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FLUORESCENCE ANALYSIS ON BLOODSTAIN

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Abstract:

Fluorimetry is one of the spectral analytical techniques, where the study of measurement of emitted radiation is carried out when electrons undergo transition from singlet excited state to singlet ground state. The fluorescence spectroscopy is versatile technique and it is mainly used for the forensics purposes. This technique rapid inexpensive and non-destructive technique. The most applicable use of this technique is determining the TSD of bloodstains. Thus, the future of fluorescent forensic analysis of bloodstains should focused on detailing out the intricacies of aging mechanism of peripheral and menstrual blood. This technique can be used in combination with other spectroscopic methods that analyses hemoglobin, which is primary component of RBC. Fluorescence is widely used in analysis than the phosphorescence. Fluorescence spectroscopy is rapid inexpensive and also used in forensic purposes also.

Blood is a complex biological sample, that is one of the most common body fluids collected during investigations of crimes. The analysis of blood stain can provide police with significant amount of information relevant to crimes. After the identification of blood stain DNA testing is carried out in order to find the victim or suspect. DNA is currently the primary use for blood stain analysis during forensic investigations as it provides most valuable information. By the use of spectroscopy, the usage of blood stains for forensic purposes increases tremendously. This technique is non - destructive, rapid and confirmatory methods for bloodstain identification.

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INTRODUCTION:

Fluorimetry is one of the spectral analytical techniques, where the study of measurement of emitted radiation is carried out when electrons undergo transition from singlet excited state to singlet ground state. [1]

When luminescence occurs, the system loses energy and if the emission is to be continuous, some form of energy must be supplied from elsewhere. Thus, the radioluminescence emitted from a luminous clock face is supplied by high energy particles from the radioactive material in the phosphor and the electrochemiluminescence of a gas discharge lamp is derived from the passage of an electric current through an ionized gas.

Fluorimetry characterizes the relationship between absorbed and excited photons at specified wavelength. There are mainly two manifestations of photoluminescence may constitute possible mechanisms whereby electronically excited molecules can lose energy. [2]

They are follows:

- Fluorescence
- Phospherescence

Fluorescnce:

When a beam of light is incident on certain substances, they emit visible light or radiations of longer wavelength than incident light. The energy of emitted radiations is lesser than that of incident or absorbed radiation because a part of energy is lost due to vibrational transitions. In fluorescence emission of radiation is instantaneous and also the materials emit excess radiation within 10^{-6} to 10^{-4} second of absorption. It lasts for shorter period. This occurs when there is transition from singlet excited state to singlet ground state. At room temperature most molecules occupy the lowest vibrational level of the ground electronic state, and on absorption of light they are elevated to produce excited states. Excitation can result in the molecule reaching any of the vibrational sub-levels associated with each electronic state.

Phospherescence:

The phenomenon where the emission of light is continuous by some compounds even when the incident light source is cut off is referred to as phosphorescence. In phosphorescence the emission of radiation is delayed, the materials emit excess radiation within 10^{-4} to 20 seconds. It lasts for longer period. This occurs when there is transition from triplet state to single ground state. When exposed to light of a shorter wavelength, a phosphorescent substance will glow, absorbing the light and reemitting it at a longer wavelength. Unlike fluorescence, a phosphorescent material does not immediately reemit the radiation it absorbs.

Fluorescence is widely used in analysis than the phosphorescence. [3] Fluorescence spectroscopy is rapid inexpensive and also used in forensic purposes also.

Blood is a complex biological sample, that is one of the most common body fluids collected during investigations of crimes. The analysis of blood stain can provide police with significant amount of information relevant to crimes. [4] After the identification of blood stain DNA testing is carried out in order to find the victim or suspect. DNA is currently the primary use for blood stain analysis during forensic investigations as it provides most valuable information. By the use of spectroscopy, the usage of blood stains for forensic purposes increases tremendously. This technique is non - destructive, rapid and confirmatory methods for bloodstain identification. Fluorescence spectroscopy can investigate the endogenous fluorophores within blood stain at trace deposition amount.

Additionally, determining the time since deposition (TSD) can assist investigators in establishing when the crime occurred. [5] Bloodstain collection and analysis can provide useful information to investigation. Spectroscopy as a means of detecting, differentiating, and estimating the age of bloodstains. Many techniques for the identification and analysis of blood are destructive, highly subjective, and only provide presumptive results. [6] Fluorescence was shown to be a promising technique to evaluate the age of a bloodstain in a crime scene within first week. This method is fast (approximately 20 min), reproducible, concentration independent and requires only minimum amount of bloodstain with minimum sample preparation. [7]

Principle of fluorimetry:

Absorption of UV or visible radiation causes transition of electrons from singlet groundstate to the singlet excited state. As this state is not stable, it emits energy in the form of UV or visible radiation and return to singlet ground state.[8] Principle involved in fluorimetry Is understand by the basic electronic states

Singlet ground state:

A state in which all the electrons in a molecule are

paired

Double state:
A state in which unpaired electron is present. e.g., free radical

Triplet state:
A state in which unpaired electrons of same spin are present.

Once a molecule has absorbed energy in the form of electromagnetic radiation, there are a number of routes by which it can return to ground state. The following graphic, termed a jablonski diagram.

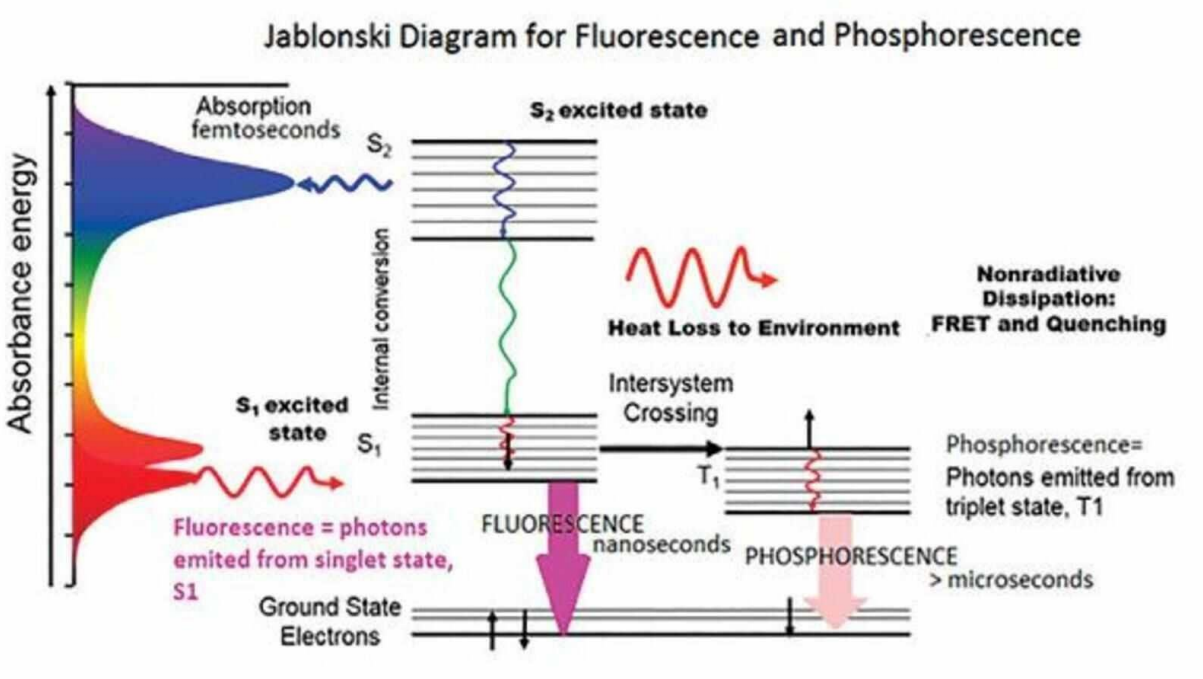


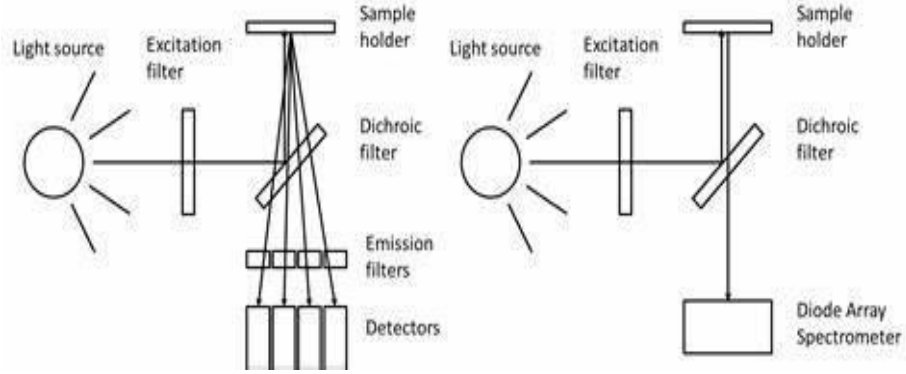
FIGURE 1. (JABLONSKI DIAGRAM)

Fig.1 shows the Jablonski diagram, a schematic of the transition of electronic state of a molecule during the fluorescence phenomenon.

The process that happens between excitationand emission is illustrated using Jablonski diagram (named after the father of fluorescence spectroscopy, Alexander Jablonski). The left axis shows increasing energy, where a typical fluorescent molecule has an absorbent spectrum. This spectrum shows the energy or wavelength, where the molecule will absorb light. In conventional fluorescence, photons are emitted at higher wavelengths that the photons are emitted that are absorbed. If the incident light is at a wavelength where the molecule will absorb the photon, the molecule is then excited from the electronic ground state to a higher excited state, denoted S_2 here.

A Jablonski diagram is basically an energy diagram, arranged with energy on a vertical axis. The energy level can be quantitatively denoted, but most of these diagrams use energy levels schematically. The rest of diagram arranged into columns. Every column usually represents a specific spin multiplicity for a particular species. Some diagrams divide energy levels within the same spin multiplicity into different columns. The first transition in most Jablonski diagrams is the absorbance of a photon of a particular energy by the molecule of interest.

This type of photoluminescence occurs when there is a long delay between the excitation and emission of light. A long delay means about 10^{-6} seconds or longer. When the delay between excitation and emission is shorter, the result is fluorescence.



Fluorescence on the other hand, has a quick flash of emission (around 10 nanoseconds, but sometimes shorter than 1 nanosecond). Because of this, sophisticated electronics and optics arenowadays used to detect and measure fluorescence. [9]

As soon as the energy input from the photon stops, the fluorophore molecule relaxes into lowest vibrational level of the excited electronic state. The fluorophore remains in this state for some time and then returns to electronic ground state. This return to ground state is associated with the release of energy known as fluorescence emission. [10]

Instrumentation of fluorescence spectroscopy:

Fluorescence spectroscopy is a type of electromagnetic spectroscopy that analyses fluorescence from a sample. It involves using a beam of light, usually ultraviolet light, that excites the electrons in molecules of certain compounds and causes them to emit light. A complementary technique is absorption spectroscopy. In the special case of single molecule fluorescence spectroscopy, intensity fluctuations from the emitted light are measured from either single fluorophores or pairs of fluorophores.

There are basically two types of fluorescence spectroscopy instrument configurations

- Transmissive
- Reflective

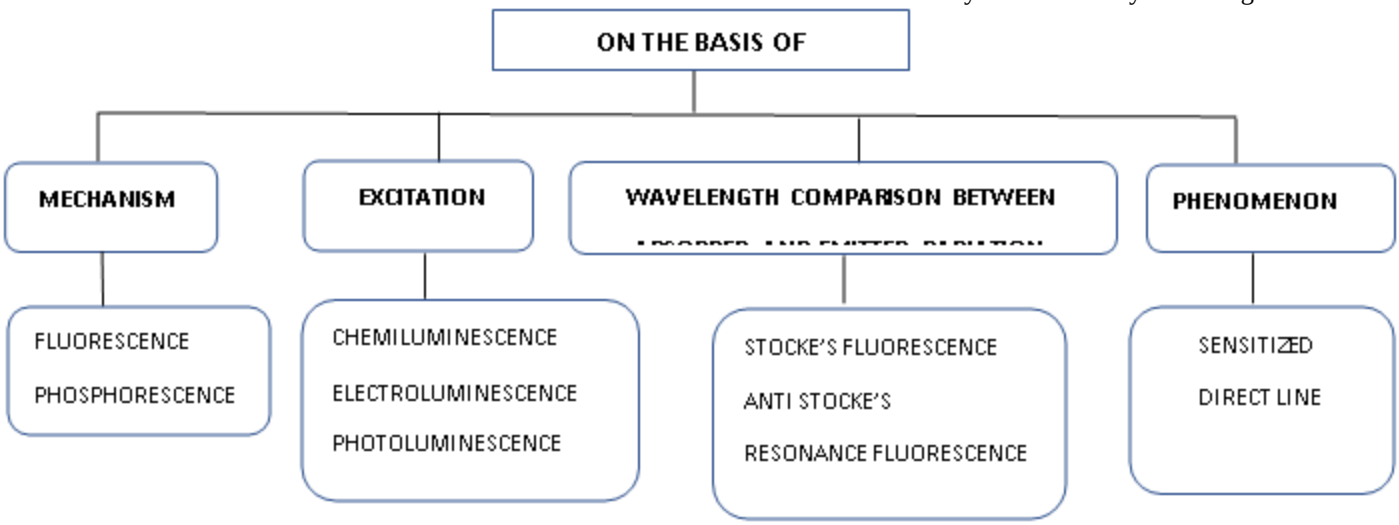
FIGURE:2 (FLUORESCENCE INSTRUMENTATION)

The only real difference between the two that in the reflective version a dichroic mirror is used to direct the excitation light to the sample and collect the emission light from the sample whereas in the transmission configuration the sample is excited directly from the light source and the emission light is collected at an angle of 90 degree from the excitation light.

A fluorescence instrument consists of the following basic components:

- Light sources
- Excitation filters
- Sample holder
- Dichroic mirror (in reflective configuration)
- Emission filters

course the flexibility to choose any wavelength but



- Detectors

Light sources:
Almost all type of light source can be used for fluorescence spectroscopy so the best choice depends on the actual requirements to spectral wavelength coverage, intensity, size, cost, efficiency and whether the light source needs to pulsed.

In general LEDs (light-emitting diodes) are a great choice if want to be build a low cost, compact fluorescence instrument for analysing a limited number of known fluorophores. Pulsed table top lasers are often the preferred choice for accurate time resolved measurements with nanosecond timing. Broadband sources are the best choice for the flexibility to analyses a large set of unknown fluorophores.

Next light source that is used in fluorescence spectroscopy is tungsten-halogen. They are wavelength about 320-2500nm and the UV and visible light range is emitted from one bulb. Its intensity is low. It is easy to replace. And it is more uniform intensity than arc-lamps. It became relatively expensive. Its intensity can be too low to image weak fluorophores. At high temperature the wavelengths emitted are shorter.

Excitation filters:
Variable optical band pass filters are mostly realized as scanning grating monochromators and are used together with broadband light sources to select the right excitation wavelength. The main benefits are of the drawback is a high cost, large size, need for electronics control and stability issues due to the moving parts inside the monochromator.

Sample holder:
The choice of sample holder really depends on the application. The main thing to consider is that in the case a transmissive cuvette or flow cell you must ensure that the sample holder material is transparent for both your excitation and emission wavelength. This is especially important for UV wavelengths where most glasses absorb light so special types of materials needs to be used**.**

Dichroic filter:
The dichroic filter is used in a 45-degree configuration. The function of the dichroic filter is to reflect the excitation light and transmit the emission light. For really simple systemsthe dichroic filter can actually function as both the excitation and emission filter.

Detectors:
In fluorescence spectroscopy it is common to use Photo Multiplying Tubes (PMT) as detectors due to the high sensitivity and fast response of these detectors. Silicon-based solid- state detectors can also be used. The number of detectors needed depends on the system configuration. If you are using a variable band pass filter (like a monochromator) you only need one detector. If you are using fixed bandpass filters, you need one detector per filter

Type of flourescence:
It is classified:

Classified by mechanism:

Fluorescence:

- Fluorescence is a type of radiative emission that occurs when a molecule absorbs energy at a wavelength where it has a transition dipole moment.
- The excitation energy provided to the molecule at the ground state promotes photons to an excited singlet state, when they then decay to the lowest vibrational energy level of this excited singlet state.

Phosphorescence:

It is a type of photoluminescence related to fluorescence.

- When exposed to light of a shorter wavelength, a phosphorescent substance will glow, absorbing the light and reemitting it at a longer wavelength. Unlike fluorescence, a phosphorescent material does not immediately reemit the radiation it absorbs.
- Instead, a phosphorescent material absorbs some of the radiation energy and reemits itfor a much longer time the radiation source is removed.
- e.g., of phosphorescent materials include glow-in-the-dark stars, some safety signs andglowing paint.

Classified by excitation:

Chemiluminescence:

- Excitation by chemicals.
- It describes the emission of light that occurs as a result of certain chemical reactions that produce high amounts of energy lost in the form of photons when electronically excited product molecules relax to their stable ground state.
  e.g., glowsticks, the vibrant party favours are also a form of chemiluminescence.

Electrochemiluminiscence:

Electroluminescence is a phenomenon through which a substance emits light becauseof an electric current/field being induced.

- Electrochemiluminescence (ECL) sensors are a combination of electrochemistry and measurement of visual luminescence.
- When a potential is applied onto an electrode, the electrode surface is excited. So, an electron transfer is produced between molecules, and resulting emitted light is measured.
- Materials widely used to build ECL sensors could be organic molecule e.g., luminol, inorganic complexes, semiconductor nanocrystals, nanoparticles.
  e.g., powdered zinc sulphide doped with copper (producing greenish light) or silver (producing bright blue light).

Photoluminescence:

- Excitation by electromagnetic radiation. [13]
- Photoluminescence is light emission from any form of matter after the absorption of photons.
- It is one of many forms of luminescence and is initiated by photoexcitation.
- It is mainly used for measuring the purity and crystalline quality of semiconductors.

Based on wavelength of emitted radiation when compared toabsorbed radiation:

Stocke's flourescence:

- The wavelength of the emitted radiation is longer than the absorbed radiation. e.g., conventional fluorometric experiments.

Anti stocke's fluorescence:

- The wavelength of emitted radiation is shorter than the absorbed radiation. e.g., Thermally assisted fluorescence

Resonance fluorescence:

- When the wavelength of the emitted radiation is equal to the absorbed radiation. e.g., Mercury vapour at 254 nm.

Based upon the phenomenon:

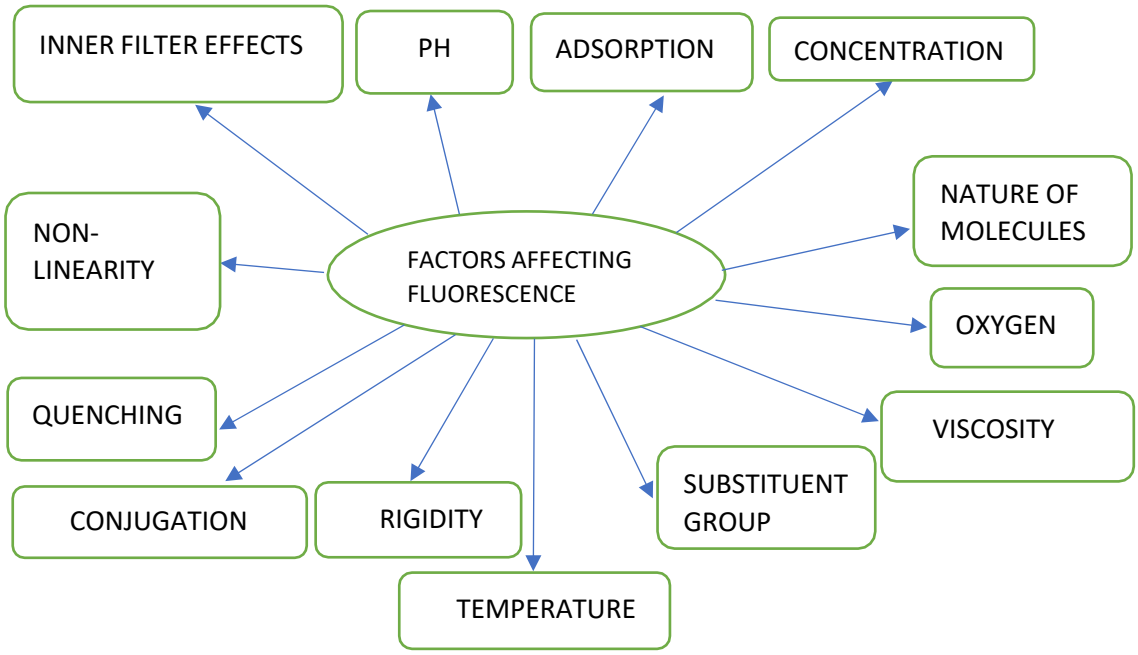
Sensitized fluorescence:

- When elements like zinc, cadmium or an alkali metal are added to mercury vapour, theelements are sensitized and thus give fluorescence.
- Sensitized fluorescence can be defined as the increase of the intrinsic fluorescence of an organic compound after formation of chelates capable of participating in energy transfer mechanism

Direct line fluorescence:

- Where, even after the emission of radiation, the molecules remain in metastable state and finally come to ground state after loss of energy by vibrational transition, with respect to atomic fluorescence spectroscopy the fluorescence that is emitted by an atom at a spectral line of a wavelength that is longer than that of the stimulating radiation.

Factors of fluorescence:
There are certain factors which are affected the fluorescence are given below:



Conjugation:

- Molecule must have unsaturation that is it must have π electrons so that UV or visible radiation can be absorbed.
- If there is no absorption of radiation, there will not be fluorescence.
- The π conjugation length affects the fluorescence emission efficiency is elucidated by examination of the theoretical and experimental relationship between absolute quantum yield and magnitude of the pi conjugation length in excited singlet state.
- Molecules with a greater extent of conjugated double bonds absorbs greater amount of light, thus causing more intense luminescence.[15]
- Molecule must have unsaturation, absorbed radiation due to presence of π electrons, that produces fluorescence.

Rigidity of structures:
Rigidity structures will produce more fluorescence, while flexible structure willproduce less fluorescence

- High degree of flexibility will trend to decrease the fluorescence.
- More rigid structure has lower collision, thus have more fluorescence potential.
- Photoluminescent compounds are those compounds in which the energetic levels within the compounds favor de-excitation by emission of UV-visible radiation rather than by loss of rotational or vibrational energy.
- Fluorescing and phosphorescing compounds usually have a rigid planar structure.
- The rigidity of the molecule prevents loss of energy through rotational and vibrational energetic level changes.
- Any subsistent on a luminescent molecule that can cause increased vibration or rotation can quench the fluorescence.
- Organic compounds that do luminesce generally consist of rings with alternative singleand double bonds between the atoms.

Nature of substituent group:

- Electron donating groups like amino, hydroxyl groups enhance fluorescenceactivity.
- Electron withdrawing groups like nitro, carboxylic group reduce fluorescence. Groups like SO_3H or on NH_4^+ have no effect on fluorescence intensity.
- Electron donating group like NH_2, OH which enhances fluorescence intensity
- Electron withdrawing group like NO_2, COOH which reduces fluorescenceintensity

Effect of temperature:

- Increase in temperature leads to increase in collisions of molecules and decrease in fluorescence intensity while decrease in

temperature leads to decrease in collisions of molecules and increased fluorescence intensity.

- Intensity of fluorescence is inversely proportional to the increasing of temperature.
- increasing temperature a 1/F.

So, increasing temperature leads to increase collision of molecule

Decrease F1

Decreasing temperature, leads to decrease collision of molecule

Increase F1

.

Chemical composition of blood:

Blood is a significant component of human body. Almost any animals that possesses a circulatory system has blood. Blood is a fluid connective tissue that consists of plasma, blood cells and platelets.

Blood is composed of two components that is, blood plasma and formed elements. Blood is about 45% formed elements and 55% blood plasma. Formed elements composed of red blood cells, white blood cells and platelets. RBC and WBC are whole cells, platelets are cell fragments. The other biochemical components of blood such as proteins, nucleic acids, lipid and carbohydrates.

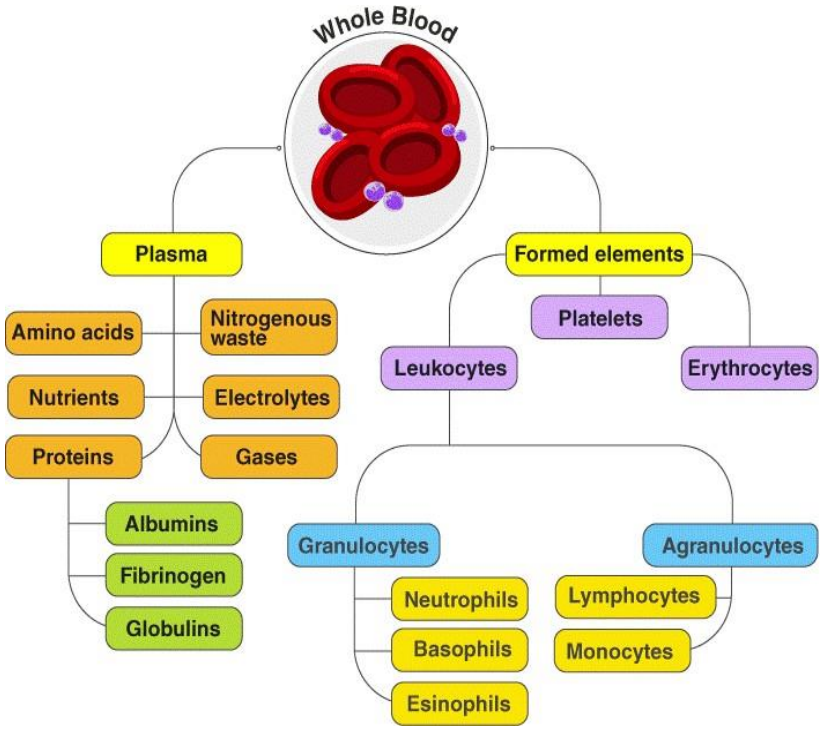


FIGURE:3 (COMPONENTS OF THE BLOOD)

Red blood cells constitute approximately 50% of whole blood and are responsible for transporting oxygen from lungs to rest of body. Red blood cell does not contain any DNA due to lack of nuclei, and they mostly composed of hemoglobin, a protein is responsible for the transport of the oxygen. RBC lack mitochondria and generate ATP anaerobically; they do not use up any of the oxygen they transport. [19] The process by which the formed elements of blood develop is called hemopoiesis.

Fluorescence spectroscopy for blood stain analysis:

After the discovery of fluorescent spectroscopy in early 19th century it was applied to the analysis of bloodstains. In 1985, Wolfbeis and Leiner utilized fluorescence spectroscopy to characterize human blood serum. The human sera were collected from human donors, by isolation from the red and white blood cells, and then diluted in a phosphate buffer.

Using a range of excitation and emission wavelengths, they were able to create a full topographic profile of human serum.

Topographic approach to characterizing the fluorescence profile of serum proved to be a useful technique for the analysis of blood by the identification of the fluorophores.

Wolfbeis and Leiner also noted additional uses for this method. As fluorescence spectroscopyis a highly sensitive technique, the location and relative intensity of the peaks are dependent on a donor's health status. Therefore, they hypothesized that by measuring the total fluorescence spectra of blood serum scientists could distinguish between healthy and non- healthy donors. As the blood serum is a complex matrix, the evaluation of the fluorescence between by the human eye.

The analysis of blood is a complex ordeal, due to the large number of components and their variable fluorescent intensities. Unless each component of blood is isolated and analysed, it is improvable that a complete fluorescent profile can be obtained. This is because fluorophores with lower fluorescent intensities can be masked by the stronger components.

This occurs both when analysing human serum or whole blood.
Menstrual blood (MB) is a common and important type of forensic evidence, especially in sexually assault cases. Menstrual blood is composed of peripheral blood (PB), vaginal fluid and endometrial cells of the uterine wall. In forensic investigations, the differentiation of MB and PB can determine whether the blood present is a result of tissue damage from an assault or a natural cause and thus help to reconstruct the event.

Fluorescence spectroscopy, a promising spectroscopic method for bloodstain analysis, was used to probe the biochemical changes that occur over time in menstrual bloodstains. It was found that steady-state fluorescence spectra underwent significant changes over first nine hours post deposition. The underlying mechanism of fluorescence changes was proposed to involve the kinetic transformation of three fluorophores: tryptophan, nicotinamide adenine dinucleotide and flavins.

Li et al was done that the measured that the excitation and emission spectra of whole human blood and determined that the endogenous fluorophores were tryptophan, NAD(P)H and FAD. Their maximum wavelengths for the emission and excitation spectra of these components were consistent with those experimentally measured by Wolfbeis and Leiner. Using the spectral characteristics of these fluorophores, researches have been able to perform more in-depth analysis of blood. Fluorescence spectroscopy to determine the time since deposition of bloodstains.

Time since deposition:
Steady state fluorescence:
Steady state fluorescence spectroscopy is a versatile analytical chemistry technique capable of characterizing the components of the whole blood. During forensic investigations there are several crucial questions that need to be answered to assist investigators. A primary question being how long a bloodstain has been at a scene. This has been termed the time sincedeposition (TSD) of a stain. TSD can aid in crime scene reconstruction, witness statements and assist investigators to determine which stains are relevant to the crime.

Using steady state fluorescence spectroscopy, Weber et al created a fluorescent profilefor human peripheral blood up to 24hour deposition. Samples were collected and fresh for 6 trials to ensure that there is no contamination or additional chemicals present and to have control over the time of bleeding.

It was determined that the fluorescence spectra of each fluorophore in blood that showssignificant monotonic changes over the first 24hour post deposition. These intensity changes were consistent in both peripheral and menstrual blood and included a decrease of tryptophan peaks and an increase of NADH peaks over time. The flavin peaks presented a more complicated trend, a two-stage process that first involved a decrease in the intensity during first3hours, followed by a large increase in the remainder of aging trail.

Fluorescence lifetime measurements:

- Fluorescence lifetime spectroscopy is used to measure the lifetime of excitedstates. Within the blood, tryptophan is a prominent amino acid that is present in the two proteins albumin and γ-globulin.
- These proteins account for over 95% of the protein mass within whole blood.
- The fluorescent lifetime of tryptophan is well documented, and the amino acid is sensitive to changes to the protein conformation.
- Therefore, when conducting fluorescence

lifetime measurements, tryptophan was determined to be ideal for measuring changes to blood overtime.

- The Berezin research laboratory were the first to utilize fluorescent lifetime measurements to determine the TSD of blood.

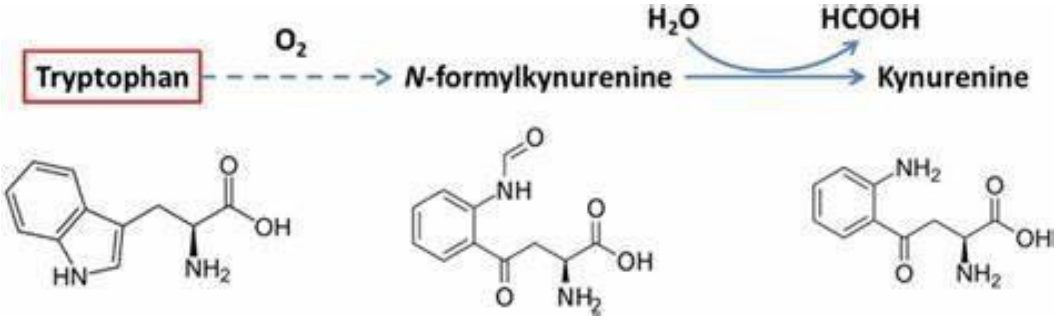


FIGURE:6 (OXIDATION OF TRYPTOPHAN), A fluorescent compound is marked withred.

Guo et al. reported on the capabilities of fluorescence lifetime spectroscopy in 2012. Blood samples were collected from different species of dogs and deposited into petri dishes to age under ambient conditions. Samples were prepared by prepared by collecting a portion of dried blood and dissolve it in phosphate buffer saline solution. For spectroscopic measurements 295nm sub nanosecond pulsed LED in conjunction with the time-correlated single-photon counting (TCSPC) technique was used to measure the lifetime of tryptophan. Tryptophan in fresh blood has a fluorescent lifetime of approximately 4ns, which then declined during the aging process, decreasing down to approximately 2.7ns after 2 weeks post deposition. After concluding that the technique promises for determining the TSD of bloodstains. This method was found to be rapid, reproducible, and non-dependent on concentration.

In 2013, Guo et al continued his work for the analysis of bloodstains with fluorescence spectroscopy to show that this technique could be utilized for different species. Isolated human, rat, mouse was used for the analysis. Additionally whole blood and isolated hemoglobin werealso required for fluorescence lifetime measurements. In testing samples from several species,they determined all the blood showed comparable optical properties, which indicated that this method for estimating the TSD of blood can be used not only on human blood, but other speciesas well.

Work done by Guo et al. primarily utilized bloodstains and isolated albumin from non- human species. For fluorescent lifetime measurements to be usable for forensic purposes, it was necessary to show that this method will work with human bloodstains.

Research by Mc shine et al. investigated the applicability of fluorescence lifetime measurements to determine the TSD of biological stains, with a focus on blood. Peripheral blood was collected from 6 human donors, into tubes that were not treated with anticoagulants. The samples were then deposited onto petri dished and left to age under ambient conditions. Samples were prepared by placing the blood into tube of PBS to create a blood solution. By the studies of previous works, it was determined that the fluorescence lifetime of tryptophan decreases during the aging process. During the first 91-hour post deposition the fluorescence lifetime decreased at an exponential rate, after which it plateaued. This allows for a clear distinction between fresh and old bloodstains, which can be crucial information to know during forensic investigations.

Further species identification work was later conducted by Gan et al, in 2019, where they used a deep belief neutral network model to classify blood from doves, chickens, mice and sheep.

Beyond the ability to estimate the TSD of blood, fluorescence spectroscopy, when paired with advanced statistical analysis, can discriminate between different species of donors. Fluorescence lifetime measurements studies show the potential for this technique to determine the TSD of bloodstains for forensic purposes. Steady state fluorescence spectroscopy has been used to identify the components within the blood. Of the research done thus far, most of themwere proofs of concept works. Thus, by increasing the number of samples tested for each form of analysis, the reliability and reproducibility of the methods could be solidified.

Phenotype research:

- The capabilities and uses of fluorescence spectroscopy have been greatly expanded upon since its discovery.
- Beyond the ability to estimate the TSD of blood, fluorescence spectroscopy, when paired with advanced statistical analysis, can discriminate between different species donors.
- In 2017, Lu et al. utilized support vector machine discriminate analysis (SVMDA) to differentiate between human and non-human blood.
- They collected samples from four species of animals (pigeon, chicken, mouse, and sheep) and using unique features of the spectra, selected by SVMDA, were able to identify human verses non-human whole blood with 100% accuracy.

Limitations of current work:

- Fluorescence spectroscopy is a sensitive technique capable of analysing minute traces of bloodstains.
- Steady-state fluorescence spectroscopy has been used to identify the components within the blood.
- Thus, by increasing the number of samples tested for each form of analysis, the reliability and reproducibility of the methods could be solidified.
- Fluorescent spectroscopy has been widely used to characterize dissolved organic matter (DOM) in engineering systems, such as drinking water, municipal waste water and industrial water treatment.
- By plotting the changes of intensity of the fluorescence peaks over time, a kinetic curve was able to calculated for the fluorophore's tryptophan and NADH understand the aging mechanism of peripheral and menstrual blood.
- These techniques have shown a strong capacity for analysing bloodstain. Also, this technique able to provide the probative information about the blood traces to investigators, such as the TSD of a stain.
- The main limitation of fluorescence spectroscopy in bloodstain is specificity. The limited specificity of fluorescence spectroscopy is highlighted in the analysis of flavins, the group of molecules composed of FAD, FMN and riboflavin.

CONCLUSION:

The fluorescence spectroscopy is versatile technique and it is mainly used for the forensics purposes. This technique rapid inexpensive and non-destructive technique. The most applicable use of this technique is determining the TSD of bloodstains. Thus, the future of fluorescent forensic analysis of bloodstains should focused on detailing out the intricacies of aging mechanism of peripheral and menstrual blood. This technique can be used in combination with other spectroscopic methods that analyses hemoglobin, which is primary component of RBC. Bloodstains discovered from the crime scenes can provide crucial information to an investigation. Fluorescence spectroscopy analysis of bloodstain is discussed by with steady state fluorescence and fluorescence lifetime measurements. Transition of electronic state of fluorescence was described by Jablonski diagram. Mainly it is used in forensic, when the crime occurred, the bloodstain present is related to the investigated event.

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