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Trends of chemometrics in bloodstain investigations

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ABSTRACT

The potential to ascertain the origin and aging of bloodstains have various important applications in the forensic serological investigation. The individualization of bloodstain is necessary as both human and animals can be involved in the crimes. The foremost advantages of these types of investigation are: the expert significantly differentiates between human and animal blood which ultimately helps in the decipherment of criminal associated with the crimes; the aging of bloodstain assists in the determination of approximate time and date of incidence of crime.

In recent time, significant advancement in analytical methods has been done to investigate the complex evidence collected from crime scene such as bloodstain. Along with modern analytical methods, the multivariate methods are also obtaining popularity in forensic science, especially in the bloodstain investigations. This review emphasizes the importance of analytical and chemometric models that have been utilized for the identification and aging of bloodstains. The reliability and sensitivity of these models are compared.

1. Introduction

Biological fluids including blood, saliva, semen, urine, mucus, and sweat, etc. are generally found at the crime scene and can prove vital evidence in the forensic investigations. Among all body fluids, the most important and studied biological evidence is the blood. Its accurate examination is having great importance to the investigator due to its frequent existence at crime place and its individuality in spite of its complex structure [1].

Blood investigation is also helpful in the determination of the origin of blood i.e. whether it comes from human or animal. There are many preliminary chemical tests which are frequently used in biological/chemical laboratories to detect the blood samples such as phenolphthalein test [2], Kastle-Meyer test [3], and luminol reagent [4] etc. but these tests are destructive, don't give any confirmatory result and can react with environmental contaminants and animal/vegetable proteins etc.

Later on, Teichman and Takayama crystal test are established which provide a confirmatory blood test [5]. However, these tests are also destructive and do not differentiate between animal and human bloodstains. These problems are recently overcome by the development of advanced analytical methods such as Mass spectrometry [6], high-performance liquid chromatography (HPLC) [7], X-ray fluorescence (XRF) [8], Raman spectroscopy [9], Fourier transform infrared spectroscopy (FTIR) [10], UV–Visible spectroscopy [1], etc. which are successfully used for the examination of the bloodstains. These techniques coupled with chemometric methods further proved to be more reliable, accurate, sensitive and less time-consuming. The outcome of the investigation becomes more objective with statistical confidence and hence, easily acceptable in the court of law.

The main purpose of this review is to explain the potentiality of modern techniques and chemometric modeling for the individualization and dating of bloodstains. We have discussed the reliability of both destructive as well as non-destructive methodologies reported in the literature. However, in forensic perspective, the techniques which are non-destructive and require minimum samples preparation are highly preferred; yet, the destructive methods are still practiced in some divisions of forensic science laboratories where there is no such non-destructive techniques are available. The review also highlights the important chemometric methods used for the investigation of bloodstains followed by some vital considerations and future challenges that one should keep in mind before examining the bloodstains.

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Abbreviations

AFM	Atomic Force Microscopy
ANN	Artificial Neural Networks
ATR-FTIR	Attenuated Total Reflectance Fourier Transform Infrared
CCR	Correct Classification Rate
DNA	Deoxyribonucleic Acid
FTIR	Fourier Transform Infrared
GC-FID	Gas Chromatography-Flame Ionization Detector
GA	Genetic Algorithm
HCA	Hierarchical Cluster Analysis
HPLC	High Pressure Liquid Chromatography
IFRG	International Fingerprint Research Group
kNN	k-Nearest Neighbor
LDA	Linear Discriminant Analysis
LS-SVM	Least Squares Support Vector Machine
MLR	Multiple Linear Regression
MSC	Multiplicative Scattering Corrections
MSP	Microspectrophotometry
MALDI-MS	Matrix-assisted Laser Desorption/Ionization Mass Spectrometry
NIR	Near Infrared
NMR	Nuclear Magnetic Resonance
PCA	Principal Component Analysis
PCR	Principal Component Regression
PLSR	Partial Least Squares Regression
PLS-DA	Partial Least Squares-Discriminant Analysis
RBC	Red Blood Cells
RMSE	Root Mean Square Error
RPD	Residual Predictive Deviation
SIMCA	Soft Independent Modeling of Class Analogy
SNV	Standard Normal Variate
SPA	Successive Projection Algorithm
WBC	White Blood Cells

2. Blood and its composition

Blood is a fluid connective tissue having a complex mixture of cells, enzymes, and proteins. It is a continuously circulating fluid and provides the body with nourishment, O_2, regulation of hormones, help in homeostatic, major role in the immune system and elimination of waste materials from the body. It comprises 6–8% of body weight. The major part of blood consists of liquid, with proteins suspended and several cells in it, making blood "denser" than pure water. The pH of normal blood ranges between 7.35 and 7.45 [11].

Blood is composed of mainly blood plasma (the fluid in which corpuscles/fat globules are suspended) and blood cells (RBCs, WBCs, and platelets). Plasma is a liquid which forms about 50% of the blood content. It comprises proteins that are useful in clotting of blood, circulates substances through the blood. Glucose and other dissolved nutrients are some of the constituents of blood plasma. The serum is blood plasma without clotting factors; in other words, it is the liquid that separates from blood when a clot is formed. The half volume of blood comprises blood cells: Red blood cells (RBC), White blood cells (WBC), and Platelets [12]. The detailed classification of blood and its components are shown in Fig. 1.

2.1. Forensic significance of blood

Blood is the most valuable forensic evidence which can be recovered from the crime scene. The analysis of bloodstain patterns can help in the evaluation of the modus operandi of crime which ultimately facilitates the reconstruction of crime event. This type of information can lead the investigation to the right and fruitful direction.

The serological expert, through the examination of dimension, scattering, amount, and position of the bloodstains can postulate about possible causes of the crime. Such investigation uses the principles of physics (cohesion, capillary action, and velocity), biology (behavior of blood) and mathematics (geometry, distance, and angle) to assist the expert in answering the questions that help in the reconstruction of crime scene such as:

- Whether it's antemortem or postmortem injuries?
- Cause of the wounds?
- The direction of the wounded victim?
- Positions of the victim/s and perpetrator/s?
- A number of potential perpetrators present during the crime?

The type of injury inflicted decides the different ways of the blood coming out from the body. It can be in the form of a pool of blood, spurt, drip, spray, seep, gush or just exudation from the wounds. Earlier to the invention of DNA typing, blood stain is associated with a source by A-B-O typing for inclusion and exclusion of suspect/victim. These methods are now replaced by the new methods such as DNA technology. However, DNA technology is having its own disadvantages as the technique is quite expensive and examination of mixed blood stains is tedious and time-consuming, etc. The detailed layout of blood stain reconstruction is represented in Fig. 2.

In order to answer each and every query, new methods and technology are required for the detailed investigation of bloodstains. There are numerous research articles reported in the literature which explain the differentiation between human and animal blood. In the year 2015, F. Zapata et al. published a review based on examination of body fluids by spectroscopy techniques [13]. Earlier, the aging behavior of bloodstains is explained by measuring the changes in the chemical composition and physical morphology of blood components such as RBC, WBC and blood plasma [14–17]. R. H. Bremmer et al. published a review in 2012 that highlights such aspects of blood aging along with other analytical techniques [18]. Recently, a review on age determination of bloodstain based on spectroscopic methods has published by G. Zadora et al. [19]. However, many important aspects regarding blood classification and aging are not covered in the aforementioned reviews. Some of them are;

F. Zapata et al. have not discussed the basics of the statistical method used for the discrimination, classification and aging of bloodstain otherwise could help forensic experts who are not professional in the mathematical modeling/statistics. Moreover, the age estimation of bloodstain which is the frontline area of blood analysis has not been discussed here. R.H. Bremmer et al. haven't discussed the multivariate based statistical approaches used for the estimation of the age of bloodstains which can deliver more accuracy and repeatability. Similarly, G. Zadora et al. review has some limitations like (i) lacks in the explanation of basic chemometric methods which ultimately helps the non-professional experts in understanding of interpretation of their results based upon these modeling (ii) Classification based methods are not explained (iii) only spectroscopic methods are discussed, and not included the