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RESEARCH ARTICLE

IDENTIFICATION OF BLOOD STAIN ON BURNT CLOTH DEBRIS THROUGH FTIR

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ABSTRACT

Bloodstains and bloodstain are some of the most common form of physical evidence encountered during the forensic investigation of incidents involving violence against a person. Blood and blood stains are a very important entity in medico legal practices as factors like source of blood and their stains help in solving the crime of violence, accidental cases. This study was carried out at Sam Higginbottom University of Agriculture, Technology and Sciences, and Ewing Christian College of Allahabad. In the present study, 5 different sample of blood were collected from central pathology lab and three different type of cloth were also been used. Cotton, Nylon and mixed cotton and nylon cloth were used. The purpose of this study was for the Identification of Blood stain on burnt cloth debris through FTIR and this was carried out with objective to find out the sensitivity of Luminol and Benzidine test for the identification of blood at higher temperature (500-10000c). Analysis on Cotton, Nylon, and Simple Cloth surfaces were considered. Blood is one of the important biological evidence commonly encountered by the investigators at the scene of violence crime. Thus it becomes essential to determine the sensitivity of reagents on blood stains on any surface. But in most cases of violence the presence of blood on the different Cloths surfaces leads to false positive or negative tests which in turns results in no option by the experts. So it's very necessary to examine blood which assists the forensic analyst in an enhanced way.

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INTRODUCTION

Blood is a fluid connective tissue. Its cells are quite distinct from other connective tissue cells both in structure and function. The extracellular material in blood is a fluid devoid of fibers. Fluids outside the cell are generally called extracellular Fluid (ECF). Blood is heavier than water. The extracellular material in blood is a straw colored, slightly alkaline (pH=7.4) aqueous fluid called plasma. Constituents, having characteristic forms, float in the plasma. They are collectively called the Formed Elements of Blood. They include the blood cells and blood platelets. Blood cells are of two type–Erythrocytes and Leukocytes. Blood circulates within blood vessels in higher animals. But other extracellular fluids such as cerebrospinal fluid, interstitial fluid, lymph and aqueous humor outside blood vessels.

Composition of Blood

Plasma contains three major classes of plasma proteins viz. serum albumin, serum globulins and fibrinogen. Plasma proteins serve as a source of proteins for tissue cells. Tissue cells may utilize plasma proteins for forming their cellular proteins. Additionally, albumin and globulins retain water in blood plasma by their osmotic effects. A fall in plasma proteins leads to movement of excessive volumes of water from blood to tissues. That is why hands and feet get swollen with accumulated fluid (oedema) in persons suffering from dietary deficiency of proteins. Albumins and globulins also transport many substances such as thyroxin and Fe^{3+} in combination with them. One class of globulin, called immunoglobulin, act as Antibodies. Plasma portions also maintain the blood pH by neutralizing strong acids and bases. Thus, they act as Acid-Base-Buffers.

Function of Plasma Proteins

1. Prevention of blood loss fibrinogen and prothrombin play a role in blood clothing.
2. Retention of fluid in the blood albumin in osmotic balance.
3. Body immunity certain globulins called act as antibodies in blood and tissue fluid. Antibodies belong to a class of portions called as immunoglobulin.
4. Maintenance of ph plasma portion serves as acid base buffers. It means they maintain ph of the blood by neutralizing acids and bases.

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5. Transport of certain materials thyroxin is bound to albumin or specific globulin for transport in the plasma.
6. Distribution of heat plasma potions help in uniform distribution of heat all over the body.

Blood groups

ABO blood group: Karl Landsteiner reported for the first time ABO blood groups in the human beings A, B and O blood groups were discovered by Landsteiner (1900) while AB blood group was found out by De Costello and steini (1902). Agglutinogens (antigen) are present on the surface of red blood corpuscles and agglutinins (antibodies) are found in the blood plasma. Both antigens and antibodies are proteins. When two different type of blood are mixed the red blood corpuscles form a clump the clumping of red blood corpuscles is called agglutination.

MATERIALS AND METHODS

Sample preparation

1. Blood were spread on cloth.
2. The stained cloth were cut into pieces and placed in crucible.
3. The cloth were burnt in muffle furnace at (500-1000^{0}C).
4. After burning clothes were cooled at room temperature and packed in cellophane bag.
5. The burnt debris were tested for chemical test and for FTIR.

Procedure for Chemical test with samples

Reagent- 1

Procedure for Benzidine test

To the burnt debris a drop of TMB solution was placed, followed by a drop of 3% Hydrogen Peroxide. An immediate bluish green color is a positive test for peroxidase activity, indicative of blood however this is not a confirmatory test for blood.

Reagent-2

2. Procedure for Luminol test

To the burnt debris freshly prepared Luminol reagent was sprayed and observed in a dark chamber presence of luminescence of Luminol is indicative of blood.

How does this test work?

The reaction that triggers Luminal, s chemiluminescence has to be catalyzed. Luminol solution also contained an oxidizing agent, such as hydrogen peroxide, and a base. In the presence of a catalyst, the reaction produced energy, promoting electrons in the product to higher energy levels, before they fall back down and release their excess energy as light.

Procedure for FTIR analysis

Preparation of samples

First, spectral grade pure KBr power was dried in an oven up to 60^{0}c for 24 hours. Then 1gm powder was taken in an agate motor and was ground until it becomes fin powder. The ground powder was mixed with blood sample and transferred into the bore of cylindrical so that it was distributed across the polished face of lower plate. The polished face of the second plate towards the power was inserted in to the bore by a plunger. The die assembly was connected to a vacuum pump and was kept under vacuum for approximately 2 min so as to remove air from the sample disk. The die was dismantled and the KBr disk was removed without touching its faces. Here, FTIR spectrometer of make Perkin Elmer was used.

RESULTS AND DISCUSSION

The work entitled Identification of Blood stain on burnt cloth debris through FTIR was carried out in the serology laboratory of Department of Forensic Science with objective to find out the sensitivity of Benzidine and Luminol reagent and of FTIR for identification of blood at higher temperature (500-1000^{0}C). Blood spread on clothes were heat treated in a muffle furnace after burning the cloth containing blood spot were tested for identification of blood the results of the test are given below from table 1 to 4.

Result of Benzidine Test

Table 1. Benzidine test result for blood samples

Sample Number	Cotton Cloth	Nylon Mixed Cloth	Colure observed
1	Positive	Positive	Bluish green
2	Positive	Positive	Bluish green
3	Positive	Positive	Bluish green
4	Positive	Positive	Bluish green
5	Positive	Positive	Bluish green
6	Positive	Positive	Bluish green
7	Positive	Positive	Bluish green
8	Positive	Positive	Bluish green
9	Positive	Positive	Bluish green
10	Positive	Positive	Bluish green

Result of Luminol Test

Table 2. Luminol test result for blood samples

Sample Number	Cotton Cloth	Nylon Mixed Cloth	Colure observed
1	Positive	Positive	Blue
2	Positive	Positive	Blue
3	Positive	Positive	Blue
4	Positive	Positive	Blue
5	Positive	Positive	Blue
6	Positive	Positive	Blue
7	Positive	Positive	Blue
8	Positive	Positive	Blue
9	Positive	Positive	Blue
10	Positive	Positive	Blue

FTIR Test result

The IR spectra showed in Figure 3. Presents FTIR spectrum of Human blood which reveals a series of bands with different intensities and the spectral data is shown in table 1. For the systematic analysis, IR spectrum is divided into three regions. Region I is form 4000 to 3000 cm^{-1}, Connected with water and hydroxyl group. This region is of considerable interest, because it reveals the nature of hydrogen bonding. Region 2 is 3000 to 1500 cm^{-1}, wherein bands for functional groups are observed. In this region, major IR absorption pertaining to fibrinogen occurs. Region 3 is 1500 – 200 cm$^{-1,}$ which has significant importance in the context of biological minerals and

their combinations. The spectra of human blood indicate the presence of bands characteristics of water molecule and also of some functional groups concerned with proteins and liquids. The IR band at a wave numbers 3294 cm^{-1} and 3065 cm^{-1} are related to amide A and amide B respectively. The dominating band at 1396 cm^{-1} maybe originated due to the important protein of blood Fibrinogen. This band is related to the stretching C=O symmetric stretching vibrations of COO^-. A band around 2960 cm^{-1} is due to the –C-H asymmetric stretching of $–CH_3$ in Fatty acids, Phospholipids and Cholesterol esters. The band at 1106 cm^{-1} is related to HbO_2, exhibits $\nu_{(02)}$ bond. The two most intensive bands are centered at 1652cm^{-1} and 1547 cm^{-1} in the FTIR spectrum of human blood. They correspond to the Amide 1 peak arises from C=O hydrogen bonded stretching vibrations, and Amide 2 is attributed to C-N stretching; NH and CH_2 bending modes. Amide 1 and 2 absorption bands are associated also with specific secondary sub- structure, such as α- helix, β- sheet and random coil. The bands at 1307 cm^{-1} and 1248 cm^{-1} are related to Amide 3 bond component of proteins (C-N). The bond at 1170 cm^{-1} corresponds to C-O-C asymmetric stretching vibrations of phospholipids. The bands at 1106 cm^{-1}, 1170 cm^{-1} and 1248 cm^{-1} are associated with triglycerides of human blood. The band at 2936 cm^{-1} is related to platelets due to –C-H symmetric stretching of $–CH_2$.

FTIR analysis of blood

Table 3. Major Peaks of whole Blood analyzed by FTIR

List of Peak Area/Height		
Peak Name	X (cm^{-1})	Y (% T)
1	3411.99(s,b)	13.47
2	1434.62(m)	15.73

Table 3 shows that peak no.1 observed that 3411.99 corresponded with the O-H H-bonded stretch having functional group alcohols, phenols and peak no 2 observed that 1434.62 (m) corresponded with C-C stretch (in ring) having functional group Alkanes specific for blood. (Gunasekarant *et al.,* 2008)

FTIR analysis of Plain Cotton Cloth blood

Table 4. Major Peaks of Cotton Cloth with blood analyzed by FTIR

List of Peak Area/Height		
Peak Name	X (cm^{-1})	Y (% T)
1	3428.7	7.38
2	1574.31	11.9
3	1453.43	10.55
4	1107.68	13
5	884.07	16.43

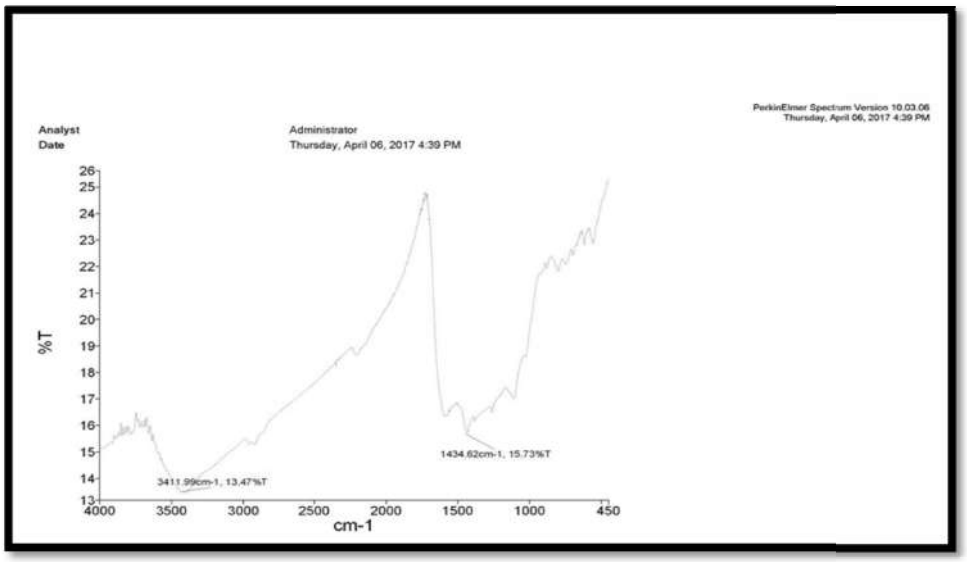

Figure 3. FTIR Spectra of Whole Blood

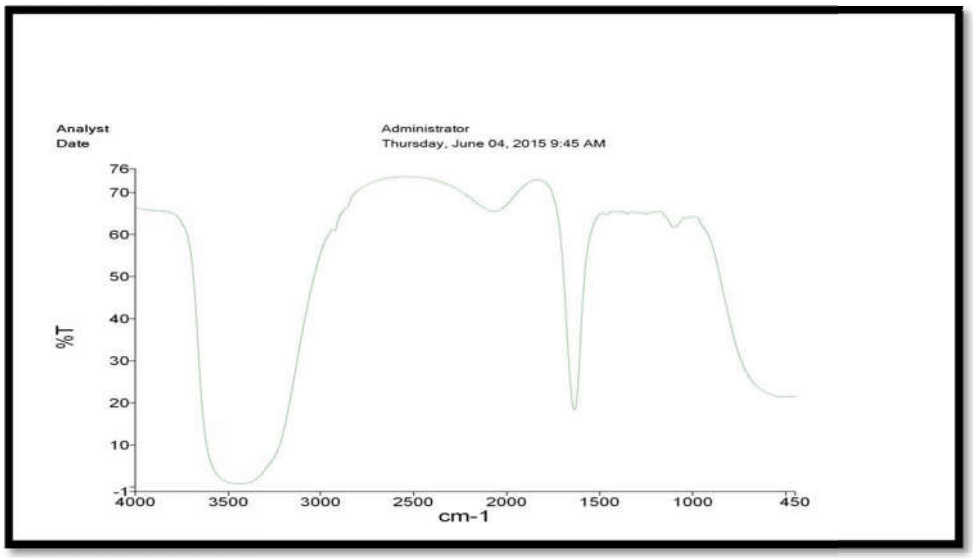

Figure 4. FTIR Spectra of Cotton Cloth

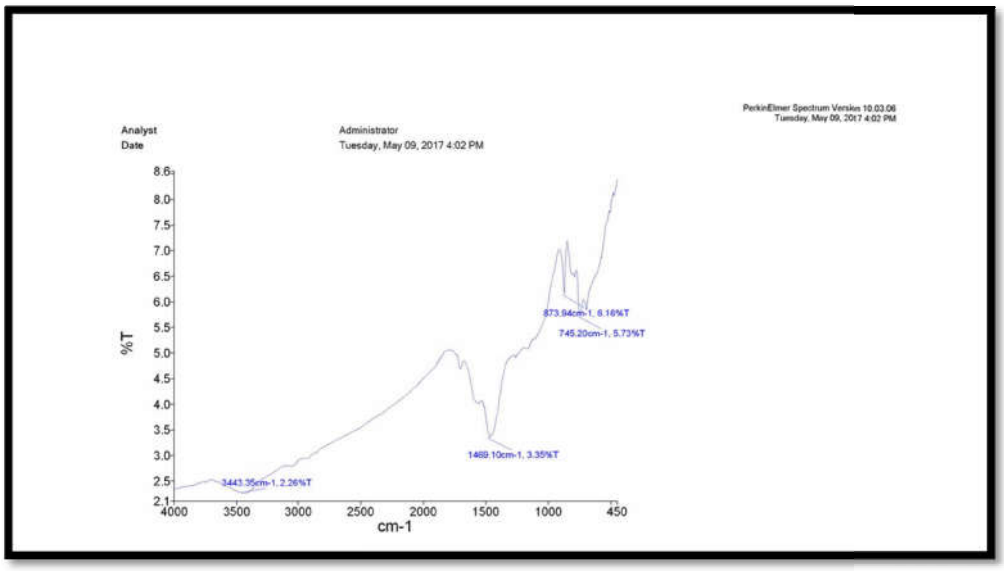

Figure 5. FTIR Spectra of Nylon Cloth

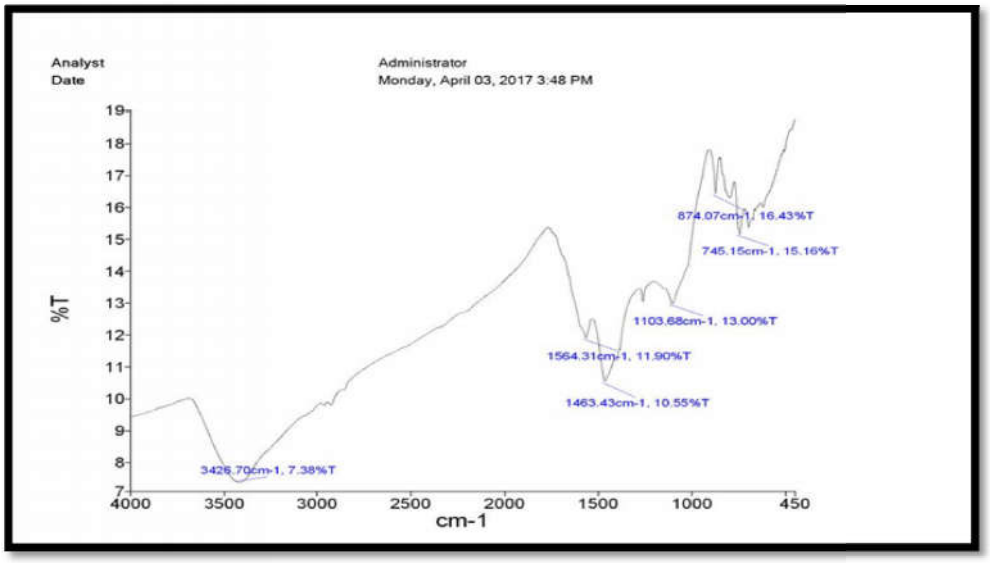

Figure 6. FTIR Spectra of mix cloth (nylon cotton) with Blood

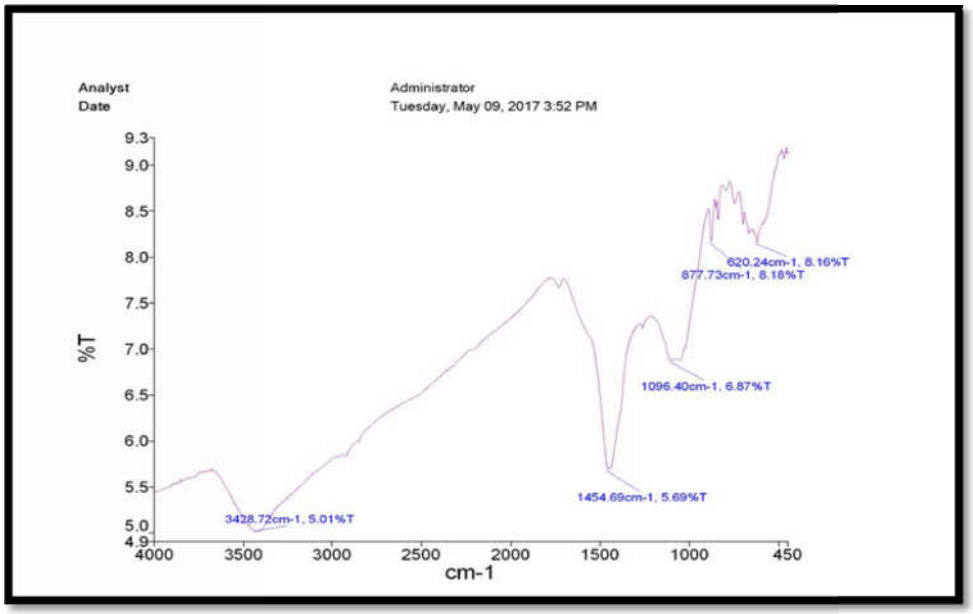

Figure 7. FTIR Spectra of Nylon Cloth with Blood

Table 4 shows that the peak no 1. Observed 3428.7 corresponded with the O-H stretch, H-bonded having functional group Alcohols, phenols. Peak no 2 observed that 1574.31 is not vary from human blood frequency. Peak no 3 observed that 1453.43 corresponded with C-H bend having functional group Alkanes, peak no 4. Observed that 1107.68 corresponded with C-H stretch having functional group Aliphatic amines, peak no 5 884.07 corresponded with C-H oop) having functional group Aromatics specific for blood. (Gunasekarant *et al.,* 2008)

FTIR analysis of Plain Nylon Cloth

Table 5. Major Peaks of Nylon Cloth analyzed by FTIR

List of Peak Area/Height		
Peak Name	X (cm^{-1})	Y (% T)
1	3443.35	2.26
2	1469.1	3.35
3	873.94	6.16
4	745.2	5.73

Table 5 shows that peak no 1. 3443.35, peak no 2. 1469.1, peak no 3. 837.94 And peak no 4. 745.2 is not match from standard human blood frequency. (Gunasekarant *et al.,* 2008)

FTIR analysis of mix cloth (nylon cotton) with Blood

Table 6. Major Peaks of mix cloth Cloth (nylon cotton) with analyzed by FTIR

List of Peak Area/Height		
Peak Name	X (cm^{-1})	Y (% T)
1	3426.7	7.38
2	1564.31	11.9
3	1463.43	10.55
4	1103.68	13
5	874.07	16.43
6	745.15	15.16

Table no 6 shows that the peak no 1. 3426.7 corresponded with the O-H stretch H- bond, having functional group alcohols, phenols. Peak no 2. 1564.31 not math to the human blood frequency. Peak no 3. Observed that 1463.43 C-H bend having functional group Alkanes. Peak no.4 observed 1103.68 corresponded with the C-H stretch and having functional group aliphatic amines. Peak no 5 observed that C- Cl stretch having functional group alkyl halides and peak no 6 observed that C-Cl stretch having functional group alkyl halides (Gunasekarant *et al.,* 2008).

FTIR analysis of Nylon Cloth with Blood

Table 7. Major Peaks of Nylon Cloth with analyzed by FTIR

List of Peak Area/Height		
Peak Name	X (cm^{-1})	Y (% T)
1	3428.72	5.01
2	1454.69	5.69
3	1096.4	6..87
4	877.73	8.18
5	620.24	8.16

Table 7 shows that the observed peak no 1. 3428 .72 corresponded with the O-H stretch, H- bonded and having functional group alcohols, phenols. Peak no 2. 1454.69 C-H bends having functional group Alkanes. Peak no 3 Observed 1069. 4 corresponded with the C-N stretch bond having functional group aliphatic amines. Peak no 4 Observed that 877.73 corresponded with the C-H "oop" bond having functional group aromatic. And peak no 5 observed that 620.24 corresponded with –C≡C-H: C-H bend, having functional group Alkynes. Specific for blood (Gunasekarant *et al.,* 2008).

Conclusion

The study concludes that

1. Presumptive tests are effective for identification of blood stains on burnt debris
2. Luminol test is more effective in comparison to Benzidine test for detection of blood stain on burnt debris
3. FTIR spectra is another sensitive technique to identify the blood stains on burnt debris, and gives conclusive results for detection of blood.

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