Section: Research Paper
ISSN 2063-5346

ASSESSMENT OF BLOOD CONTAMINATION IN BIOLOGICAL FLUIDS USING ULTRAVIOLET VISIBLE SPECTROMETRY

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ABSTRACT

The proteome profile of bodily fluids can be influenced by blood contamination during the collecting of samples. Visual inspection and counting of red blood cells in the sample are commonly used in proteomics investigations to rule out tainted samples; particular blood-derived protein analysis is less common. We devised a rapid and sensitive approach for detecting blood contamination in fundamental biological fluids based on a unique blood-derived protein, haemoglobin detection by ultraviolet visible spectrometry, to bridge the gap. The reference approach, UV spectrometry, was found to be less sensitive. The absorption and scattering properties of particle suspensions are represented in the UV spectrum. This data can be utilised to decipher the spectrum in terms of particle size distribution, particle shape, and chemical composition of the sample material. Because of the existence of various chromospheres found in proteins, strong plasma absorption in the UV area can offer information about these proteins from the plasma. The current method has several advantages, including being rapid, effective, and sensitive, requiring a small sample size, and being able to detect blood contamination in a range of physiological fluids collected for proteomics study.

Keywords: UV-spectrometry, blood contamination, haemoglobin detection, Biological fluid, Raman spectrometer

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INTRODUCTION

Blood is the most common kind of biological evidence collected, consisting of a heterogeneous mixture of water, proteins, mineral ions, amino acids, hormones, blood cells, and other factors. Haemoglobin, which transports oxygen and carbon dioxide and gives blood its red colour, is the most important sign for forensic purposes.[1] Haemoglobin detection is used in a wide range of screening and confirmatory techniques. Bodily fluid evidence can be very important in forensic investigations. Bloodstains are widespread in criminal investigations, and determining the difference between menstrual and peripheral blood is crucial in rape and sexual assault cases. [10] To detect specific menstrual blood components, the bulk of current regimens require complex laboratory tests. Blood and other bodily fluids that have been exposed to blood can spread the hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) (HIV). These viruses can be spread by sperm and vaginal secretions.

Hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) can all be spread by blood and other bodily fluids that have been exposed to blood (HIV). These viruses can be transmitted through sperm and vaginal fluids. For non-invasive BHL measurements, the proper wavelength will be employed. Unlike traditional phlebotomy methods, which are painful, this non-invasive method is painless. This approach avoids the use of needles and reduces the risk of infection. The analysis of haemoglobin ‘trends' can be quite useful in determining blood transfusion decisions, as it generates data on Hb concentration measurements in real time. Pulse oximetry, opt acoustic, diffuse reflectance spectroscopy, and photoplethysmography are some examples of non-invasive procedures (PPG). Pulse oximetry evaluates oxygen saturation by lighting the skin and measuring variations in the absorption of light by oxygenated (oxyhemoglobin) and deoxygenated (reduced haemoglobin) blood. Ultrasonic wave absorption technique with opt acoustic methodology (absorbance).

ATR FT-IR spectroscopy was used in this study as a non-destructive approach for distinguishing menstrual and peripheral blood traces. [1] This method was subjected to both internal and external validation testing, as well as statistical analysis. Internal validation showed that menstruation and peripheral blood samples could be distinguished very well. External validation provided 100 percent accuracy in determining if a sample was peripheral or menstrual blood. [2] This work describes a low-cost technique for measuring the -blocker timolol maleate (TM) quickly and accurately using differential pulse anodic stripping voltammetry (DP ASV) at a bare glassy carbon electrode (GCE). At pH of 7.2 and varied analytical circumstances, a well-defined oxidation peak was seen at 0.85 V vs Ag/AgCl electrode (accumulation time, deposition potential, scan rate, and pulse amplitude). The recommended sensor had excellent precision, selectivity, and reproducibility when it came to TM determination.[3] A tin oxide (SnO_2) quantum dot (QD) fluorine copolymer heterojunction photo detector (FPPD) is used to provide a quick and effective opt electrical approach for determining blood components. Using two different wavelengths of UV light, the optical characteristics of blood components, as well as interactions between the photo detector and blood components, are studied systemically. The relationship between photocurrent signal and absorbance qualities of various blood component mixtures is

thoroughly explored.[4] It is customary to conduct forensic investigation of paper, which serves as the foundation for many papers. Office paper samples from various manufacturers were artificially aged in a climatic chamber for varied periods of time, and infrared and Raman spectroscopy were used to analyse them. [5] The carboxycellulose nanofibers (CNF) were made from raw jute fibres using a one-step nitro-oxidation process based on nitric acid/sodium nitrite. Extracted CNF has a low crystallinity of 35%, a high carboxylate content of 1.15 mmol/g, and a negative surface charge of 70 mV, all of which make it an excellent nanomaterial for removing Pb(II) from water.

As a family of possible carcinogens, benzophenone-type UV filters (BPs) are commonly found in natural surroundings and in organisms. A important stage in the carcinogenic process is the interaction of malignant chemicals with DNA to form adducts. [6] With quenching constants ranging from 0.93 103 to 5.89 103 L/mol, only BPs with hydroxyl groups could bind to DNA in the groove binding mode. Substituted BPs were favoured for binding to thymine. [7] The new iron oxide nanoparticles have a 1.96 mg/g adsorption capacity, a bigger surface area, and improved catalytic degradation abilities. chevalier The removal efficiency of arsenic by iron oxide magnetite nano catalyst (adsorbent) on real water samples was 81.09 percent, whereas the removal efficiency of synthetic water was 81.09 percent, according to ICP-MS equipment. [8 The release of haemoglobin from red blood cells into the supernatant was measured using a spectrophotometric technique. At 340 nm wavelength, the ratio of fluorescence emission intensity of protein extract from irradiated whole blood or red blood cells was not different from that of the non-irradiated control. [9] Surface enhancement of blood serum C. neoformans infection in the lungs was detected and assessed using Raman spectroscopy (SERS). Infected mice were separated into four groups based on infection duration. [10] Hb and DNA degradation in forensic blood samples kept under different settings were investigated using spectroscopy (tabulated and graphically). The quality of the samples was then assessed using DNA analysis under various preservation circumstances. Finally, our research provides a way for conserving samples that is both quick and effective.

II MATERIALS AND METHODS

Forensic blood gauzes are collected at random from male victims at numerous government hospitals and delivered to the State Forensic Science Laboratory (SFSL). Researchers investigated the effects of storage length and storage conditions (dry/wet) on Hb and DNA quality. These samples were initially subjected to Hb (Hb) and DNA extraction, followed by spectroscopic inspection, to ensure their quality and amount. The forensic samples' serology (ABO blood grouping) and DNA were then examined.

2.1 SAMPLES

The 38 blood gauze samples utilised in this investigation were sent to the State Forensic Science Laboratory in Rohini, New Delhi, for serological and DNA fingerprinting. The amount of solute compared to the solvent was 3990 l after 400 times diluting blood samples in distilled water. Following that, the Wiggens vortex3000 tool was used to homogenise the samples. Using a UV-Vis spectrophotometer, the absorbance of the dissolved materials was

measured in a 3-4 ml cuvette. Before use, the samples were frozen, and 5 litres of whole blood were placed on an aluminium-coated glass slide. The slide was placed in a petri dish and allowed to dry overnight to avoid contamination.

2.1.1 INSTRUMENTATION

A UV spectrometer with WiRE 3.2 software and a Lecia research grade microscope were used to obtain the UV of the blood samples. To confirm that the device was properly calibrated, the spectrum of a silicon standard was obtained prior to sample collection. The laser beam on the samples was focused using a 20 objective. With a laser power of 5%, a 785 nm excitation wavelength was employed. Over a 300 - 1800 cm1 range, 20 ten-second accumulations for each spectrum were obtained. To account for intrasample variability, 10 to 20 spectra were taken from different places inside the same bloodstain.

2.1.2 UV ANALYSIS

The absorbance of the band at 275 nm, which is characteristic of DEHP, was used to determine the amount of DEHP in the film and solution. The DEHP content of the film was determined by analysing it directly with air as the reference material and comparing its absorbance to that of a film bearing a ratio of DEHP of r = 0.4. A double-beam Jasco V-550 UV spectrophotometer was used. Water or ethanol was used as a reference sample for DEHP measurement in the surrounding liquid.

2.1.3 UV MEASUREMENT

The samples were measured with a 2 nm resolution across the entire wavelength range of 180 to 820 nm. The generated extinction spectra, which adjusted intensity effects due to red cell concentration, were used to analyse and compare the uv spectral data. Extinction spectra were created with in-house software. Five additional dilutions of the sample were measured to calculate the error associated with the extinction spectra. The first two dilutions entailed adding solvent to the cuvette, mixing thoroughly, then measuring the result.

2.2 UV SPECTROMETRY

Our research improved UV spectrometry for examining crude biological fluids without sample pre-treatment and identifying Hb concentration to identify blood contamination. The improved test is quick, accurate, and sensitive (LOD 0.12 nM), and it only takes a tiny amount of sample (1 lL). The latter is particularly useful when evaluating biological samples, which are typically small. UV spectrometry assays can also be employed for high-throughput screening by using a robotic workstation to automate UV spot preparation. In comparison to UV spectroscopy, other approaches are either less exact (visual inspection, for example) or require a larger sample volume. As a comparison approach, MALDI-TOF MS was used in this investigation. Although the NanoDrop ND-1000 and comparable equipment have a small sample volume and are simple to use, NanoDrop has

been demonstrated to be less sensitive than classical MALDI-TOF MS spectrometry. A UV-2410PC spectrophotometer was used for UV spectrometry analysis, as were 50 lL Eppendorf disposable cuvettes. Between 515 and 615 nm, the absorption of Hb was detected.

2.2.1 UV SPECTROSCOPY OF HB & DNA

UV spectroscopic profiles of Hb and DNA were obtained using the ND 1000 Nano-drop Spectrophotometer. For testing, a 1mL sample was obtained. Using 0.2 M TrisHCl, all spectrum data for Hb was blank corrected. From 350 to 550 nm, spectral data was gathered. Changes in the iron bound ligand have a big impact on the Hb spectrum. The structure and function of haem proteins are altered as a result of these alterations. The Soret Band is a strong transition at 400 nm that spans the porphyry's cyclic tetrapyrrole ring. Using 1X TE buffer, all spectral data from DNA was blank corrected. The spectral data ranged from 190 to 840 nm.

2.2.2 CAPABILITIES OF UV SPECTROPHOTOMETRY

UV spectrophotometry can characterise whole blood samples. The primary components of whole blood include red blood cells (RBCs), white blood cells (WBCs), platelets, and plasma, all of which contribute to the whole blood spectrum. Due to physical variables that govern its optical behaviour, each component has its own spectrum features. The cumulative spectral features of a particle are determined by a mixture of essential criteria (size, shape, and chemical composition) known as the joint property distribution (JPD).

III RESULT AND DISCUSSION

When comparing the best preserved samples to the remaining samples, UV spectroscopic research revealed that the quantity and quality of DNA (A260/A280) was rather high. Even in the worst preserved samples, high to intermediate quality DNA could be used to produce DNA STR profiles. According to the data, most blood samples submitted for forensic DNA testing were damp gauzes housed in a polythene/plastic container or greenish gauzes. Only a small percentage of blood gauzes are dried and preserved in reddish/brownish paper envelopes (Figure 2). The wavelength shift/intensity in several conserved samples was tested using data from the lmax Hb Soret band. Changes in the 'a' and 'b' bands were also found in samples kept under different circumstances.

Absorbance in wavelength		Hb level (gr/dL)		Different in
525 nm	570 nm	Prodia	ZunZunSite3	Hb level
0,254	0,357	13,2	13,2603931	0,06039311
0,256	0,374	12,9	13,0195863	0,11958632
0,157	0,23	12,1	11,2614429	-0,8385571
0,181	0,268	14,5	12,1055761	-2,3944239
0,231	0,343	15,8	14,4352938	-1,3647062
0,177	0,264	11,3	11,9732511	0,67325109
0,213	0,3	13,6	12,934629	-0,665371
0,197	0,278	12,5	12,5136423	0,01364227
0,197	0,278	12,2	12,5136423	0,31364227
0,197	0,288	12,8	12,6182407	-0,1817593
RMSE				0,9666641

Table 1. Hb level comparison of test data from Prodia and ZunZunSite3.

The BHL value of Prodia Kedoya will be compared to this value. The difference between two BHL values was used to get the RMSE (Table 1). The RMSE number is used to assess the precision of the data as well as the degree of error when measuring LED concentration.

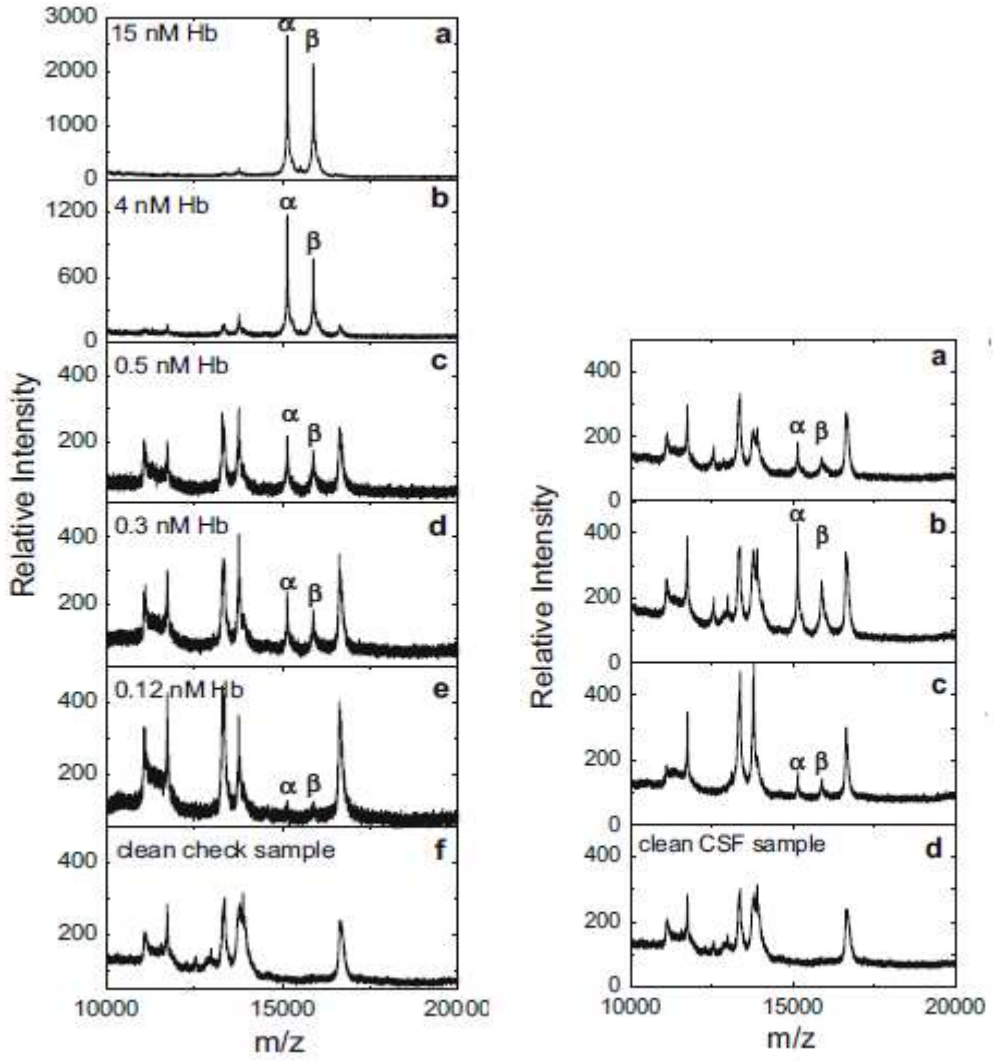

Fig. 2 Contamination of biological fluids with blood a) CSF UV spectra CSF (A–e) that has been contaminated artificially. The Hb a- and b-chains are represented by A and B. B) UV spectroscopic spectrum of CSF. Contaminated CSF samples (a–c)

Three of the 25 evaluated CSF samples (12%) had distinct Hb peaks, indicating that these samples had been contaminated with blood (Fig. 2, II, a-c). When utilising UV to compare identical contaminated CSF samples to clean CSF samples, only minor differences in Hb absorbance were identified, supporting the UV spectroscopic –based assay's greater sensitivity. The optimised assay was also used to test FF samples in order to establish its appropriateness for the study of other bodily fluids. In 31 of the 131 samples examined, Hb was found (24%).

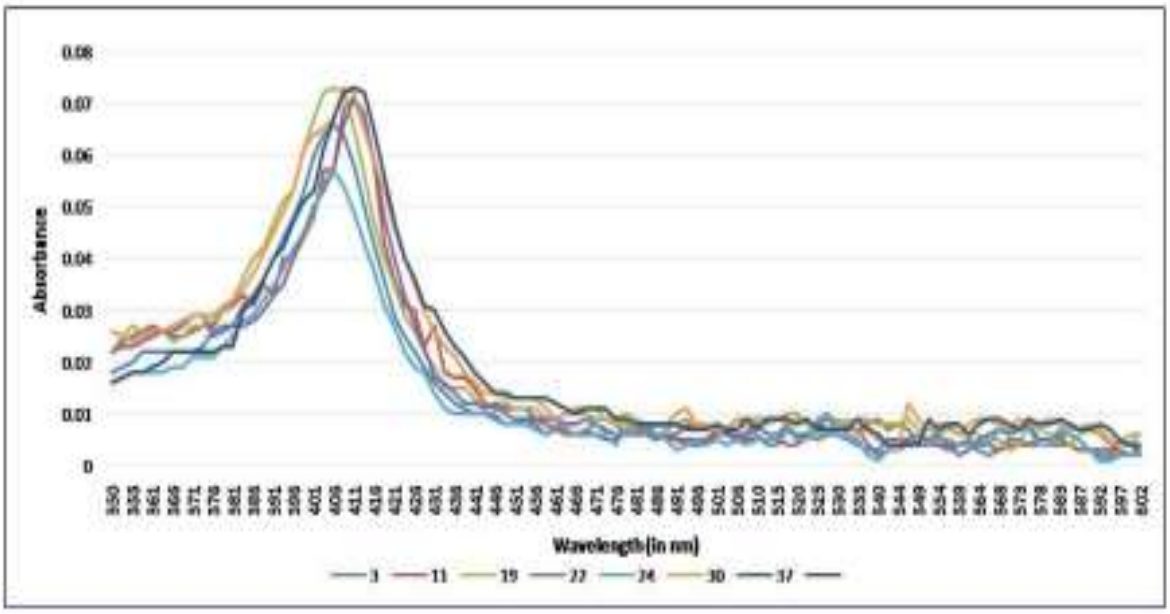

Fig. 2. The UV spectral spectrum of the best maintained samples

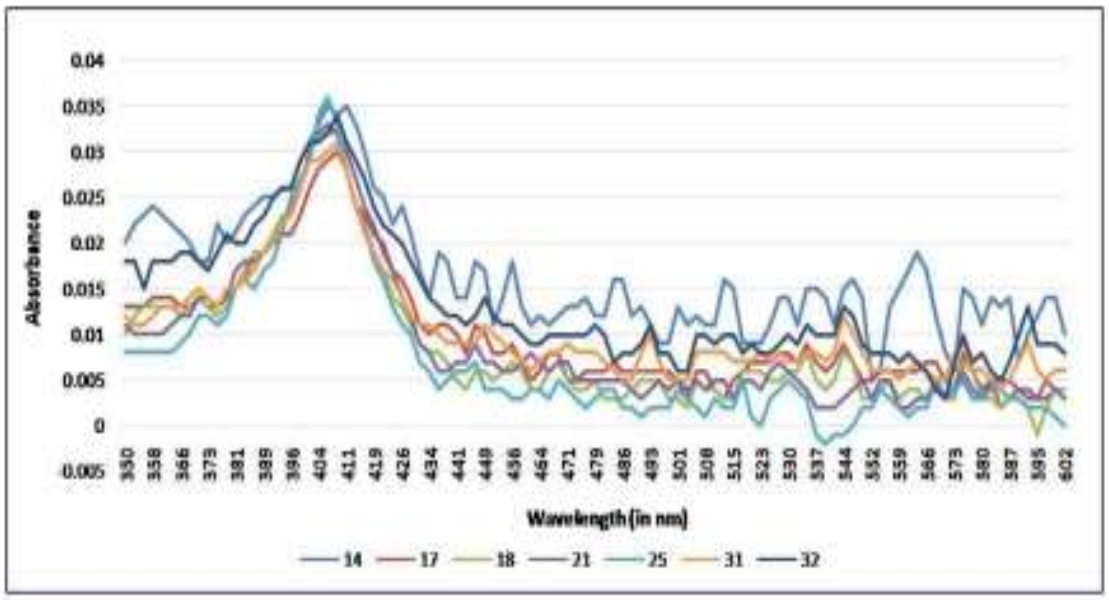

Fig. 3. The UV spectral spectrum of moderately preserved materials is presented.

Extra peaks in the Soret bands at 541 (Alpha) and 578 (Beta) that our investigation could not detect. Among the best-preserved samples, delta Abs Soret values range from 0.05 to above, with a delta lambda Soret peak about 410 nm (Fig. 2). The majority of the samples in this group were also notable for their reasonably smooth peak curvatureAt the delta lambda, the delta Abs Soret values vary between 0.03 and 0.05. According to spectral properties, soret peak around 410 nm in moderately preserved gauzes (Fig. 3).

CONCLUSION

In this paper, showed that a UV-spectrometry-based approach for detecting blood contamination is quick, sensitive, requires a small amount of sample (only 1 lL), and can be used on a variety of samples. As a result, we believe that this method can be used to quickly screen biological fluids for blood contamination prior to proteomic analysis, avoiding blood proteome interference and the danger of incorrectly interpreting analytical data . A variety of contamination sources must be considered when calculating the "normal values" of trace elements in human bodily fluids. It is only possible to compare concentrations in body fluids from unexposed and exposed people if all samples are obtained and prepared using the same methods.

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