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THE IDENTIFICATION OF THE MIXTURE OF SEMEN WITH BLOOD SAMPLES BY RAMAN SPECTROSCOPY FOR FORENSIC PURPOSES

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Abstract

Background & Objectives: Raman spectroscopy was shown to be a panacea for identifying body fluids, marking a watershed event in the field of serology. Raman spectra of certain fluids can be used for more than just identification; they can also be analyzed using chemometrics to learn things like the fluid's origin, the donor's race and gender, the donor's age, and the amount of time since depositing. This study aimed to determine the ability of the Raman Spectroscopy to identify the semen-blood mixture. **Subjects & Methods:** The present work is using Raman Spectroscopy to identify semen-blood mixture samples. Fifteen body fluids samples were obtained, and mixed together. All of these samples were examined using a 3D confocal Raman imaging microscope with a 50x long-range objective (numerical aperture = 0.75) and a WiTec alpha300 R confocal Raman spectrometer. **Results**: We found that the average Raman spectra of dry traces of semen-blood mixture constitutes mainly of hemoglobin, choline, and spermine phosphate hexahydrate. This proof of concept approach shows the potential for Raman spectroscopy to identify semen mixtures with blood during forensic analysis. **Conclusion:** Different mixtures of semen with blood can be distinguished from each other using Raman spectroscopy couple with chemometrics.

Keywords: Raman spectroscopy; Semen; Body fluid mixtures; chemometrics; Principal component analysis

1. Introduction

Blood is the most researched biological evidence among all body fluids. Due to its uniqueness despite its high complexity and prevalence at crime scenes, a thorough examination of this object is crucial to the investigator's work.**(Sharma & Kumar, 2018)**.

Locating blood stains on white or other light surfaces could be simple. Finding an old bloodstain on dark clothing can be incredibly challenging because of the low contrast between the stain and the fabric. Moreover, recognizing the stains can be difficult because other chemicals may have a similar appearance, or the support has been washed to remove the stain. **(Indalecio-Céspedes et al., 2021).** Human or animal blood must be identified first before forensic DNA profiling may begin. **(Fujihara et al., 2017).**

In most cases, the examination of biological evidence starts with a preliminary screening to determine whether or not body fluids are present. Enzymatic and immunological assays are typically used in the traditional procedures for identifying body fluids; however, some of these tests have low level of specificity. In the past ten years, several new techniques for determining the identity of body fluids have been developed. These techniques include tissue-specific DNA methylation, microbial forensics, proteomics, and RNA profiling. **(Dørum et al., 2018).**

A few tests may be applied appropriately at the crime scene to detect or confirm the presence of blood traces. Visual inspections with the use of microscopy, chemical inspections such as the well-known Luminol or KastleMeyer tests, Takayama or Teichmann microcrystal inspections, spectroscopic inspections such as alternating light sources or UV–vis absorption, and immunological inspections such as the antibodies test kits RSIDTM or ABA Card®Hematrace® are some examples of these types of tests. **(Pereira et al., 2017).** Luminol is frequently used in identifying blood stains due to the emission of light as a result of an oxidation of luminol enhanced by iron in hemoglobin and its derivatives in blood, trace amounts of blood can be detected by chemiluminescence of a blue-green color. **(An et al., 2012)**

Lednev's 2008 publication showed Raman spectroscopy's potential. After this initial expedition, Raman spectroscopic mapping and statistical analysis examined body fluid signals. Blood was analysed and differentiated between species. Raman spectroscopy distinguished peripheral and menstrual blood. Raman spectra of blood deposited on various substrates were also obtained. **(Khandasammy et al., 2018**).

The aim of this study is to identify the semen-blood mixture samples using Raman spectroscopy.

2. Material and Methods

2.1 Sample Preparation

The samples were collected from different volunteers after obtaining informed consent. Inclusion criteria are different body fluids obtained from donors from different age groups and both sex. Exclusion criteria are any donor with abnormalities in semen analysis or blood diseases. We collected fifteen samples of semen and blood to the study. A semen-and-blood mixture was made by shaking together 150 μL of semen and 5 μL of blood for 20 seconds. The next day, we wiped a 30 μL sample of the solution of a microscope slide and let it dry at room temperature.

2.2 Raman Spectroscopy

A 3D confocal Raman imaging microscope with a 50x long-range objective (numerical apertures = 0.75) and a WiTec alpha 300 R confocal Raman spectrometer we conducted to examine the samples. The spectrometer was calibrated with a reference silicon sample, and the resulting readings had a resolution of 1 cm^{-1}. In semen mixed with blood, the laser power was around 1.5 mW.

2.3 Data Processing and Chemometrics

In order to process the data retrieved from the Raman Spectrometry, we used the OriginLab software**.** The peak analysis of the spectrum was done without any manipulation of the graph. We applied Principal component analysis (PCA) which is a multivariate analysis method that can transform a set of answers or quality qualities into a linear connection of the components that are not connected with one another.

3. Results

The present work is a prospective cross-sectional analytical study in which we use Raman Spectroscopy to identify fifteen semen mixture with blood samples. The following figure **(1)** was detected by the (CCD) camera of the Raman Spectroscopy of the semen-blood mixture before the analysis by the device.

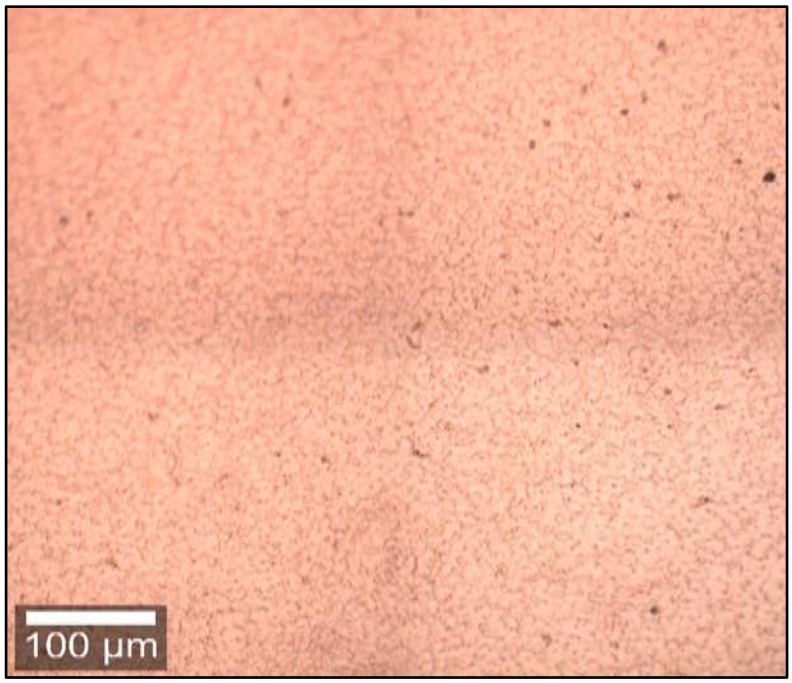

Figure (1): The dried semen stain mixed with blood detected by the charge-coupled device (CCD) camera.

The analysis of the peaks of the dried semen stain mixed with blood are showed in figure **(2)** (716, 754, 930, 959, 1003, 1226, 1268, 1356, 1447, 1619, and 1639 cm^{-1}).

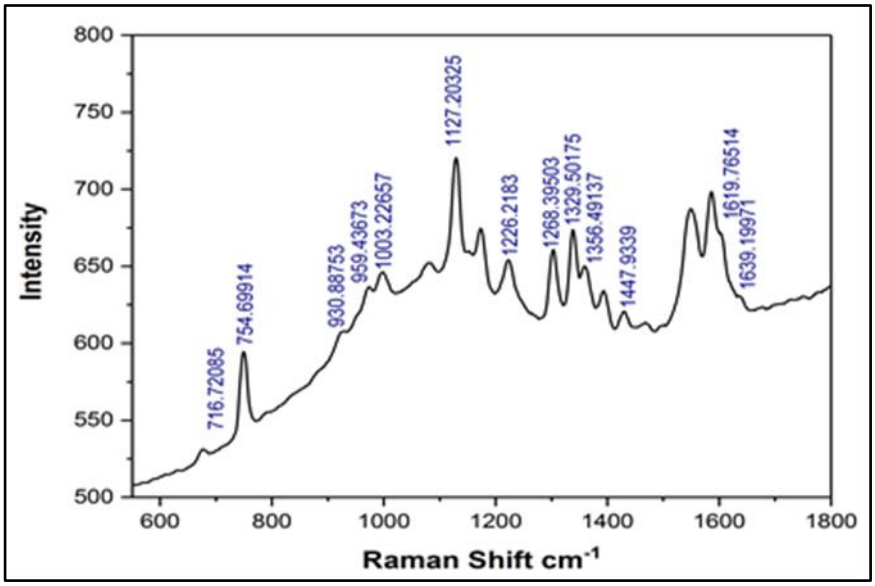

Figure (2): Showing the different peaks detected in the spectrum of the dried semen stain mixed with blood.

The following figures are shown the variations in the intensity of the spectra between the different donors as pure Raman spectra and the spectra after baseline subtraction. Figure **(3)** shows the averaged raw spectra of dry samples of semen mixed with blood obtained from different donors using 532-nm laser light for excitation and laser power of 1.5 mW. We took note of the various intensities that were present in the spectrum.

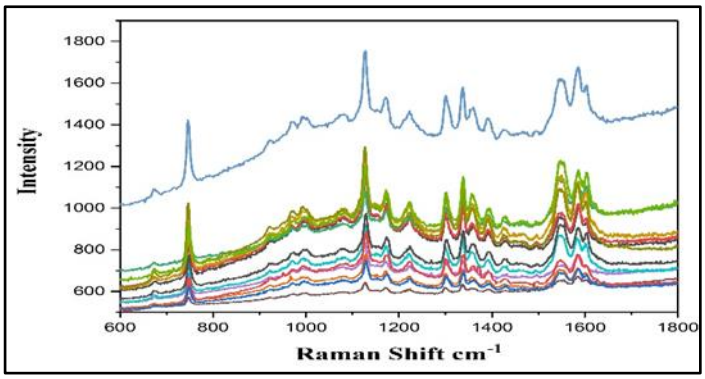

Figure (3): Variation in the raw Raman spectra of dried semen stains including blood from 15 distinct donors.

Figure **(4)** shows the averaged Raman spectra of dry samples of semen mixed with blood obtained from different donors after baseline subtraction.

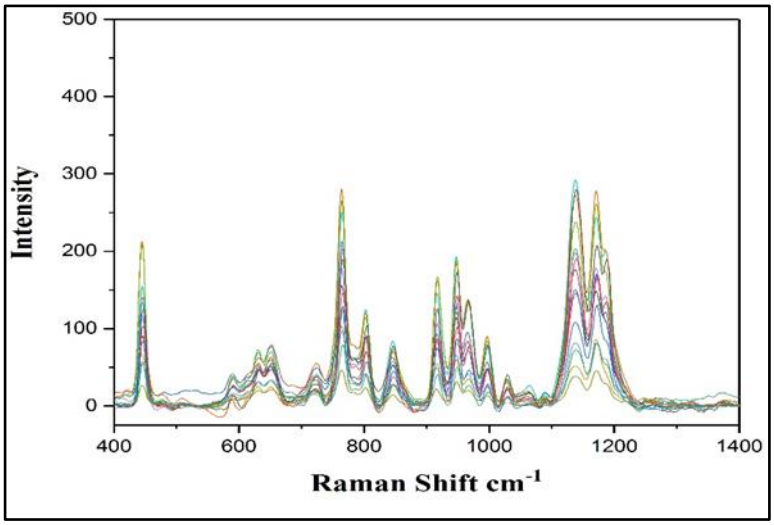

Figure (4): Subtracting the baseline from Raman spectra of dried semen reconstituted with blood.

By using OriginLab software, we did PCA of the Raman spectra of the dried semen-blood stains. We obtained from PCA the correlation matrix between samples, the total variance explained in PCA, the scree plot, the loading plot and the spectral components of PCA.

Table (1): Illustration the total variance explained in the principal component analysis of all 15 relevant semen samples mixed with blood.

	Eigenvalue	Percentage of Variance	Cumulative
1	13.67066	91.14%	91.14%
2	0.51967	3.46%	94.60%
3	0.27036	1.80%	96.40%
4	0.16465	1.10%	97.50%
5	0.1035	0.69%	98.19%
6	0.09534	0.64%	98.83%
7	0.04922	0.33%	99.16%
8	0.03351	0.22%	99.38%
9	0.02187	0.15%	99.53%
10	0.01786	0.12%	99.64%
11	0.01537	0.10%	99.75%
12	0.01199	0.08%	99.83%
13	0.01058	0.07%	99.90%
14	0.00878	0.06%	99.96%
15	0.00664	0.04%	100.00%

4. Discussion

Raman spectroscopy was shown to be a panacea for identifying body fluids, marking a watershed event in the field of serology. Raman spectra of certain fluids can be used for more than just identification; they can also be analyzed using chemometrics to learn things like the fluid's origin, the donor's race and gender, the donor's age, and the amount of time since depositing. **(McLaughlin et al., 2019).**

While there have been numerous advances in techniques for identifying body fluids, there are currently no nondestructive tests that can be done at a crime scene. The most promising nondestructive techniques for verifying the identity of body fluids are fluorescence and Raman spectroscopies. **(Sikirzhytski et al., 2010).**

The examination and prosecution of a case sometimes depend on the ability to identify body fluids in a biological stain recovered from a crime scene. The presence of semen, for instance, might be actual proof in a rape prosecution. **(Ingold et al., 2020).** Some body fluids provide very accurate timelines of occurrences. Semen or vaginal fluid detection, for instance, may point to a sexual encounter or assault that has taken place. Crime scenes typically contain the usual assortment of blood, semen, saliva, vaginal fluid, urine, and sweat. (An et al., 2012).

The present study aimed to determine the ability of the Raman Spectroscopy to identify the semen-blood mixture in fifteen different donors.

In the present study, we analyzed the Raman spectra of the dried semen stain mixed with blood. The Raman bands of the blood (754, 1226, 1356, 1619, and 1639 cm^{-1}). These bands points to hemoglobin which is the main component of blood. The Raman peaks of the semen also found in the spectrum of the semen stain mixed with blood as we found peaks at 716, 959, 1003,1268, 1329, and 1619 cm^{-1}).

The interpretation of our results are in line with the results of ***Atkins et al.,(2017)*** who assigned peaks at 1223,1335 and 1636 cm^{-1} to be corresponded to hemoglobin.

Sikirzhytski et al.,(2012) also interpreted the peaks at 754, 1003, 1226, and 1619 cm^{-1} to be the Raman bands of blood. The combination of semen and blood, with a minor presence of blood, caused the mixture to take on a pinkish color. This occurrence was also noted in a prior study performed by ***(Sikirzhytski et al., 2012).*** Alongside the hemoglobin peaks, significant peaks were identified for choline, and spermine phosphate hexahydrate. The Raman analysis of the pure blood stain did not reveal the presence of those peaks***(Fujihara et al., 2017; Mclaughlin et al., 2013; Muro et al., 2016; Reese et al., 2021; Sikirzhytskaya et al., 2014, 2016).*** In this way, the presence of choline and spermine phosphate hexahydrate makes the spectrum specific to the mixture of semen and blood.

5. Conclusion and Recommendations

In cases of sexual assault, the evidence of semen can be very important and can even make up a large part of the corpus delecti (Latin for "body of the crime") .Therefore, semen must be properly identified. Seminal fluid and sperm are the two main components of semen. Vibrational spectroscopy techniques, such as Raman spectroscopy, have shown great promise in biomedical investigations due to their ability to provide molecularly specific biochemical information without the use of external labels and without damaging the evidence under investigation. Raman spectroscopy and chemometrics can be used to differentiate between different body fluids. This has been done before with great success **.**In order to discriminate and identify semen-blood mixtures, we have evaluated the effectiveness of these techniques **.** Haemoglobin, albumin, spermine phosphate hexahydrate and tyrosine were the main

components of the average Raman spectra of dry traces of semen mixed with blood **.**Principal component analysis of the semen/blood mixture showed that the first spectral component was formed by haemoglobin, albumin and spermine phosphate hexahydrate.

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8. Disclosure of conflict of interest

All authors declared that there is no conflict of interest reported in this paper.

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