Al-Kufa University Journal For Biology: 2024. Vol. 16 No. 1 P: 72-77
ISSN (Print)2073-8854 ISSN (Online): 2311-6544

Original Research Paper : *Journal hompage : https://journal.uokufa.edu.iq/index.php/ajb/index*

Estimating The Age of Human Bloodstains by FTIR Spectroscopy in Forensic Laboratories

Yousif M. Jabor, Baneen M. Yusef, Anfal S. Hassan, Jaafar I. Ibrahim, zahra M. dekhel, Baneen H. Neama, Bashaier E. kamel, Haider A. Mahdi, Alaa M. Muhammad, Amany H. Kazem, Baneen S. Rasul and Jassim M. Atwan

1 Department of Forensic Technologies, Najaf Technical Institute, Al-Furat Al-Awsat Technical University

Article history
Received: 20 / 11/2023
Revised: 10 / 1 /2024
Accepted: 26 / 1 /2024
DOI:
10.36320/ajb/v16.i1.13435

*Corresponding Author:
Email:
yousuf1991mo@gmail.com

Abstract: *In forensic techniques, determining the age of human blood spots is crucial, but it can be challenging because there is no widely used, trusted method for doing so. An innovative technology called Fourier transform infrared (FTIR) was utilized to calculate the age of blood spots from one day to 42 days. Six healthy male and female donors provided six blood samples, the ages of the donors ranged from (14-32) years, and samples were taken at different times, the blood splatter storage conditions closely resembled actual crime scenes. When analyzing the samples, it was observed that there were peaks related to amide A, I, II and III in all samples, which are considered diagnostic features of human blood using the FTIR device. In samples 1, 2, and 3, it was observed that there were beaks (3564 – 3960) indicating the presence of moisture or water particles in the sample, and the reason for this may be due to the failure to dry the sample properly, or that it. It was also observed that there were beaks (1968 – 2973) related to lipids in plasma and (1055-1062) related to glucose in samples 1-2-3, which were preserved for a period of (10-42) days, and their absence in samples that were kept for a period of (1-3) days, and this may indicate decomposition of blood samples as time progressed and breakage. The fast, simple, and non-destructive properties of FTIR spectroscopy make it an excellent tool for forensic medicine. This is one of the most recent studies showing how FTIR spectroscopy can be a useful technique for determining the age of bloodstains in made-up crime scenes. Global bio spectral information from aging blood spots can be extracted and analyzed via chemical analysis, which has proven to be successful.*

.**Keywords: FTIR Spectroscopy. Bloodstrain. Age of blood human.Forensic Laboratories**

1.Introduction

Body fluids contain chemical and biological elements that can be examined to provide data at various degrees of identification. Blood, saliva, semen, and vaginal fluids can all be the subject of various tests. However, from a forensic standpoint, the most important

analyses are hypothetical tests that reveal the presence of certain bodily fluids and DNA profiling that establishes the identification of the person who deposited body fluids. The major body fluids' composition must be understood to apply the proper analysis methods (1).

In a crime scene, bodily fluid traces can be removed in several ways. The identification of certain bodily fluids can help with the criminal procedure investigation by giving the investigator contextual information. Body fluid analysis has a bearing, deoxyribonucleic acid (DNA) has a significant impact on characterizing an individual's identity; nevertheless, forensic experts must be able to recognize the biological evidence's source before the DNA may be recognized. Blood is one of the most often extracted body fluids among all others. DNA can be easily found at crime scenes in body fluids (2,3). Its examination of stained fibers, however, is still a relatively untapped market. There are some restrictions with regard to spotting blood on materials. Blood samples' subsequent extraction from dirty or overlapping fibers could result in contamination The hue of the cloth might make blood difficult to visually identify, making identification of blood challenging and time-consuming, if not impossible. In some cases, a blood microscope's actual fiber count is constrained, which makes it more challenging to identify blood because there isn't enough of it. A critical need exists for additional study, analysis, and the application of technologies that can identify the presence of blood while also removing the interference brought on by substrates and colors (4).

It has also become possible to analyze body fluids using vibrational spectroscopy methods, such FTIR spectroscopy. It has been demonstrated that FTIR spectroscopy yields positive outcomes. Its intrinsic surface sensitivity and lack of penetration depth have been regarded as characteristics that make it a tool with significant potential for the detection of blood spots on tissue substrates. This method is sensitive, non-destructive, ecologically benign, low-cost, quick, and sure (qualitatively and quantitatively). During analysis, no sample preparation is necessary. The key benefit of this technology is that handheld devices are also easily accessible in forensic laboratories for spot identification (5,6).

FT-IR spectroscopy has received a lot of interest recently for its use in forensic identification and in differentiating between various body fluid types. This method has also been used to identify species and determine the age of human body fluids. Blood absorption bands in the middle of the infrared range of 4000-6000 cm-1 are attributed to Amide A (3300 cm-1), Amide B (2800-3000 cm-1), Amide I (1650 cm-1), Amide II (1540 cm-1), and Amide III (1200-1350 cm-1). FT-IR spectroscopy was employed to make this result. Additionally, FT-IR spectroscopy offers a penetration depth of less than 10 micrometers that makes it possible to find blood traces on surfaces with a high absorption rate. Results from this method are displayed as strips or peaks, representative functional groups, and positions from the peak specific to specific interactions with it through molecular bonds that provide precise details on the biological composition (7).

Because it is a safe, non-destructive method for the sample, spectral analysis will enable the preservation of samples and make it easier for samples to undergo further examinations, the goal of this study is to use the FT-IR method in examining blood spots taken from crime scenes and to study the effect of the age of the spot taken from the crime scene.

2.Mathodology[17]

Six healthy male and female donors provided six blood samples. Before collecting samples, each subject gave their written informed consent. For all samples, blood (3 ml) was drawn using a Butterfly 23G needle and put in test tubes without an anticoagulant. Hepatitis C antigen, HIV, and HBs all came

back negative in every participant's test. Each donor provided a single sample. The ages of the donors ranged from (14-32) years, and samples were taken at different times, as follows:

1. The first sample was withdrawn from a 23-year-old donor, The sample was dried for 42 days before the date of the examination by exposing it to air at room temperature.
2. The second sample was drawn from a 26-year-old donor, and the sample was dried in the same way as above for 36 days before the date of the examination.
3. The third sample was withdrawn from a 14-year-old donor, and the sample was dried in the same way as above for ten days before the date of the examination.
4. The fourth sample was withdrawn from a 17-year-old donor, and the was dried in the same way as above for three days before the date of the examination.
5. The fifth sample was withdrawn from a 24-year-old donor, and the sample was dried in the same way as above for two days before the date of the examination.
6. The sixth sample was withdrawn from a 32-year-old donor, and the sample was dried in the same way as above for one day before the date of the examination.

Sample preservation conditions

The sample was kept in a clean, dry, and temperature-controlled laboratory environment at room temperature and was exposed to the air continuously throughout the drying days.

Characterization of samples by FT- IR.

The transmittance of the produced formulations was measured using an FT-IR spectrophotometer with a 4 cm-1 resolution and a spectral range of 4000-400 cm-1.

3.Results and Discussion

The "bio fingerprint region," which is the 1800–900 cm-1 studied spectral range in the present work, offers the most details on the chemical components of biological samples, including lipid esters (1800–1700 cm-1), amide I, II, and III proteins (1700–1500 cm-1, 1350–1200 cm-1), nucleic acids, and carbohydrates (1200–900 cm-1) (47,48,49). The matching infrared spectra for bloodstains show a strong representation of hemoglobin, which makes up 97% of the dry blood content (8).

Fourier Transform Infrared (FTIR)

Fourier transform infrared spectroscopy (FTIR) was used to determine the functional groups of the blood samples. Figure 1 displays the findings from the FTIR analysis of the two samples, and Table 1 lists the functional groups. Analysis of the FTIR data based on (9,10).

Peaks in the absorption present in the sample 1 (42 days) were (3960, 3905.32, 3773.33, 3732.17, 3648.55 and 3565.46 cm^{-1} relates to the O–H stretching and H-bonded) and (3360.59 , 3235.46 and 3201.41 cm^{-1} relates to symmetric N-H stretching (amideA)) and (2973.13, 2374.91, 2310.43, 2242.31, 2197.76, 2134.93 and 1979.45 cm^{-1} relates to the CH_3 stretching (plasma lipids)) and (1513.94 cm^{-1}relates to N-H bending coupled to C-N stretching (amide II)) and (1390.20 cm^{-1} relates to C-N stretching (amide III)) and (1058.49 cm^{-1} relates to symmetric C-O stretching (glucose)) and (750.24, 715.86 and 635.17 cm^{-1}related to amide IV).

The absorbance peaks present in the sample 2 (36 days) were (3899.23, 3855.71 and 3737.23 cm^{-1} relates to the O–H stretching and H-bonded) and (3487.65 , 3431.42 and 3275.22 cm^{-1} relates to symmetric N-H stretching (amide A)) and (2929.08, 2367.09, 2312.93, 2103.01 and 1980.88 cm^{-1} relates to the CH_3 stretching (plasma lipids)) and (1633.99 cm^{-1} relates to C-O stretching (amide I) (1514.14 cm^{-1}relates to N-H bending coupled to C-N stretching (amide II)) and (1390.52 and 1236.75 cm^{-1} relates to C-N stretching (amide III)) and (1062.07 cm^{-1} relates to symmetric C-O stretching (glucose)) and (676.41 and 634.04 cm^{-1}related to amide IV).

The absorbance peaks present in sample 3 (10 days) were (3900.02, 3826.59, 3740.31, 3671.83, 3593.41 and 3564.32 cm^{-1} relates to the O–H stretching and H-bonded) and (2363.04 and 3276.69 cm^{-1} relates to

symmetric N-H stretching (amide A)) and (2942.91, 2377.73, 2312.21, 2237.44, 2196.55, 2064.10 and 1968.50 cm^{-1} relates to the CH_3 stretching (plasma lipids)) and (1637.52 cm^{-1} relates to C-O stretching (amide I) (1517.17 cm^{-1}relates to N-H bending coupled to C-N stretching (amide II)) and (1392.75 cm^{-1} relates to C-N stretching (amide III)) and (1055.53 cm^{-1} relates to symmetric C-O stretching (glucose)) and (776.45, 669.10 and 634.93 cm^{-1}related to amide IV).

The absorbance peaks present in sample 4 (3 days) were (3278.89 cm^{-1} relates to symmetric N-H stretching (amide A)) and (1631.60 cm^{-1} relates to C-O stretching (amide I) and (1541.14 cm^{-1}relates to N-H bending coupled to C-N stretching (amide II)) and (1396.44 cm^{-1} relates to C-N stretching (amide III)) and (680.65 cm^{-1}related to amide IV).

The absorbance peaks present in sample 5 (2 days) were (3273.74 cm^{-1} relates to symmetric N-H stretching (amide A)) and (1630.89 cm^{-1} relate to C-O stretching (amide I) and (1543.85 cm^{-1}relates to N-H bending coupled to C-N stretching (amide II)) and (630.72 cm^{-1}related to amide IV).

The absorbance peaks present in sample 6 (1 day) were (3273.97 cm^{-1} relates to symmetric N-H stretching (amide A)) and (1637.87 cm^{-1}relates to C-O stretching (amide I) and (1545.20 cm^{-1}relates to N-H bending coupled to C-N stretching (amide II)) and (673.91 cm^{-1}relates to amide IV).

Table(1): Peaks, bonding, and functional group blood samples

No.	The absorbance peak ($cm-1$)	Bonding and type of vibration	Functional groups
1	3564 - 3960	O–H stretching and H-bonded	water
2	3201 - 3487	symmetric N-H stretching.	amide A.
3	1968 - 2973	CH_3 stretching	plasma lipids
4	1630 - 1637	C-O stretching.	amide I.
5	1513 - 1545	N-H bending coupled to C-N stretching.	amide II.
6	1236 - 1396	C-N stretching	amide III
7	1055-1062	symmetric C-O stretching	glucose
8	630 - 776	N-H bending coupled to C-N stretching.	amide IV

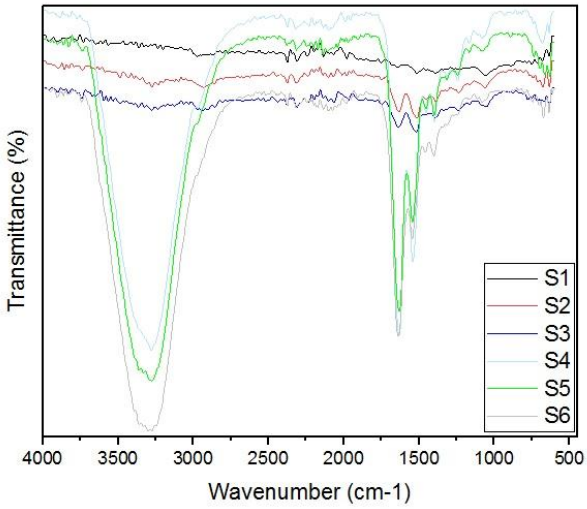

Figure 1. Fourier Transform Infrared Spectroscopy averaged spectra of human bloodstains

When analyzing the above results, it was observed that there were peaks related to amide A, I, II and III in all samples, which are considered diagnostic features of human blood using the FTIR device (10, 11, 12, 13). In samples 1, 2, and 3, it was observed that there were beaks (3564 – 3960) indicating the presence of moisture or water particles in the sample, and the reason for this may be due to the failure to dry the sample properly, or that it had absorbed moisture while preparing the sample for examination, and therefore the presence of these beaks was observed (13, 14). It was also observed that there were beaks (1968 – 2973) related to lipids in plasma and (1055-1062) related to glucose in samples 1-2-3 (15), which were preserved for a period of (10-42) days, and their absence in samples that were kept for a period of (1-3) days, and this may indicate decomposition of blood samples as time progressed and breakage. Compounds and cells that lead to decomposition and then aggregation of biochemical compounds again as time progresses and thus increase absorption (16)

Also it was noticed that there were no beaks of the functional group amide 3in samples 4 and 5, which were preserved for a period of (1-2) days, and their appearance in the samples that were preserved for a period of (3-42) days, and this may be considered an indication that the presence of beaks of this active group indicates the passage of time from 3 days upwards to sample preservation.

Conclusion

The fast, simple, and non-destructive properties of FTIR spectroscopy make it an excellent tool for forensic medicine. There have been earlier reports on its use in identifying species and bloodstains. To our knowledge, however, this is one of the most recent studies showing how FTIR spectroscopy can be a useful technique for determining the age of bloodstains in made-up crime scenes. Global bio spectral information from aging blood spots can be extracted and analyzed via chemical analysis, which has proven to be successful.

Note: The consent of the people donating blood was obtained before conducting the examination

References

1- Jackson, A.R.W. & Jackson, J.M. (2010). Forensic Science, 3rd Edition. Essex, Pearson Education Ltd.

2. Frumkin, D., Wasserstrom, A., Davidson, A. and Grafit, A., 2010. Authentication of forensic DNA samples. Forensic science international: genetics, 4(2), pp.95-103.

3- Muro, C.K., Doty, K.C., de Souza Fernandes, L. and Lednev, I.K., 2016. Forensic body fluid identification and differentiation by Raman spectroscopy. Forensic Chemistry, 1, pp.31-38.

4- An, J.H., Shin, K.J., Yang, W.I. and Lee, H.Y., 2012. Body fluid identification in forensics.

5- Muro, C.K., Doty, K.C., Bueno, J., Halamkova, L. and Lednev, I.K., 2015. Vibrational spectroscopy: recent developments to revolutionize forensic science. Analytical chemistry, 87(1), pp.306-327.

6- Zapata, F., de la Ossa, M.Á.F. and García-Ruiz, C., 2016. Differentiation of body fluid stains on fabrics using external reflection Fourier transform infrared spectroscopy (FT-IR) and chemometrics. Applied Spectroscopy, 70(4), pp.654-665.

7- Sharma, S., Jossan, J.K. and Singh, R., 2020. ATR FT-IR Spectroscopy: A Novel and Non-Destructive Approach for the Detection of Blood on a Single Fiber. Arab Journal of Forensic Sciences & Forensic Medicine, 2(1), pp.22-27

8- Bremmer, R.H., De Bruin, K.G., Van Gemert, M.J., Van Leeuwen, T.G. and Aalders, M.C., 2012. Forensic quest for age determination of bloodstains. Forensic science international, 216(1-3), pp.1-11.

9- Nandiyanto, A.B.D., Oktiani, R. and Ragadhita, R., (2019). How to read and interpret FTIR spectroscope of organic material. Indonesian Journal of Science and Technology, 4(1), pp.97-118.

10- Orphanou, C.M., 2015. The detection and discrimination of human body fluids using ATR FT-IR spectroscopy. Forensic science international, 252, pp.e10-e16.

11- Orphanou, C.M.R., 2015. A bioanalytical approach to forensic body fluid identification & age determination (Doctoral dissertation, Staffordshire University).

12- Sharma, S., Chophi, R., Jossan, J.K. and Singh, R., 2021. Detection of bloodstains using attenuated total reflectance-Fourier transform infrared spectroscopy supported with PCA and PCA–LDA. Medicine, Science and the Law, 61(4), pp.292-301.

13- Cano Trujillo, C.P., Saldaña López, J., Ortega Ojeda, F.E., García Ruiz, C. and Montalvo García, G., 2023. ATR FTIR spectroscopy for the forensic tracing of blood stain age.

14- Mistek, E. and Lednev, I.K., 2015. Identification of species' blood by attenuated total reflection (ATR) Fourier transform

infrared (FT-IR) spectroscopy. Analytical and bioanalytical chemistry, 407, pp.7435-7442.
15- Voronina, L., Leonardo, C., Mueller-Reif, J.B., Geyer, P.E., Huber, M., Trubetskov, M., Kepesidis, K.V., Behr, J., Mann, M., Krausz, F. and Zigman, M., 2021. Molecular origin of blood-based infrared fingerprints. arXiv preprint arXiv:2102.00765.
16- Perez-Guaita, D., Ventura-Gayete, J., Pérez-Rambla, C., Sancho-Andreu, M., Garrigues, S. and De La Guardia, M., 2012. Protein determination in serum and whole blood by attenuated total reflectance infrared spectroscopy. Analytical and bioanalytical chemistry, 404, pp.649-656.
17- Edelman, G., Manti, V., van Ruth, S.M., van Leeuwen, T. and Aalders, M., 2012. Identification and age estimation of blood stains on colored backgrounds by near infrared spectroscopy. Forensic science international, 220(1-3), pp.239-244.