software.

3. Results

a. There are observable changes with time and temperature

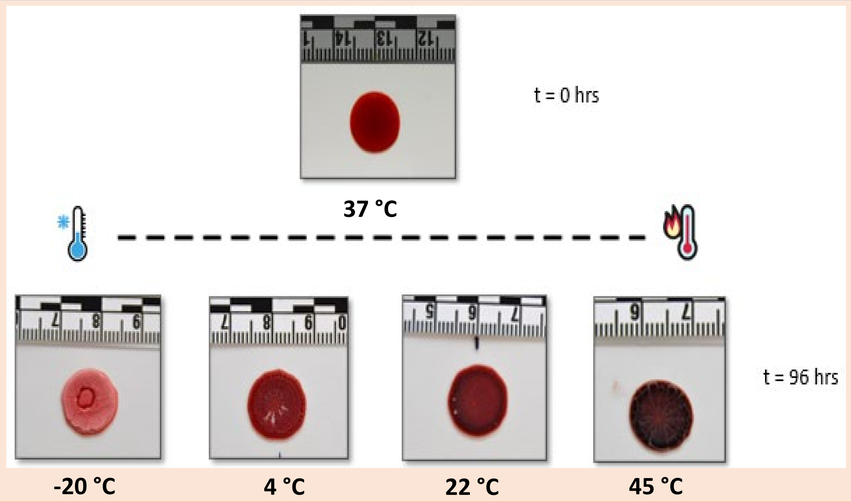

Figure 1: Color changes observed as bloodstains of 45.0 µL dried over time (t = 0 - 96 hrs) in four different temperature conditions (-20°C, 4°C, 22°C, 45°C).

b. There are spectral changes with time and temperature

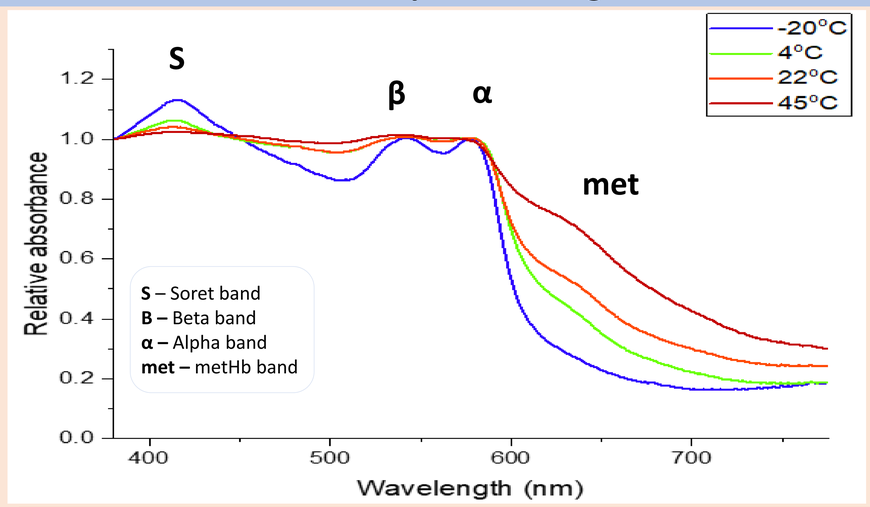

Figure 2: Average spectra (n = 4 per temperature replicate) of bloodstains of 45.0 µL in different temperature conditions (-20°C, 4°C, 22°C, 45°C) at 96 hrs.

- Peak identification is seen at low temperatures (-20 °C), especially in the Soret band region.
- Interestingly, we also see differences in the metHb bands, where relative absorbance is higher with increased temperature.

c. The spectral changes have statistical differences with time and temperature

- **PC1** had the **highest separation of temperature over time**, and was **mostly** contributed by wavelengths 601- 690 nm, corresponding to **metHb signatures**. [1]

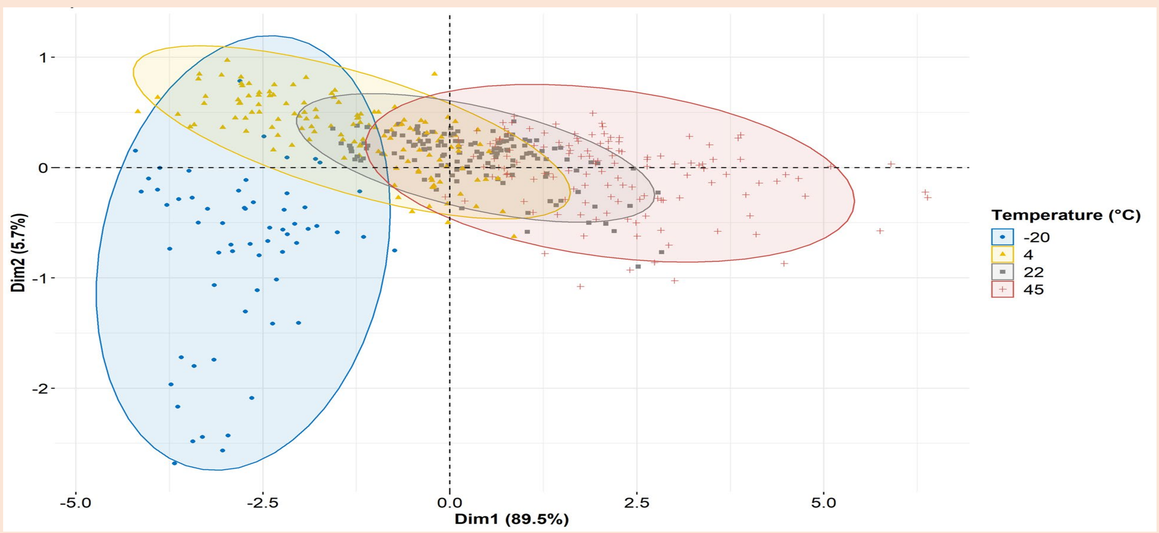

Figure 3: Principal component plot of Dimension 1 showing groupings related to temperature-dependent changes at all time-points during experimentation.

- **Inter-donor variability** present but **not significant** in predicting TSD signatures: marginal $R^2 = 0.78$, conditional $R^2 = 0.82$.

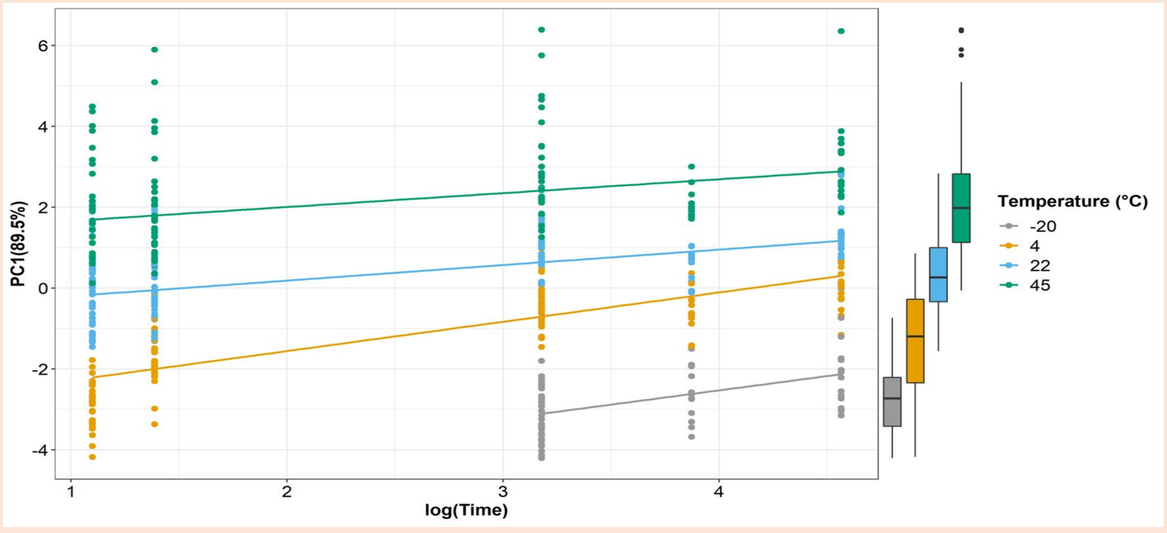

Figure 4: Combination plots showing linear mixed-models relating principal component 1 (PC1) to log(time) and temperature conditions.

4. Discussion & Future Works

- **Color change** of bloodstains in solid – state supports the **oxidative changes occurring in Hb** as bloodstains degrade.
- **Highest data variation** contributed by **metHb signatures in contrast to the Soret band**; when **temperature is known, metHb signatures can be used to predict TSD** of a bloodstain.
- **Inter-donor variability** was **not significant**, but is **still important** when considering TSD estimates.
- **Future works**: (i) Long-term studies using solid-state UV-VIS spectroscopy. (ii) Using temperature ranges closer to yearly averages for better TSD estimates

5. References

[1] G. Zadora, A. Menżyk, In the pursuit of the holy grail of forensic science – Spectroscopic studies on the estimation of time since deposition of bloodstains, TrAC Trends Anal. Chem. 105 (2018) 137–165. **[2]** E.K. Hanson, J. Ballantyne, A Blue Spectral Shift of the Hemoglobin Soret Band Correlates with the Age (Time Since Deposition) of Dried Bloodstains, PLoS One. **[3]** R.H. Bremmer, G. Edelman, T.D. Vegter, T. Bijvoets, M.C.G. Aalders, Remote Spectroscopic Identification of Bloodstains*, J. Forensic Sci. 56 (2011) 1471–1475. **[4]** R.H. Bremmer, D.M. de Bruin, M. de Joode, W.J. Buma, T.G. van Leeuwen, M.C.G. Aalders, Biphasic Oxidation of Oxy-Hemoglobin in Bloodstains, PLoS One. 6 (2011) e21845. **[5]** H. Sun, Y. Dong, P. Zhang, Y. Meng, W. Wen, N. Li, Z. Guo, Accurate Age Estimation of Bloodstains Based on Visible Reflectance Spectroscopy and Chemometrics Methods, IEEE Photonics J. 9 (2017).