International Journal of Innovative Technology and Exploring Engineering (IJITEE)
ISSN: 2278-3075 (Online), Volume-9 Issue-1, November 2019

Estimation of The Age of Bloodstains on Soil Matrices By ATR-FTIR Spectroscopy and Chemometrics

Nik Fakhuruddin Nik Hassan, Durga Devi Sandran, Masitah Mohamad, Yusmazura Zakaria, Noor Zuhartini Md Muslim

Abstract*: Estimating the accurate time of a crime occurred is one of the priceless information in forensics practice and for the investigation purposes. There are profuse of evidence can be found at the crime scene and each of the evidence will give an important information for the investigation purposes. In this study, the Attenuated Total Reflection (ATR)- Fourier Transform Infrared (FTIR) technique combined with advanced chemometrics method was deployed. For the purpose of determining the age of the bloodstain, two storage conditions; indoor and outdoor were set up to simulate real crime scene scenario and bloodstains on soil matrices were exposed and analyzed for selected time intervals for up to 63 days. Six partial least squares regression-discriminant analysis (PLSR-DA) models were constructed-indoor and outdoor models with 1-63 days-exhibited good performance with acceptable values of predictive root mean squared error (7.04-16.0) and r2 values (0.45-0.89), respectively. Using these models, correct classification of the aged bloodstains was calculated up to 70%. In conclusion, the multivariate analysis based on PLS-DA models indicates that ATR-FTIR spectroscopy, coupled with chemometrics provides acceptable discrimination for rapid and non-destructive determination the age of bloodstains on soil matrices in particularly for outdoor and very aged bloodstains.

***Keywords:* ATR-FTIR spectroscopy, Bloodstains, Chemometrics, Forensic science, PLS-DA**

I. INTRODUCTION

Biological evidence is the most frequent sample that found at crime scene especially bloodstains and semen. There are a lot of information can be obtained from the bloodstains itself such as DNA for individualization and to reconstruct the crime scene event. This kind of sample usually can be found in violent crime scene such as murder or sexually abused cases [1, 2]. Understanding the properties of blood is imperative for study of bloodstain evidence and for interpretation [3]. In most situations especially at outdoor violent-related crime scenes, human biological evidence from the suspect or victim may be deposited in the soil [4]. This evidence can be analyzed to link the suspect or victim to the crime scene and to provide information on the temporal aspects of a crime. The accurate estimation time of bloodstain can provide colossal information or clue for the police as well as forensic investigator to determine the exact time of the crime happen and to reconstruct the crime scene [5]. When the exact time of the bloodstain deposition is known, it can link with the other information and information to give additional knowledge and intelligence about the case to the investigation officers [6, 7].

The aim of this study was to determine the feasibility and efficiency of ATR-FTIR spectroscopy and Chemometrics in estimating the ageing of human bloodstains on soil matrices such as peat, coarse and beach soils. The effectiveness of the crime scene process relies on the information obtained from each of the sample that are discovered and recovered from the scene until being analysed at the laboratory [8]. In the case of interest such as murder and assault, knowing the age of bloodstain found at the crime scene can provide a valuable information to the investigators to establish the time of the crime happen and to isolate the other bloodstain that is not related to the case [9].

Usually the amount of the sample found at the crime scene was inadequate, contaminated, degraded and not sufficient for the laboratory purpose [10]. Soil as a transfer evidence can provide an important information towards forensic investigation. In most cases soil will be transferred to the suspect or victim involved in that particular case when contact has occurred [11]. Soil is a complex mixture with a variety of mineralogical, biological, physical and chemical properties. Soil texture depends on the amount of each size of particle in the soil. Soil is composed of particles of different sizes. The texture is determined by the amount of sand, silt and clay particles in the soil, each of which is a different size [12, 13].

Blood can be identified and detected by chemical and physical methods.

Revised Manuscript Received on November 30, 2019.
***** Correspondence Author
Nik Fakhuruddin Nik Hassan*, Forensic Science Programme, School of Health Sciences, Universiti Sains Malaysia, Health Campus, Kelantan, Malaysia.
Durga Devi Sandran, Forensic Science Programme, School of Health Sciences, Universiti Sains Malaysia, Health Campus, Kelantan, Malaysia
Masitah Mohamad, Forensic Science Programme, School of Health Sciences, Universiti Sains Malaysia, Health Campus, Kelantan, Malaysia. Royal Malaysia Police (PDRM)
Yusmazura Zakaria, Biomedical Science Programme, School of Health Sciences, Universiti Sains Malaysia, Health Campus, Kelantan, Malaysia.
Noor Zuhartini Md Muslim, Forensic Science Programme, School of Health Sciences, Universiti Sains Malaysia, Health Campus, Kelantan, Malaysia.

© The Authors. Published by Blue Eyes Intelligence Engineering and Sciences Publication (BEIESP). This is an open access article under the CC-BY-NC-ND license http://creativecommons.org/licenses/by-nc-nd/4.0/

Spectroscopic, microscopy, immunological and chemical are the current technique in identification of biological fluid. Previous studies have demonstrated that vibrational spectroscopy is feasible technique to determine the bloodstain age when combined with Chemometrics method [14, 15].Vibrational spectroscopy techniques are becoming more and more popular in forensic science because of their non-destructive, rapid, quantitative, and confirmatory features [16]. ATR-FTIR showed an ability to determine bloodstain age, especially when combined with chemometric methods [17]. However, most of the studies were conducted using simulated bloodstain samples under ideal laboratory conditions [18]. In real-world case work, varying ambient conditions will affect the process of bloodstain denaturation and aggregation and ultimately contribute to the complexity and difficulty of bloodstain age estimation [19-21]. In our study, an approach combining ATR-FTIR spectroscopy with chemometric methods was established to estimating the age of bloodstains up to 63-days with sample created and stored in indoor and outdoor environments, with the storage conditions mimicked the actual crime scenes. Chemometric methods are efficient to extracting useful information from complex spectral datasets to yield more comprehensive and accurate results.

II. MATERIALS AND METHOD

A. Sample preparation

In this study, human ethical approval was obtained to collect human blood from the The Human Research Ethics Committee (JEPeM) University Sains Malaysia (USM) Kubang Kerian, Kelantan (USM/JEPeM/19030199) to collect the blood samples from the volunteers and the written consent form was obtained from all blood donors. 5 ml of fresh blood were collected from 6 volunteers and kept in a tube with Ethylenediaminetetraacetic acid (EDTA) deposited immediately onto selected soil matrices; beach soil, coarse soil and peat soil for the ageing purposes. For estimation of aged bloodstain studies, 25 µl of blood sample were deposited on each soil surface with total of 12 spots. Twelve-time points were set: 0, 3, 5, 7, 14, 21, 28, 35, 42, 49, 56 and 63 days. Each standardized spot represented the time interval for this study. Six replicates of bloodstain samples were prepared for each time interval, of which three samples were stored in an indoor environment (room temperature with dim sunlight during the day and no light at night) and other three sets in an outdoor environment.

The outdoor samples were exposed to the sunlight, heat, and outdoor humidity but being preserved from raining. 20 µl of fresh blood was deposited directly on the clean ATR-FTIR crystal surface and IR measurement was collected. The spectra provided information to correlate to the peak intensity and position. The IR spectrum of each soil matric was also carried out that acted as negative controls by depositing the soil on ATR-FTIR crystal prior to the IR measurements.

In the analysis of aged blood, samples were analyzed in a dried-state (bloodstain) using ATR-FTIR Spectrophotometer. Specifically, the blood samples were deposited onto the selected soil surfaces which were coarse, beach and peat soils before being exposed to indoor and outdoor environments in order to allow the samples to age according to the specific time intervals as previously stated. Prior to each measurement for each time interval, one spot of bloodstain sample containing 25 µl blood was collected using a tweezer and placed in sterile Eppendorf tube and mixed with 15 µl of normal saline and let to sit for 2 minutes. Then, 1 µl of the extracted sample was deposited on the ATR crystal face using micropipette, and the spectra were recorded. A total of 487 bloodstain samples were ultimately collected, encompassing an indoor training group of 246 samples and an outdoor training group of 241 samples. These two groups were used for chemometric model constructions.

B. Spectral collection and data pre-processing

Spectral procurement was implemented using FTIR Spectrophotometer (Bruker Tensor 27 equipped with an ATR accessory from Germany. This equipment contains approximately 2 mm in diameter a crystal face ZnSe. A chemical "fingerprint" of bloodstain was obtained by FTIR spectroscopy to identify the frequent peaks that can be used to determine the age of the bloodstain. With reference to optimized parameters. All samples were scanned 32 times and averaged within 4000-550 cm^{-1} at 4 cm^{-1} resolutions. The absorbance was used as the measurement of the data obtained. The raw spectral data then imported into Minitab version 17 software (Minitab Incorporated, State College, USA) to perform chemometrics analysis; Partial Least Square Regression (PLSR) and Partial Least Square-Discriminant Analysis (PLS-DA). The spectra data points were truncated in the range of 1800-1000 cm-1 for chemometrics analysis. Data pre-processing technique ie. standardization was carried out on data set to minimize error due to larger variance by subtracting the mean and dividing standard deviation prior to PLSR and PLS-DA. Ten latent variables were selected for the analysis. Prior to the chemometrics analyses, the spectral data were collected using OPUS version 7.5 software package (Bruker Optic GmbH) and recorded in a Microsoft Excel spreadsheet. Root mean square error (RMSE) and regression determination (r^2) as the main parameters of the model's predicted results, were used to evaluate the regression model reliabilities. High values of r^2 and a low value of RMSE demonstrate a well-established PLS regression model.

III. RESULTS AND DISCUSSION

In this age determination of bloodstain study, the examined spectral range of 1800-900 cm^{-1}, also called the "biofingerprint region", provides the most information on the chemical compounds of such biological specimens. The typical IR spectrum of human blood is depicted in Fig. 1. However, it is not practical to estimate the age of a bloodstain with the selection of one or several absorption peaks by visualizing intensity changes because of the overlapping spectral features of bloodstain. Thorough visual examination between spectra indicated that highly varied vibrational bands were at 1650 cm^{-1} (corresponding to the α-helix structures of haemoglobin) and 1533 cm^{-1} (assigning to amide II). Figs. 2 to 4 illustrate the comparison of the average spectra for the indoor and outdoor bloodstains on three types of soil matrices with selected twelve-time intervals.

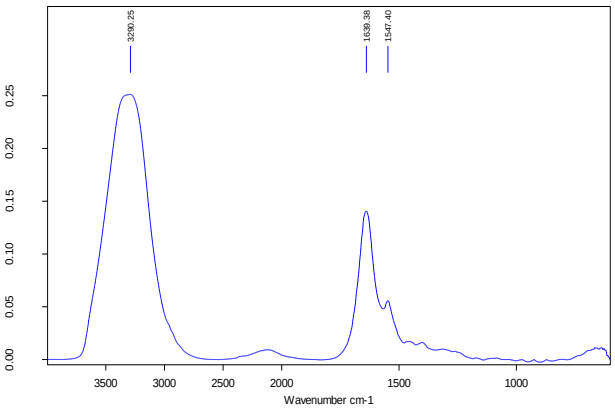

Fig. 1: IR spectrum of human blood

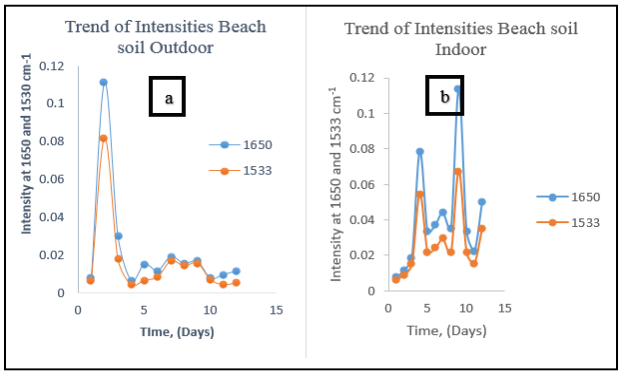

Fig. 2: Intensities trends of the peaks at 1650 and 1533 cm^{-1} of average spectra of bloodstains on beach soil according time interval

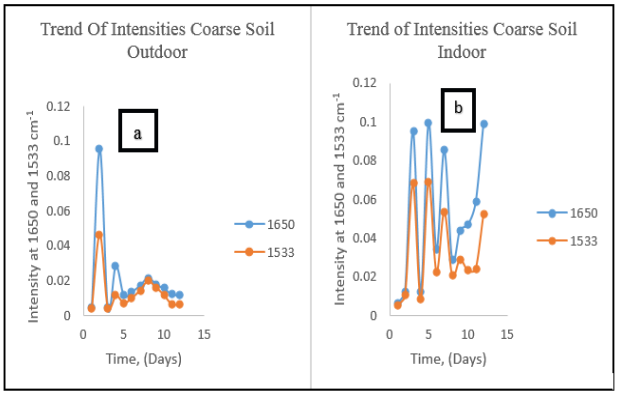

Fig. 3: Intensities trends of the peaks at 1650 and 1533 cm^{-1} of average spectra of bloodstains on coarse soil according time interval

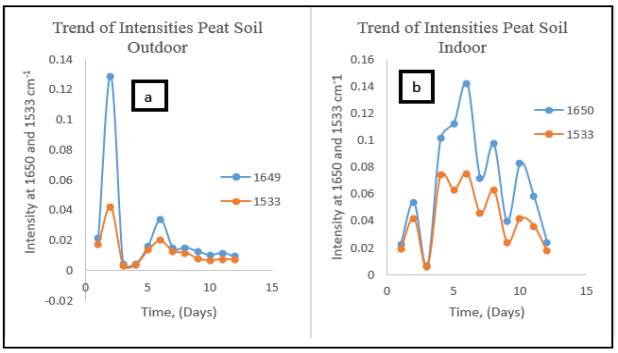

Fig. 4: Intensities trends of the peaks at 1650 and 1533 cm^{-1} of average spectra of bloodstains on peat soil according time interval

In general, the absorbance intensity at 1650 cm^{-1} shows decrease trend at first, reach the minimum values at age = 4 day and increase slowly subsequently. Although, the average intensity for absorbance at 1533 cm^{-1} shows the decreasing trend at age=9 day after deposition. These results proposed that the secondary hemoglobin structure changed persistently as the age increased, and these maybe due to association with kinetic efforts of haemoglobin during the ageing of the bloodstain (Hb- > HbO2- > met Hb-> hemichrome). Auto-oxidation of haemoglobin occur promptly when the fresh blood was exposed to the air in the environment and followed by the aggregation and denaturation as the time progressed. It can be concluded that the process of the degradation of the bloodstain started immediately and can be detected after a few hours and over the longer time period. The age of the bloodstain can also be determined by the intensity of the selected peaks from the infrared spectra that provide information about haemoglobin, which mostly contained in the dried bloodstain. Estimation of aged bloodstains based on infrared spectra was observed to be reliable if samples were exposed in outdoor condition as compared to indoor condition. This is because decreasing trends of intensities in these two peaks were noted in the former and it could be due to slow degradation of bloodstain on soil matrices when exposed in indoor environment, thus, caused the fluctuation (decreasing and increasing) of intensities of the peaks for indoor samples. In addition, other factors that affect degradation such as high temperature that was exposed to samples in outdoor conditions were not imposed to indoor samples.

Similar trends were observed for all bloodstain's regardless types of the soil matrices. Interestingly, the bloodstain can still be detected though after being exposed for 63 days. This is of paramount importance in order to assist the police officers in forensic investigation since aged body fluids if detected, can be further identified their sources/owners by DNA profiling.

In this study, PLS regression analysis was performed with 10 latent variables (LVs), to deliver satisfactory prediction performances and to build models for age estimation of indoor and outdoor bloodstains extracted from three types of soil matrices over the entire age period (1–63 days). Six partial least squares regression-(PLSR) models were constructed—three indoor and three outdoor models. Figs. 5 to 7 and Figs. 8 to 10 illustrate the calibration results of the indoor and outdoor PLSR models. The models for outdoor displayed better performance with predictive root mean squared error (7.04-14.5) and r^2 values (0.54-0.89), respectively as compared to less reliable indoor models with predictive root mean squared error (14.7-16.0) and r^2 values (0.45-0.53). A stable prediction PLSR model is expected to have a high r^2 value and a low RMSE value. In the ideal linear regression, all spectra (symbols) should lie directly on the line of best fit, and the minimal spread should be within the symbols for each age point. However, the results demonstrated that the indoor and outdoor PLSR models in the 1-7 days period were not appropriate for prediction of bloodstain with estimated error of ± 30 days and were almost off the fitting line.

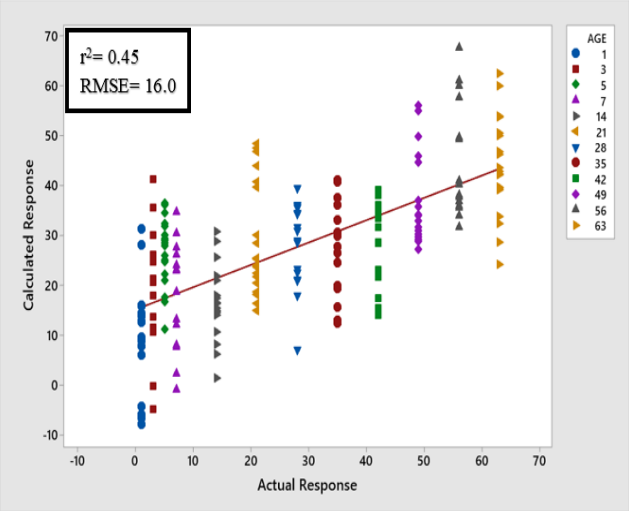

Fig. 5: PLSR plots for indoor bloodstains deposited on beach soil

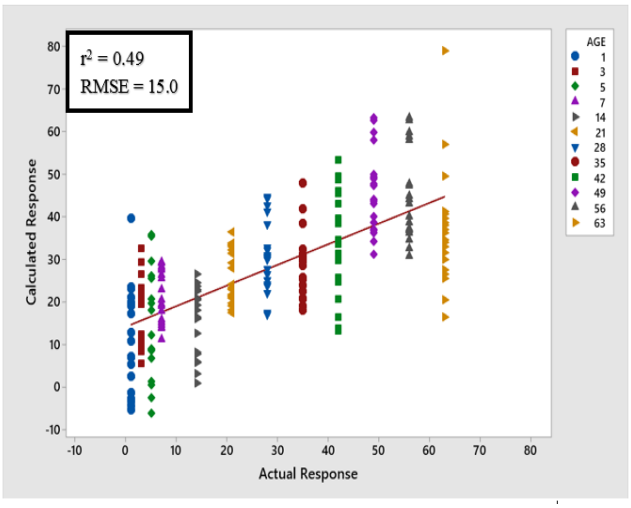

Fig. 61: PLSR plots for indoor bloodstains deposited on coarse soil

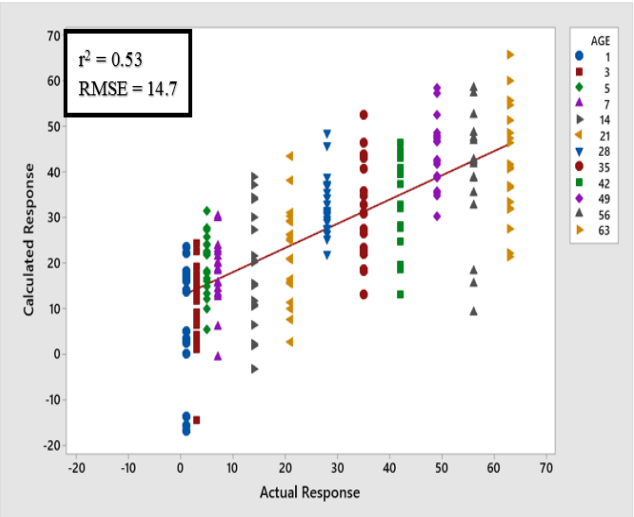

Fig. 7: PLSR plots for indoor bloodstains deposited on peat soil

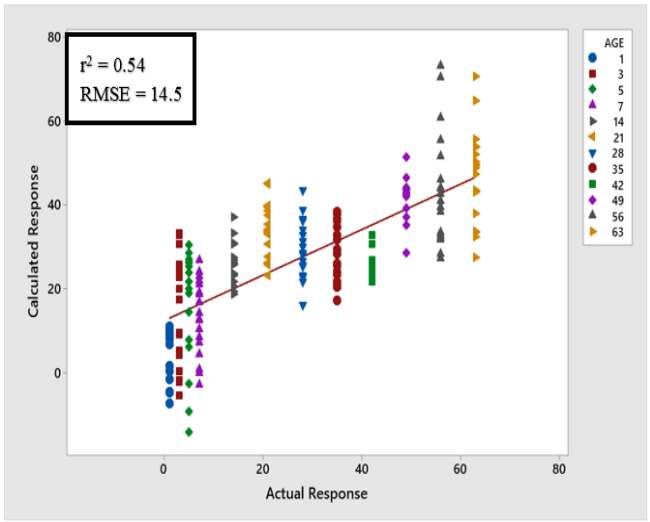

Fig. 8: PLSR plots for outdoor bloodstains deposited on beach soil

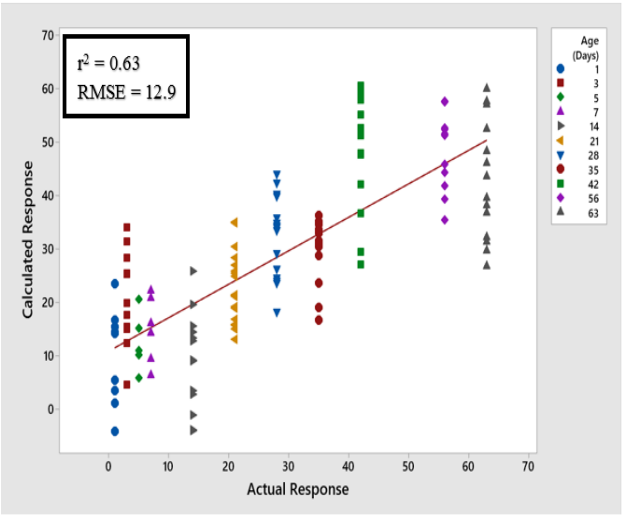

Fig. 9: PLSR plots for outdoor bloodstains deposited on coarse soil

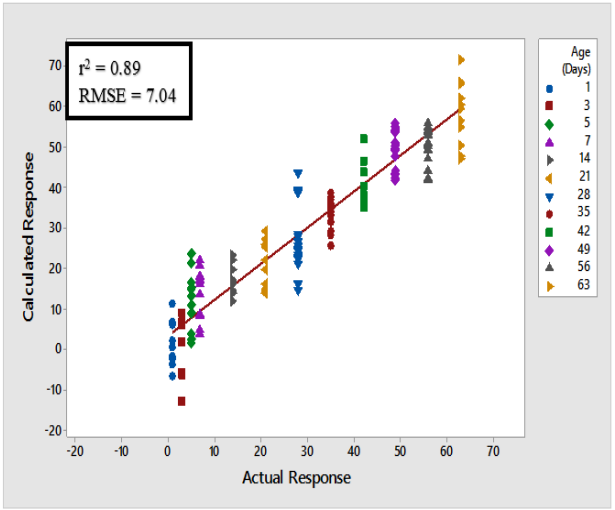

Fig. 10: PLSR plots for outdoor bloodstains deposited on peat soil

A possible explanation for the early-stage uncertainty is associated with the reaction kinetics of haemoglobin.

The autoxidation process of HbO_2 can be divided into an initial fast decay and final slow decay. The initial fast decay lasted a few hours and then transited to the slow decay. The slow decay probably lasted ten days and entered a slower decay phase subsequently. It was also reported that the rates of HbO_2 are strongly temperature-dependent and that the transition of met-Hb into hemichrome is strongly humidity-dependent. It is conceivable that the fluctuating temperature and humidity in both the indoor and outdoor environments where the bloodstains were stored resulted in the instability of the autoxidation process of HbO_2 and increased the complexity of the haemoglobin reaction kinetics in the 1- 7 days period, which was probably corresponding to the early phase of slow decay. As a consequence, the variety and relative quantity of secondary structures of haemoglobin and its derivatives changed rapidly and irregularly [22]. Large variations in the age prediction of bloodstains were also noted at certain time intervals probably due to contribution of environmental factors to the degradation of bloodstains was also large.

To further classify the samples and examine the robustness of the predictive model, PCA was carried out by projecting extracted 10 LVs as new input for PLS-DA analysis. Tables 1 to 6. tabulate the classification of indoor and outdoor aged human bloodstain on tested three soils. Using these classification models, correct classification of the aged bloodstains extracted from soil matrices was calculated up to 70%. Again, as presented in PLSR results it was evident that misclassifications were higher in the indoor groups regardless of soil types with accuracies of between 0.40 to 0.51.

Table 1: Classification of aged human bloodstain on beach soil indoor samples

	True Group											
Put into Group	1	3	5	7	14	21	28	35	42	49	56	63
1	11	0	1	0	0	0	1	1	0	0	0	0
3	0	6	0	1	2	1	2	0	1	3	0	0
5	1	2	13	3	4	5	4	6	1	7	3	2
7	5	1	1	6	5	0	1	0	1	0	0	0
14	1	2	0	1	5	0	0	1	1	0	0	0
21	0	2	2	1	1	5	0	0	0	0	0	0
28	0	2	0	3	0	0	0	3	0	1	0	0
35	0	0	1	0	1	1	1	4	0	2	0	1
42	2	0	0	0	0	0	6	1	12	1	0	2
49	0	1	0	0	0	0	0	0	0	5	0	1
56	0	0	0	0	0	2	1	0	0	0	10	0
63	0	0	2	0	0	5	0	1	3	0	4	14
Total N	20	16	20	15	18	19	16	17	19	19	17	20
N Correct	11	6	13	6	5	5	0	4	4	5	10	14
Proportion	0.55	0.38	0.65	0.4	0.28	0.26	0	0.24	0.63	0.26	0.6	700

Table 2: Classification of aged human bloodstain on coarse soil indoor samples

	True Group											
Put into Group	1	3	5	7	14	21	28	35	42	49	56	63
1	19	1	0	1	0	0	0	0	0	0	0	0
3	0	12	1	0	0	2	0	1	1	0	0	0
5	0	1	10	4	0	0	1	4	1	0	1	2
7	0	3	5	12	1	1	1	1	1	0	1	1
14	0	0	2	1	14	1	0	1	1	0	0	0
21	0	2	0	0	0	14	1	0	0	1	1	0
28	0	1	1	1	0	1	15	0	2	0	0	0
35	1	0	0	0	1	1	0	11	4	0	0	1
42	0	0	1	1	2	0	1	0	4	4	2	3
49	0	0	0	0	2	0	0	1	1	4	1	5
56	0	0	0	0	0	0	0	0	0	4	5	0
63	0	0	0	0	0	0	1	1	5	7	9	8
Total N	20	20	20	20	20	20	20	20	20	20	20	20
N correct	19	12	10	12	14	14	15	11	4	4	5	8
Proportion	0.950	0.600	0.500	0.600	0.700	0.700	0.750	0.550	0.200	0.200	0.250	0.400

Table 3: Classification of aged human bloodstain on peat soil indoor samples.

Put into	True Group										
Group	1	3	5	7	14	21	28	35	49	56	63
1	12	1	3	0	0	0	0	0	0	0	0
3	2	8	3	6	1	2	1	1	0	0	2
5	0	0	9	0	1	0	0	0	0	0	1
7	0	2	0	12	4	5	0	1	0	0	1
14	0	3	2	1	12	0	0	0	0	0	0
21	4	2	0	1	0	6	1	5	1	0	2
28	1	0	0	0	2	5	14	1	0	0	2
35	0	1	0	0	0	1	0	8	0	3	0
42	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	14	10	2
56	0	0	1	0	0	0	1	1	4	4	1
63	0	1	1	0	0	1	2	3	1	3	9
Total N	19	18	19	20	20	20	19	20	20	20	20
N correct	12	8	9	12	12	6	14	8	14	4	9
Proportion	0.63	0.44	0.47	0.6	0.6	0.3	0.74	0.4	0.7	0.2	0.45

Table 4: Classification of aged human bloodstain on beach soil outdoor samples.

	True Group											
Put into Group	1	3	5	7	14	21	28	35	42	49	56	63
1	13	0	5	1	0	0	0	0	0	0	0	0
3	0	8	2	0	1	0	0	0	0	0	0	0
5	5	2	5	2	0	0	1	0	0	0	0	1
7	0	2	2	17	0	0	1	2	0	0	1	0
14	0	1	0	0	9	4	0	0	2	0	0	0
21	0	1	6	0	2	11	1	2	2	0	2	1
28	0	0	0	0	0	0	9	1	0	1	0	0
35	0	4	0	0	0	0	2	15	0	0	1	0
42	0	0	0	0	3	0	0	0	16	0	0	0
49	0	1	0	0	1	1	4	0	0	17	2	2
56	0	0	0	0	0	3	0	0	0	0	11	4
63	0	0	0	0	1	0	0	0	0	0	2	11
Total N	18	19	20	20	17	19	18	20	20	18	19	19
N correct	13	8	5	17	9	11	9	15	16	17	11	11
Proportion	0.722	0.421	0.250	0.850	0.529	0.579	0.500	0.750	0.800	0.944	0.579	0.579

Table 5: Classification of aged human bloodstain on coarse soil outdoor samples.

	True Group										
Put into Group	1	3	5	7	14	21	28	35	42	56	63
1	11	0	0	0	0	0	0	0	0	0	0
3	0	3	0	0	0	5	0	0	0	0	0
5	1	1	5	2	1	1	0	2	0	0	0
7	0	0	0	5	2	0	0	0	2	0	0
14	0	2	0	0	8	0	0	0	0	0	0
21	0	3	0	0	2	12	0	2	0	0	1
28	0	1	0	0	0	0	15	0	0	0	0
35	0	0	0	0	0	0	0	12	0	0	0
42	0	0	0	0	0	0	0	0	9	2	1
56	0	1	0	0	0	1	0	1	3	14	4
63	0	0	0	0	0	0	0	0	2	1	9
Total N	12	11	5	7	13	19	15	17	16	17	15
N correct	11	3	5	5	8	12	15	12	9	14	9
Proportion	0.917	0.273	1.000	0.714	0.615	0.632	1.000	0.706	0.563	0.824	0.600

Table 6: Classification of aged human bloodstain on peat soil outdoor samples

	True Group											
Put into Group	1	3	5	7	14	21	28	35	42	49	56	63
1	9	0	1	0	0	0	0	0	0	0	0	0
3	1	5	2	1	0	0	0	0	0	0	0	0
5	0	2	4	0	0	1	1	0	0	0	0	0
7	0	1	2	9	0	0	0	0	0	0	0	0
14	0	0	0	0	10	3	0	0	0	0	0	0
21	0	0	3	1	3	7	2	1	0	0	0	0
28	0	0	0	0	0	0	11	0	0	0	0	0
35	0	0	0	0	0	1	1	11	3	1	0	0
42	0	0	0	0	0	0	0	3	8	0	0	0
49	0	0	0	0	0	0	1	0	2	10	6	0
56	0	0	0	0	0	0	0	0	0	8	10	4
63	0	0	0	0	0	0	0	0	0	0	0	9
Total N	10	8	12	11	13	12	16	15	13	19	16	13
N correct	9	5	4	9	10	7	11	11	8	10	10	9
Proportion	0.900	0.625	0.333	0.818	0.769	0.583	0.688	0.733	0.615	0.526	0.625	0.692

Interestingly, as observed in IR spectra previously, the coarse and beach samples exhibited better predictive classification model for this study as compared to peat soil. This again could be attributed to the properties and particle sizes of each soil that may affect their ability to retain the bloodstains.

IV. CONCLUSION

In conclusion, this study aimed to determine the suitability and efficiency of ATR-FTIR and chemometrics in estimating the aging of human bloodstains on soil matrices such as beach soil, coarse soil, and peat soil. The results have highlighted that the sampling strategy using the extraction method using normal saline produced the best quality spectra, lower the background noise, and high signal to noise ratio. Direct analysis of bloodstain on soil matrices by ATR-FTIR spectroscopy reveal some disadvantages such as the presence of soil matrix reduced the intensity of the band from blood and masked their identification. On the contrary, ATR-FTIR spectroscopy of blood utilizing the extracted method combined with chemometrics has demonstrated as a promising tool for estimating the age of the bloodstains. The combination of ATR-FTIR spectroscopy and chemometrics, particularly Partial Least Square Regression (PLSR) and Partial Least Square-Discriminant Analysis (PLS-DA) as prediction models on the truncated regions of IR spectra, facilitates the determination of the age of the bloodstains. Though excellent classification was not achieved using these models, estimation of the age of bloodstains was still feasible especially for outdoor and very aged bloodstains. More research is warranted in order to improve the confidence in the classification before any protocols can be proposed for future forensic routine use. In summary, ATR-FTIR spectroscopy coupled with chemometrics has the potential as a rapid, robust, reliable and non-destructive application for estimation of the age of bloodstains on soil matrices.

REFERENCES

1. P. Thanakiatkrai, A. Yaodam, and T. Kitpipit, "Age estimation of bloodstains using smartphones and digital image analysis". Forensic Science International, 2013. 233(1-3): p. 288-297.
2. S. H. James, P.E. Kish, and T.P. Sutton, Principles of bloodstain pattern analysis: theory and practice. 2005: CRC Press.
3. W. G. Eckert and S.H. James, Interpretation of bloodstain evidence at crime scenes. 1998: CRC press.
4. M. Kasu, and K. Shires, "The validation of forensic DNA extraction systems to utilize soil contaminated biological evidence". Legal Medicine, 2015. 17(4): p. 232-238.
5. Lin, H., et al., "Estimation of the age of human bloodstains under the simulated indoor and outdoor crime scene conditions by ATR-FTIR spectroscopy". Scientific Reports, 2017. 7(1): p. 13254.
6. J.H. An et al., Body fluid identification in forensics. 2012.
7. L. Gefrides and K. Welch, Forensic biology: Serology and DNA, in The forensic laboratory handbook procedures and practice. 2011, Springer. p. 15-50.
8. S. Harbison and R. Fleming, "Forensic body fluid identification: state of the art". Res Rep Forensic Med Sci, 2016. 6(2016): p. 11-23.
9. M. A. Jobling and P. Gill, "Encoded evidence: DNA in forensic analysis". Nature Reviews Genetics, 2004. 5(10): p. 739.
10. N. Laan et al., "Volume determination of fresh and dried bloodstains by means of optical coherence tomography". Journal of Forensic Sciences, 2014. 59(1): p. 34-41.
11. Y. Marumo, "Forensic examination of soil evidence". Japanese Journal of Science and Technology for Identification, 2003. 7(2): p. 95-111.
12. R. Sugita and Y. Marumo, "Screening of soil evidence by a combination of simple techniques: validity of particle size distribution". Forensic Science International, 2001. 122(2-3): p. 155-158.
13. K. Pye and S.J. Blott, "Particle size analysis of sediments, soils and related particulate materials for forensic purposes using laser granulometry". Forensic Science International, 2004. 144(1): p. 19-27.
14. A. Sikirzhytskaya et al., "Forensic identification of blood in the presence of contaminations using Raman microspectroscopy coupled with advanced statistics: effect of sand, dust, and soil". Journal of Forensic Sciences, 2013. 58(5): p. 1141-1148.
15. R. Kumar and V. Sharma, "Chemometrics in forensic science". TrAC Trends in Analytical Chemistry, 2018. 105: p. 191-201.
16. C. K. Muro et al., "Vibrational spectroscopy: recent developments to revolutionize forensic science". Analytical Chemistry, 2014. 87(1): p. 306-327.
17. C. M. R. Orphanou, A bioanalytical approach to forensic body fluid identification & age determination. 2015, Staffordshire University.
18. Y. Fujita et al., "Estimation of the age of human bloodstains by electron paramagnetic resonance spectroscopy: Long-term controlled experiment on the effects of environmental factors". Forensic Science International, 2005. 152(1): p. 39-43.
19. D. Patterson, "Use of reflectance measurements in assessing the colour changes of ageing bloodstains". Nature, 1960. 187(4738): p. 688.
20. H. Inoue et al., "Identification of fetal hemoglobin and simultaneous estimation of bloodstain age by high-performance liquid chromatography". International Journal of Legal Medicine, 1991. 104(3): p. 127-131.
21. J. Andrasko, "The estimation of age of bloodstains by HPLC analysis". Journal of Forensic Science, 1997. 42(4): p. 601-607.
22. R. H. Bremmer et al., "Biphasic oxidation of oxy-hemoglobin in bloodstains". PloS One, 2011. 6(7): p. e21845.