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Hydrogen isotope: An ideal tracer for forensic examination

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Abstract--Stable isotope analysis is a valuable tool in forensic investigation. Recently, isotopic investigation is in great trend, Forensic discipline is making use of multi-purpose isotopic profiles along with isotopic landscapes or isoscapes from body tissues. These isotopes help in predicting the geographical location or origin of the unidentified body. Isotope analysis is a commendable and powerful tool for geolocation and can provide investigative leads to the investigating officer as well as forensic experts. This review article basically focuses on the hydrogen isotopes and their applications in forensic science. Being a bio-element, it is ubiquitous and has immense biological and chemical significance. We have discussed the

role of hydrogen isotopes in different disciplines of forensic science such as forensic biology, wildlife forensics, forensic anthropology, forensic chemistry and toxicology etc. and the newer advancements that are employed in this discipline for making the analysis more accurate and robust. This field is still in its growing stage and hence, with more advancements, it will provide a great aid in forensic investigation.

Keywords---Stable isotope analysis, hydrogen isotopes, Forensics, geolocation.

1. Introduction

Forensic Science is a discipline which deals with the analysis and investigation of the suspected evidences encountered from the crime scene. The key concern of forensic science is to determine the degree of relation between the materials that seem identical and to collect the information regarding the origin of that particular evidence [1]. In case of any disaster, say a train crash DNA is the imperative tool for victim identification. Evidences like dental records, fingerprints etc. are checked and matched. The major drawback of DNA evidence is that it gets decomposed thus, reducing the integrity of the samples [2]. The major challenges faced by the analyst is when the recovered body is hampered or decomposed badly. This is the drawback in establishing the identity of the victim. Other distinguishing features which aid in the identification of the affected person is the personal belongings like jewellery, tattoos clothing, birth mark etc. [4]. By analysing the current scenario, it seems necessary to implement some screening tools for better identification of human beings [4]. Stable isotope analysis is acting as a promising tool in environmental, biological, ecological and geochemical fields [1, 10, 3].

The never advancement in forensic scenario is the implementation of stable isotope analysis. It is rapidly expanding technique and is used in forensic investigation and law enforcement because of its analytical approach. This technique can complement with other analytical techniques for chemical investigation [10]. Geo-location and diet of an individual influences the composition of isotopes inside the human tissues like hairs, nails, bone and teeth. Therefore it is commendable technique for human identification. Isotopic profiles of human beings can help in providing the information regarding the life history of an unidentified human being but it all depends upon the tissue available for the analysis [4]. Stable isotope analysis provides an additional fingerprint that further characterises the evidence encountered in forensic science. It is helpful in analysing the two materials having exactly same chemical composition and figure out the relativeness and differences b/w the two [15]. The ability of an instrument to measure the samples small differences and to relate that composition with that of suspected sample is the basic function of stable isotope analysis in forensic studies [13]. Unlike other evidences like Dental records, DNA fingerprints etc. the isotopic profiling does not rely on the individual specific database, it focuses on comparing the isotopic profile of one tissue with another one or it helps in figuring out the pattern of the pattern of isotope

variation, in different populations, one might infer the information regarding the individual itself for identification [4]. Stable isotope analysis is becoming common in forensic field because in addition to collection of evidences, it provides stable isotope fingerprint [1]. Moreover, it complements other applications by the addition of “Stable isotope signature” to the evidences (chemicals) identified by other methods. The main application of stable isotope ratio is sample matching with the database, sample preparation and source location [13,10]. The only condition is comprehensive databases of samples of known origin should be preferred and if fractionation groups are well known [13].

The stable isotopes that are usually employed for isotopic profiling of human remains are C, H, N, O and S. In some cases, heavy elemental metal isotopes are also helpful in providing valuable information. Some radiogenic isotopes like Sr or Pb are having high demands in the fields of archaeology and forensics [4]. National Research Council (NRC, 2009) had presented 13 recommendations for easy assessment and investigation and to overcome the challenges faced by forensic investigators during analysis in USA. Stable isotope analysis technique was not mentioned specifically in NRC, 2009 report but the stable isotope fingerprint of any sample is a unique characterization of the material and hence, it plays a significant role in analysis and investigation of forensic evidences [1].

The biological samples recovered from the same place or geographical location and at same time will have identical environmental conditions and hence, will have similar ratio for stable isotopes. Whereas, chemical processing of the material can lead to different ratio of stable isotopes because the impurities are left behind during the chemical procedure and production of derived products [10]. Stable isotopes are present in various biological materials like human hair, teeth, bones, fingernails etc. and also in drugs as well as food [10]. The concentration of these light stable isotopes (C, H, N, O, S) may vary in dietary items among the biomass in which an individual is residing [7]. This can lead to significant difference in the isotopic composition of people living in different parts of the world.

1.1 Application of Hydrogen Isotopes in Forensic Sciences

Hydrogen is one of the “bio-element” which is responsible for the making of majority of living organisms [16]. Hydrogen isotopes act as an aid in identification and understanding of chemical and biological procedure. These isotopes have immense properties of ideal tracer nuclides. As compared to carbon isotopes, hydrogen isotope labelling is quite faster, easier and is cost effective [17]. The major source of hydrogen in human body is water that is consumed through liquid intake, foods such as fruits and vegetables [4]. The stable isotopic composition of hydrogen in the human or other organic or biological materials is used for environmental, biosecurity, ecological and forensic applications etc. [3].

1.1.1 Forensic Biology:

Biological samples like hair, nails, urine, are analysed by stable hydrogen isotope technique. The isotopic composition of water consumed by any human differs geographically. The stable oxygen and hydrogen composition of these biological

samples reflect the variation in geographic location. Thus, providing the history of geographic movement of an individual [9, 14].

1.1.1.1 *Hair and Nails:*

Human hair has many potential implications with movement and in specifying regions of origin in different disciplines such as forensic science, anthropology etc.. In forensic investigations, this spatial variation is helpful in estimating the geo-location of unidentified humans. Investigators, medical examiners sometimes stuck in situations when they have no identity of victims. In that case, they can make use of spatial isotope ratio to evaluate the relation of suspected forensic material is isotopically consistent with the reference (individual of that origin) [25].

On the basis of tracer experiment [8] it was stated that nearly 30% of hydrogen is stored in human hair which is directly derived from the consumed water. Therefore, hydrogen and oxygen both are invasive source of information as isotopic composition of human hair and nails is having high correlation with that of drinking water [4]. Several studies have been conducted and they have suggested that the variation in isotope ratio can help in distinguishing the individuals on the basis of their geographical origin. Both hair and nails can provide record of dietary and information of water source and hence aid in detecting the geographic information for forensic investigation. Fingernails comprises of keratin, which is also found in hairs [10]. It has been proved that citizens from different geographic origin like England, Brazil and the United States can be individualised on the basis of isotope ratios of fingernails. The isotopic study conducted on the hair samples revealed that the hydrogen extracted from the keratin of human hair is derived majorly from food hydrogen and in minor quantity from the drinking water. Furthermore, in the case of archaeological hairs, there is a close link established between the hair and the local meteoric water in humans that do not migrate. Hydrogen isotope analysis aids in establishing link between paleo-environmental and paleodietary conditions by resolving daily to weekly isotopic signals in mammals and associating them with respect to the water and food intake [8].

Studies have revealed that the isotopic values of both nails and hair varies in the same individual. Suggesting that the nail keratin takes longer time for its formation as compared to human hair keratin [19]. Hydrogen isotopes are used specifically for analysing hair and nail keratin because $\delta^2\text{H}$ values of precipitation differs across geographical location. Moreover, serial sampling of hair can help in fetching information regarding the travel history of an individual with the use of isotopically different regions [19]. Based on the isotopic composition of 4 elements in fast growing tissues like hair and nails, forensic investigators are able to establish the victim's life history 200 days prior to death [14]. USGS42 and USGS43 are the first stable isotopes of hydrogen and oxygen. The determination of accurate isotopic composition of human hair along with the keratin present in it is complicated by the fact that human hair comprises of exchangeable hydrogen. It is a challenging task to measure same homogeneous sample in all the laboratories for yielding similar isotopic results. There was no internationally distributed stable hydrogen and oxygen reference material in isotopic form for human hair, so the US geological survey had made two such materials namely

USGS42 and USGS43 so that all the laboratories dealing with human hair worldwide can yield similar isotopic composition within analytical uncertainty [9]. By using multi-isotopic analysis, one can get more information but analysing more than one isotope at a particular time is quite challenging task because of limited amount of requirement. Secondly, by analysing multiple hair strands, the values obtained are at risk of buffering and might get delayed due to different growth histories of an individual's hairs as the hairs do not grow at same rate. Hydrogen and oxygen isotopes are used for reconstructing the movements and provenance. The geolocation using hydrogen and oxygen isotopes in conjugation do not show higher variability because they both have low spatial resolutions and in some cases, the value is similar across different parts of the world. Therefore, other isotopes like Strontium can be combined with hydrogen and oxygen isotopes to enhance the resolution of geolocation [21]. A study was conducted in USA in which 3 hair clippings which were discarded were collected from 18 states. Irrespective of origin, the analysis was performed by regression models. The hair and tap water samples were analysed to evaluate the relationship between both of the samples. Geographical maps were made with the use of geographical information system program. Arc GIS9 which was a spatially explicit map of isotope ratios of tap water. Further, these calculations were used to relate to hair and tap water information for predicting distributions of hydrogen and oxygen isotope ratios in the United States.

Case study

On September, 1971 stable isotope analysis technique was applied in the case of unidentified murder in Stanislaus country, California, US. The case was reopened in 2008 and certain strands of hair were recovered from the victim and were sent for stable isotopic analysis to clarify the travel history of women prior to death. The analysis was performed and it was found that there was little to no movement or travel was done by the victim as the isotope ratio of the hair and the drinking water did not showed much variation. Furthermore, the enamel carbonate from tooth was also analysed by same method to elucidate the childhood origin. The victim was identified as Mary Alice Willey and all the information was observed from the isotope data. It was found that Mary Alice was born and brought up in Anaheim, California and then she moved to San Francisco, California in September, 1970 to attend college. Hence, by using hair sample, it might be possible to reconstruct the movement or travel history of an individual [1].

1.1.2 Anthropological analysis:

The isotopic composition of hydrogen and oxygen in human tissues such as bones can be related to the concentration of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of the water consumed by an individual. The water is consumed in form of drinking water or other drink and by food also [16]. Careful and empirical examination of hydrogen stable isotopic signals in animals can provide an idea regarding the characterization and help in constraining the life history as geographical origin. It is the important point to keep in mind that seasonal, geographical and other variables during the lifespan of any human, animal may leave an isotopic record. In case of bone collagen, around 20% of the total hydrogen is exchangeable. The upper cascading flow of organic hydrogen in the food chain from one trophic level to the next leads to the introduction of successive isotopic patterns that are relatable with successive trophic and ecological situations. The resultant isotopic relationships are quite complex but these are analytically calibrated with the modern faunal analogues. Moreover, the hydrogen isotope concentration of bone collagen should be sensitive to the amount and dietary importance of consumed protein, which offers analytical opportunities for reconstructing diet and trophic structure [20] Stable isotope analysis is proved to be a powerful tool for screening human remains and provenance unidentified bodies from a variety of forensic contexts [24,23].

1.1.3 Microbial forensics:

Stable isotope analysis is explored as a tool for microbial analysis in forensic science. The use of this technique is highly recommended because there is direct link between the stable isotope ratios and the environment in which they reproduce. [6] Microorganisms also show the same trend, as studies have been conducted to elucidate the relationship between growth environment and cellular isotope composition. This would assist in knowing the nature of water in which the microbes had been reproduced, therefore it can also aid in identifying the genetically identical organism which is grown in same environment to get knowledge regarding the geographical origin [10,11]. Stable isotope forensic can be used in microbial forensic analysis in two ways.

1. It can act as a signature for sample matching and comparison:

In this application, two or more than two samples are compared for determining the degree of similarity between the two samples. Stable isotope ratios have a tendency to differentiate between genetically similar organisms which are reproduced in different batches but in similar conditions. Bulk isotope ratios H, O, C and N can be calculated in less time span and with cost effective technique therefore, making it a commendable and screening tool for comparison.

2. Secondly, it can associate a sample with potential media used for its growth.

Oxygen and hydrogen isotope ratio are linear function of those spores that are grown in water. Spore hydrogen isotopes correlate with those of the growth media [6].

Stable isotope analysis is proved as a promising tool in forensic science to complement studies related to genetics. For analysing the bioterrorism incidents, this technique helps in constraining the range of places or origin from where microbiological content might have originated. DNA analysis is also employed for

getting information regarding genetic information but it don't provide direct evidence of the environment in which particular microbe was grown [11]

1.1.4 Wildlife

Stable isotope analysis is used for studies of non-humans too for understanding dietary inputs. This technique showed advancement in wildlife analysis in past few decades [1]. The hydrogen composition of organic tissues of both flora and fauna reflects the hydrogen isotopes of the local season or annual precipitation and hence, it can be used for analysing the geo-location of the particular organism [3]. The application of this technique is in characterizing or comparing the evidences encountered to determine whether the sample belong to specific origin. The forensic experts usually measure minute variations in the stable isotopic composition of the suspect sample and hence, establish the linkage. The value of hydrogen isotope in the tissues of both flora and fauna can be correlated with average precipitation 'isoscares' which depicts strong latitudinal and altitudinal patterns across the world [12]. Stable isotope forensics make use of isotopes like H, C, S for migratory studies of birds and insects who have differing summering and wintering grounds. Studies have been conducted in past to understand migratory behaviour of birds and insects and the result of migrant population to habitat loss throughout their range [7]. The earliest study of stable isotope in wildlife forensics was done for investigation of butterfly origin, specifically monarchs (*Danaus plexippus*). The purpose of this study was to focus on the conservation of natal grounds. The hydrogen isotopic value of wings collected from dead butterflies were linked to the stable isotope ratio of water available in that particular location. In France, the hydrogen isotopic values of wings and chitin were employed for investigating the origin of certain insects like hoverfly. This study also focused on calculating the minimum separation distance which is required to distinguish the geo-location of origin that showed variability because of latitude as well as the isotopic value of hydrogen precipitation [1].

The recent and most effective stable isotope environmental forensic tracer involves the use of hydrogen isotopes ratios of bird feathers for tracing the movement [8,7]. In the recent times, most of the studies focus on the migration phenomenon and to compare the hydrogen isotope values of feathers of the birds with that of GS precipitation [7].

1.1.4 Forensic chemistry:

Hydrogen isotopes act as a unique tool for identification and understanding chemical as well as biological processes. Hydrogen isotopes help in detection and quantification of drugs and their allied materials. Hydrogen as a tracer can be used for longer periods of time of radiolytic decomposition can be reduced have stable Hydrogen isotopes are also employed in detection of explosives [17, 22, 1, 18] and arson.

1.1.4.1 Drug Detection

Illicit drugs are said to have stable isotopic ratio that can be used to characterise the geolocation from which the drug was originated. Therefore, stable isotope ratio value helps the investigators to link different evidences or exhibits of drugs and relate them to their common source [10]. Synthetic drugs such as Pseudoephedrine are analysed by the stable isotope technique during the investigation of such illicit drugs, it is important to know the drug precursors and

how the isotopic signals are changed or maintained during their production. Pseudoephedrine which is direct precursor to methamphetamine is formed by fermentation of pyruvic acid in the presence of benzaldehyde and further reductive amination is performed. Hydrogen isotope value of pseudoephedrine varies from synthetic to semisynthetic sources, as it got depleted than natural ephedrine [10].

Kinetic isotope effects can be employed for investigation of metabolism mediated target organ toxicity. Deuterium labelled analogue is preferred in some case as it is less fatal and provides less DNA damage as compared to 1,2- dibromomethane [17]. Stable isotope analysis or fingerprinting of Hydrogen, carbon and nitrogen is used for forensic investigation of Hexachlorocyclohexanes. Hexachlorocyclohexanes (HCH) are the persistent organic pollutants and are fatal for environment as well as human health. This method of stable isotopic analysis has less analytical uncertainties therefore is regarded as accurate and robust multi elemental technique one can easily differentiate two samples with vast majority of samples from dissimilar geo-location and production time by the use of STA if the samples have moderate amount of carbon, hydrogen and chlorine isotope composition [5].

1.1.4.2 Explosives

Neutron generators are commendable tools as compared to radioactive sources. These generators have many applications including:

- Allow timed neutron pulses
- Can be turned off
- Easy to transport
- High neutron fluxes

Deuterium – Deuterium and Deuterium Tritium generators are employed for the detection of the explosives [22, 18]. NIPS system comprising of deuterium – deuterium generator is established for detection of hidden explosives in bulk and other chemical warfare agents [22].

Several D-T and D-D generators are developed specifically for active neutron interrogation application. In order to detect explosives, these interrogation methods focus on identification of signature chemicals makeup that most of the explosive compounds passes. Nitrogen is the major component in most of the explosives [18].

1.1.4.3 Arson

Wooden matchsticks are usually the originators in arson cases. These matchsticks can be discriminated by stable isotopic analysis the stable isotopes like hydrogen, carbon and oxygen values are analysed to and hence, unburned snatches and their manufacturers are determined. Stable Isotope analysis is known to have tendency and can be employed for comparing known and unknown even if the matchstick recovered are burnt. But there is no such evidences in case of gasoline assisted fire [1].

1.1.4.4 Crude Oil And Petroleum Products

SIA is used in forensic analysis of crude oils petroleum based products. SIA is helpful in determining:-

Source rock

Oil spill source

Export or import control

Isotopic analysis helps in characterization of oils and source rocks. The imperative application of SIA in forensic petroleum analysis is to detect the environmental contamination focused on petrochemical plants in flow & outflow waters [1].

Non-radioactive also known as stable isotopes of any chemical element possess same chemical character but differs in atomic mass [2]. Stable forms of atoms do not go through radioactive decay, they generally record the in vivo chemical signatures of the organisms. They react slightly different from each other in chemical reaction, their rate of reaction varies. These stable isotope are expressed in the form of ratio: the heavy isotope to the light isotope with respect to the known standard. The report is made in per mil value and is denoted by delta symbol (δ) [24]. Stable isotopes are different from radioactive isotopes (C, H) radioactive isotopes possess limited lifetime and they also undergo decay for the formation of different element. The time required for the same varies widely [10].

Stable isotope analysis in forensics: future perspective

SIA capitulates conclusively distinctive information for forensic cases, as it adds on “stable isotope signatures” to the suspected evidences that are indistinguishable or are proved alike by other analytical approaches. The indispensable requisition of this method includes. Apart from this, forensic analysts can discern individual samples with the database created (stable isotope ratio) from the authentic samples. Moreover, sample with unknown origin or identity could be easily investigated by the database comparison. The major application that yields greater information is potential to get the geographical information system. This aids in the construction of spatial maps with predicted stable isotope ratio. By comparing the suspected sample with the database, one can easily predict the source location [10].

Stable isotope analysis proves to be the commendable technique for better identification. This technique can be used easily in variety of disciplines. Because of its analytical approach, it can be used exclusively in chemical investigation. The main and foremost feature of this technique is that it can give idea regarding the life history of any unidentified body. Secondly, it aids in differentiating the two chemical components. This stable isotope analysis technique would help the experts in gathering data for investigation as well as for performing accurate analysis.

Challenges

As Stable isotope analysis is still in its growing stage and research work is still going on in this field sufficient ample data is not available for comparison as well as library for reference has not been established yet. Moreover, it is quite tedious and troublesome task to gather information of all the areas. In addition to this, it is newly emerged field and is not much known to everyone.

Conclusion

Isotopic profiling is a prominent tool in forensic investigations. This emerging technique makes use of human tissues like teeth, hair, nails, bones etc. and can predict the life history of the deceased or the suspect. That cannot be obtained by other instruments or analytical methods. The application of stable isotopes is a completely new trend in the forensic science. This isotope data would aid the experts in investigation, including and excluding evidences as well as in establishing the link between the victim, suspect and the crime scene. In this article, we tried to cover all the applications of hydrogen isotopes in forensic science along with its functions in providing investigative lead during investigations.

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