The Detection of Animal Blood Using Green Synthesized Surface Enhanced Raman Spectroscopy (SERS)

Geraldine Monjardez[1], PhD*, Erin Bruner[2], MSc, Rajesh Balaraman[3], PhD, and Patrick Buzzini[1], PhD.

[1] *Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340*

[2] *Department of Public Safety, Houston Crime Lab, Houston, TX 77065*

[3] *Vironova, Boston, MA 02114*

MEMBER THE TEXAS STATE UNIVERSITY SYSTEM

INTRODUCTION

While DNA profiling is currently considered as the gold standard to identify individuals in a forensic case, identifying the type of body fluid recovered from crime scenes before it can be used for DNA typing may be critical to help qualifying its connection with the case.

Surface-enhanced Raman spectroscopy (SERS) has been used to detect trace level of chemical and biological analytes using their characteristic vibrational signatures as it enables the enhancement of analyte signals by several orders of magnitude compared to Raman spectroscopy.

The purpose of the following research is to develop a method of collection and identification for non-human blood using green synthesized SERS swabs (Figure 1).

Following a general movement in the natural sciences to move toward more sustainable practices, silver nanoparticles (NPs) were synthesized directly on nylon evidence swabs using a biosynthesis reaction and curcumin as the reducing agent to form the nanoparticles.

Three studies were conducted with animal whole blood: (1) Investigated the limit of detection of bovine blood, (2) Investigated the ability to collect and detect dried bovine blood with the SERS swabs and (3) Involved the collection and analysis of whole blood from three animal species (bovine, horse, and sheep) using the SERS swabs.

MATERIALS AND METHODS

Silver Nanoparticles Synthesis Procedure

- Silver nanoparticles were attached to nylon Copan 4N6FLOQswabs™ Crime Scene (Figure 1) using a protocol modified from Alsammarraie et al[1], based on the reduction of silver nitrate by curcumin to form the nanoparticles.

Figure 1. Evidence swab after synthesis reaction.

Characterization of silver nanoparticles on evidence swabs by SEM-EDS

- A Hitachi SU3500 Scanning Electron Microscope coupled with Bruker Quantax XFlash ® 6 energy dispersive spectrometer was used to capture images to confirm the presence of nanoparticles on the fibers of the swabs.

Study 1: Analysis of fresh bovine blood

- Dry swab was dipped into blood.
- Decreasing volumes of blood (50 µL, 30 µL, 20 µL, and 10 µL) were pipetted onto microscope slides and swabbed with dry SERS swabs.

Study 2: Analysis of dried bloodstains

- 200 µL bovine blood was pipetted onto two substrates, 100% cotton fabric pieces and glass microscope slides.
- Substrates were placed in petri dishes and blood was left to dry overnight.
- Bloodstains were swabbed with SERS swabs wetted with ultrapure H_2O.

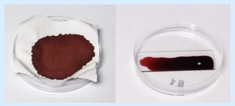

Figure 2. Dried bloodstains on 100% cotton fabric and glass microscope slide.

Study 3: Analysis of fresh bovine, horse, and sheep blood

- 50 µL of bovine, horse, and sheep blood was pipetted onto microscope slides and swabbed with dry SERS swabs.

Spectral data collection and analysis

- SERS data were measured on a Renishaw inVia™ InSpect confocal Raman microscope (20x magnification) using a 532 nm laser excitation.
- Each swab was collected with an acquisition time of 10 seconds and 10 accumulation (study 2) and 15 accumulation (studies 1 and 3).
- Spectra were processed by baseline subtraction and polynomial smoothing using WiRe 5.4 software (smooth window of 9 and a polynomial order of 3).

RESULTS AND DISCUSSION

- The SEM images (Figure 3 a) confirmed the presence of the NPs adhering to the fibers of the swabs and their rough surface texture. The NPs were observed to be dispersed on the fibers, which led to the creation of random hot spots.
- The effect of light on the synthesis of NPs was observed (Figure 3 b), with the silver aggregating in clumps instead of forming NPs alongside the fibers of the swabs when the synthesis was conducted without constant light.

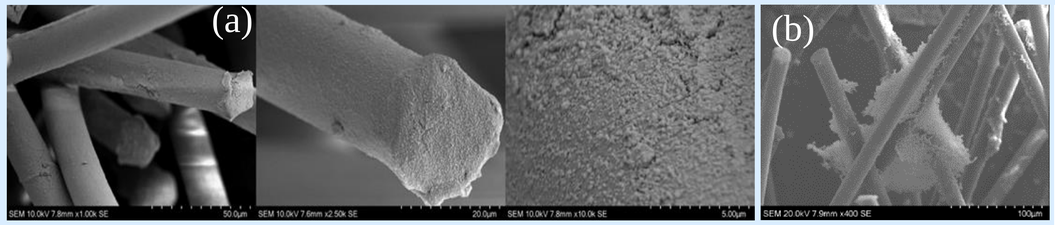

Figure 3. **(a)** SEM images obtained after 24 hr. synthesis under constant light and **(b)** without constant light.

- While swabbing 30 µL and 20 µL of bovine blood resulted in the most resolved spectra, swabbing 10 µL of blood still produced identifiable peaks, with a much lower intensity in the 1100-1500 cm^{-1} range (Figure 4).
- The following peaks characteristic of blood were identified on the spectra: 1580 and 1632 cm^{-1} (heme group), 1299, 1368, 1393 and 1559 cm^{-1} (hemoglobin) and 995 cm^{-1} (phenylalanine).
- Due to the large contribution of hemoglobin, which makes up about 95% of blood, many of the peaks seen in the spectra can be attributed to vibrational modes of hemoglobin.
- Dominant bands in some spectra also displayed a shift in dimensions of both left/right. This could be due to several factors, including the complexity and heterogeneity of blood, which could be further increased by the swabs, and the degree of dryness of the sample.

Figure 4. SERS spectra for decreasing volumes of bovine whole blood swabbed with SERS swab.

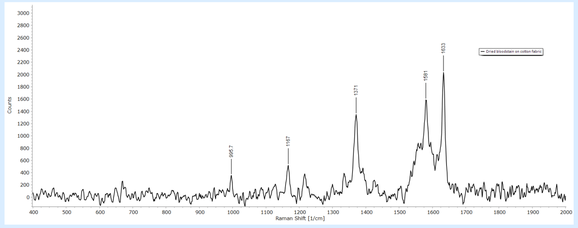

Figure 5. SERS spectrum dried bloodstain on fabric swabbed with wet SERS swab.

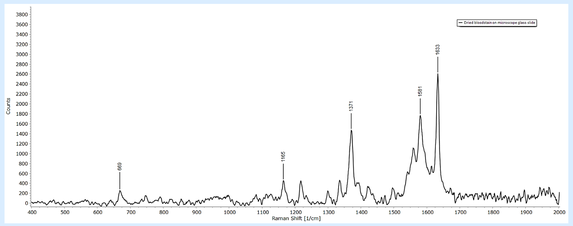

Figure 6. SERS spectrum for dried bloodstain on microscope slide swabbed with wet SERS swab.

- When comparing the spectra, it was observed that swabbing the dried bloodstain on the fabric (Figure 5) resulted in spectra with a lower signal-to-noise ratio (SNR) than when swabbing the microscope slides (Figure 6).
- This difference in spectral quality between substrates was also highlighted in a study conducted by Burleson[2] using semen, which showed that dry swabs produced high quality spectra for the glass substrate, but no SERS signal for the fabric substrate.
- The intensity of the peaks differed between the three species, but the peak positions remained consistent (Figure 7).
- The differences in intensity could be due to the complexity and heterogeneity of blood, which is further increased by the swabs.
- No peaks in any of the individual spectra could be attributed to a specific species as they were present in all spectra.
- It is hypothesized that the large contribution of hemoglobin could mask the potential differences between species.

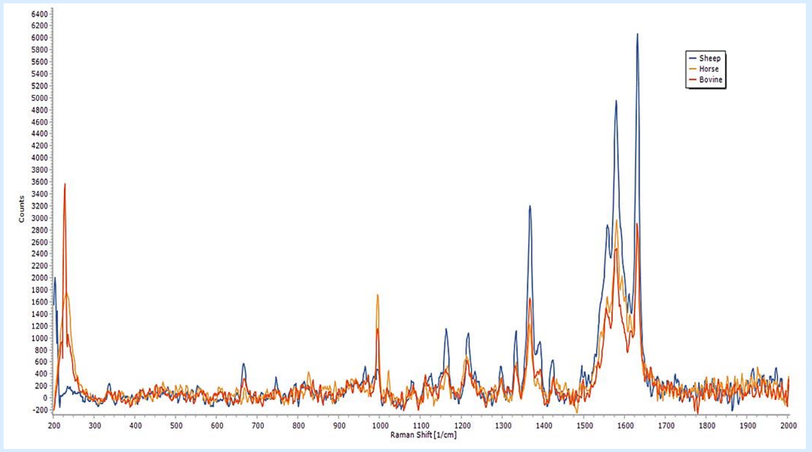

Figure 7. Overlaid SERS spectra of the three blood species; bovine (dark orange), horse (blue) and sheep (light orange).

CONCLUSIONS

- Raman bands characteristic of blood were identifiable for each specie, showing that the SERS swabs allowed the successful detection of the three animal blood species.
- This study demonstrates the efficiency of the green synthesis to grow the nanoparticles on the swabs and shows their viability to be used as SERS substrates to collect and detect animal blood using Raman spectroscopy.
- Future work will be performed to further develop the biosynthesis procedure and optimize the reproducibility of the swabs, such as varying the concentration of the silver nitrate solution and the synthesis time to investigate their effects on the nanoparticles' formation on the swabs, as well as the method by which the swabs are dried after being removed from the reaction solution (dried at room temperature vs cured in nitrogen gas at different temperatures and curing times).

REFERENCES

[1] F.K. Alsammarraie, W. Wang, P. Zhou, A. Mustapha, M. Lin, Green synthesis of silver nanoparticles using turmeric extracts and investigation of their antibacterial activities, Colloids and Surfaces B: Biointerfaces 171 (2018) 398-405.

[2] M. Burleson, SERS-Active Nylon Fiber Evidence Swabs for Forensic Applications, Department of Chemistry & Physics, Western Carolina University, (2016).

ACKNOWLEDGEMENTS

SEM images were acquired with the help of the SHSU imaging facility and Dr. Rajesh Balaraman.