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*Original Article*

# Anodic Stripping Voltammetry Determination of Total Arsenic in Urine Using Gold Rotating Disc Electrode: A Method Validation

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**Abstract**

The salts of Arsenic (As) are of great toxicological importance and can cause poisoning. The quantitative determination of traces of arsenic and its compounds is important to assess its harmful levels in environmental and biological samples. Therefore quantitative determination of traces of Arsenic in urine is very essential. Routinely, inductively coupled plasma, atomic absorption spectrometry, graphite furnace atomic absorption spectrometry were used for analysis of Arsenic. An attempt has been made to develop for determination of traces of Arsenic in urine using anodic stripping voltammetry. The analysis utilizes three electrode systems, Gold rotating disc electrode (RDE) as a working electrode, Ag/AgCl as a reference electrode and Glassy carbon electrode as an auxillary electrode. Urine was processed by closed digestion method using 34.5% nitric acid ($HNO_3$). Determination of Arsenic was made by primary solution with a sweep rate of 20mV/s and pulse amplitude 50 mV by standard addition method. The solution was purged with nitrogen gas and cleaning was done at -1200 mV for 60 seconds and the potential was scanned from 300 mv to 400 mv on RDE with stirrer speed 2000 rpm. As (III) and As (IV) in HCl were reduced at -1200 mV by nascent hydrogen to $As^0$ and was deposited at 0 mv for 10 sec. The deposited metal was sweeped by scanning the potential from -200 mV to 300 mV using DP mode. The stripping current arising was correlated with the concentration of the metal in the sample. The peak potential for Arsenic is 50 mV. The detection limit of Arsenic by this method was 1.0µg/l.

**Keywords**: Anodic Stripping Voltammetry (ASV); Gold Rotating Disc Electrode; RDE; Arsenic; Urine; Glassy Carbon Electrode.

## Introduction

Arsenic is widely distributed throughout Earth's crust, generally as arsenic sulfide or as metal arsenates and arsenides. The normal level of arsenic in whole blood concentration should be less than 50µg/L. Level of arsenic in urine measured in a 24 hour collection, following 48 hours without eating seafood exceeds 100µg/L in people with arsenic poisoning. In nails and hair it should be less than one ppm [1-3]. Drinking Arsenic contaminated water poses a variety of health problems such as skin lesions include melanosis, leucomelanosis and keratosis. Other problems are high blood pressure, diabetes mellitus, lung disorders and peripheral neuropathy [4-8].

Arsenic has been very popular from centuries as a homicidal agent. It can cause instant poisoning and death if consumed in large amounts. In cases of acute poisoning it causes nausea, vomiting and diarrhea1. Chronic exposure to Arsenic may lead to hyperkeratosis and skin pigmentation. Dermal

exposure causes arsenicosis which is the first symptom of arsenic exposure. Dermatitis is common in arsenic exposure. When it is inhaled it causes primarily the lung cancers and secondarily the liver, skin and digestive tract cancers have also been observed through various studies. Areas where water is contaminated of Arsenic, skin tumors are the most common types of cancer. Oral exposure increases the risk of other kinds of cancer such as bladder, lung, liver, kidney and prostate. Arsenic is also known to cause cancer of skin, lung and bladder [9-12].

Arsenic exists in the environment in two forms arsenic (III) and arsenic (IV). Among these two, arsenic (III) is the more potent and hazardous [13-14]. Due to its toxic effects arsenic is also known as the "king of poisons". Through water it is transported mainly by blood [15], after entering into the blood, As (III) undergoes methylation inside the hepatic cells. It is methylated to form MMA (V) which further undergoes reduction to form MMA (III). It undergoes subsequent oxidative methylations to form DMA (V) which is the primary excretory product after arsenic exposure16.

Measuring total amount of arsenic in urine is the most reliable means of detecting recent arsenic exposures [17]. The tests conducted in urine gives total amount of arsenic present in the body. Routinely, inductive coupled plasma, atomic absorption spectrometry, graphite furnace atomic absorption spectrometry have been used for the analysis of arsenic [18-21]. An attempt has been made to develop a new method for determination of total arsenic in urine by using Gold Rotating Disc Electrode (RDE). One of the major advantages of this technique is that the running cost of instrument is low, compared to any other technique. In the present study, determination of arsenic was made in hydrochloric acid medium with a sweep rate of 20 mV/s and pulse amplitude 50 mV by Gold Rotating Disc Electrode.

## Materials and Methods

*Apparatus and Accessories*

1. Trace metal analyzer model 797 VA Computrace from Metrohm AG Ltd, Switzerland (Fig 1) was used, which contains following electrodes:

| | | |
|---|---|---|
| Working Electrode | - | Gold Rotating Disc Electrode (RDE) (Fig 2) |
| Auxillary Electrode | - | Glassy Carbon (Fig 3) |
| Reference Electrode | - | Ag/ AgCl (Ammonia buffer) |

2. Nitrogen gas of purity 99.99% from laser gases, India was used.
3. Micropipette of Eppendorf make of volume 10 - 100ìl and 100- 1000 ìl were used.

**Fig. 1:** 797 VA Computrace (Trace Metal Analyzer from Metrohm)

**Fig. 2:** Rotating disc Electrode (RDE) (From Metrohm)

**Fig. 3:** Glassy Carbon Electrode (From Metrohm)

*Reagents and Chemicals*

Suprapur hydrochloric acid (HCl), 1000 ppm arsenic standard solution (traceable to SRM from NIST H2AsO3 in HNO3, 0.5mol/l) from Merck, Germany and Ultrapure water from Rion India were used.

*Glasswares*

Volumetric flask of 50 ml capacity from Borosil India was used. The glasswares were thoroughly washed and rinsed 2-3 times with ultrapure water and dried in digital oven.

*Urine Sample*

Urine sample of the OPD patient of AIIMS New Delhi was used.

*Preparation of 1 ppm Standard Arsenic Solution*

1 ppm solution of arsenic was prepared by diluting 1000 ppm of standard solution.

*Preparation of 30% HCl solution*

30 ml of suprapure HCl was taken in 100 ml volumetric flask and make up to mark.

*Preparation of 34.5% HNO3*

Equal volume of concentrated nitric acid and ultrapure water were mixed.

*Sample Preparation*

Liner vessels of microwave digestion system were cleaned with HNO3 and water mixture (1:1) followed by water and dried. 10 ml of urine sample was placed in the liner vessel. 15 ml of 34.5% HNO3 was added in each liner vessel in fume hood. In the reference vessel, 1 ml of water (instead of sample) was added along with 15 ml HNO3 for sample blank. Vessel carrousels were loaded in the microwave digestion oven and the microwave was run with the program given in Table 1. After digestion, the samples were allowed to cool down and then, each vessel was opened in the fume hood. Digested sample was transferred to 50 ml volumetric flask and then the volume was made up to the mark with the help of ultrapure water.

**Table 1:** Programming Condition for Microwave Digester

| Step | Time (sec) | Starting temp ($^0$C) | Ending temp ($^0$C) |
|---|---|---|---|
| 1 | 200 | 28 | 100 |
| 2 | 400 | 100 | 160 |
| 3 | 400 | 160 | 170 |

*Conditioning of the Gold Electrode*

In order to obtain reproducible curves the gold electrode must be electrochemically conditioned. This should be done every day before starting the measurements and also when the standing current varies strongly from measurement to measurement. Generally the standing current should as small as possible, which should be between 0.5 and 1.5 µA at -200mV. For conditioning 10 ml of ultrapure water and 1ml of 30% of HCl was taken in voltammetric vessel of the instrument and conditioning was done under the condition given in the Table 2.

**Table 2**: Parameter for conditioning of gold electrode

| Parameter | Value |
|---|---|
| **General** | |
| Working electrode | RDE |
| Stirrer speed | 2000 rpm |
| Purge time | 300 s |
| **Conditioning Cycle** | |
| Cleaning potential 1 | -1500 mV |
| Cleaning time 1 | 30 s |
| Cleaning potential 2 | + 400 mV |
| Cleaning time 2 | 60 s |
| No. of repetition cycles | 16 |
| **Sweep** | |
| Equilibration time | 5 s |
| Start potential | -200mV |
| End potential | + 300 mV |
| Voltage step time | 0.3 s |
| Sweep rate | 20 mV/s |

*Determination of Blank Value of the Reagents*

It is very difficult to obtain reagents that are completely arsenic free. The content can also vary from batch to batch. Owing to the high sensitivity of this method it is therefore absolutely necessary to determine the blank value of the reagents used. This was determined in 10ml ultrapure water +1 ml 30% of HCl solution in the same way as the arsenic content of the sample is determined.

*Anodic Stripping Voltammetry (ASV) Determination of Arsenic*

The electrode was washed with ultrapure water. 10 ml of ultrapure water and 1 ml 30% HCl were taken in voltammetric vessel and voltammogram was recorded under the condition given in Table 3. After completion of voltammogram, 0.1 ml of digested urine sample was added in voltammetric vessel and voltammogram was recorded under same condition. After completion of the sample Voltammogram, 0.1 ml of 1 ppm standard solution of As was added and voltammogram was recorded. Again, 0.1 ml of 1 ppm standard solution was added in the same vessel and voltammogram was recorded second time. The concentration of the analyte was calculated by linear regression method (standard addition). All the measurements were done by standard addition technique to avoid the sample matrices effect. The voltammogram of the standard and sample was given in fig. 4(a) and 5(a) along with their extrapolation graphs 4(b) and 5(b).

**Table 3**: Parameters for ASV Determination of Arsenic

| Parameter | Value |
|---|---|
| **General** | |
| Working electrode | RDE |
| Stirrer speed | 2000 rpm |
| Mode | DP |
| Purge time | 130 s |
| **Deposition** | |
| Cleaning potential (Deposition potential 1) | -1200 mV |
| Cleaning time (Deposition time 1) | 60 s |
| Deposition potential (Deposition potential 2) | 0 mV |
| Deposition time (Deposition time 2) | 10 s |
| **Sweep** | |
| Pulse amplitude | 50 mV |
| Start potential | -200mV |
| End potential | +300 mV |
| Voltage step | 6 mV |
| Voltage step time | 0.3s |
| Sweep rate | 20 mV/s |
| Pulse amplitude | 50mV |
| Pulse time | 0.40s |
| Peak potential As | +50 mV |
| Equilibration time | 5 s |

**Result and Discussion**

Voltammetry is a technique in which the current flowing through an electrode is measured while a potential scan is superimposed on that electrode. Voltammetry is a three electrode system consisting of a working electrode, a reference electrode and an auxillary electrode which involves two steps. In the first step metals/metal ions are deposited on the electrode and in the second step metals/metal ions are stripped out of the electrode. The metal/metal ions stripped out are directly proportional to the current, greater the current greater will be concentration of metals and lesser will be current lesser the concentration of metals. The total arsenic content is determined at a gold electrode with the electrode surface on its sides. As (III) and as (IV) in HCl were reduced at 1200 mV by nascent hydrogen to As0 and deposited. Deposited metals are anodically striped by scanning the potential from -

200 mV to 300mV. All the measurements done by standard addition technique in which first sample is taken into the voltammetric vessels and current is measured, then standard of known concentration is added twice to the sample solution and the current is measured. After all the measurements extrapolation curve is plotted between current vs. concentration. The extrapolation curve will show the amount of metals present in the sample solution. All the analysis was done with automatic blank subtraction, which is feature of the instruments.

Voltamogramme of As obtained from standard addition technique is given in (Fig. 4(a) and 5(a). The sensitivity was calibrated by standard additions to the sample and the initial metal concentrations were calculated by extrapolation given in Fig. 4(b) and 5(b). Consequently, the linear calibration range was automatically obtained as being related to quantitative mode of the Voltammetric unit. Under these conditions, Concentration of arsenic in the sample was 116.75µg/L. Several papers [10, 11, 18] have discussed the determination of Arsenic in matrices other than the urine. The advantages of proposed voltammetric method over the other known techniques include the sensitivity of the method besides the other features such as the rapidity, cost effectiveness and sophistication of the method.

**Fig. 4a:** Voltammogram of Arsenic Standard

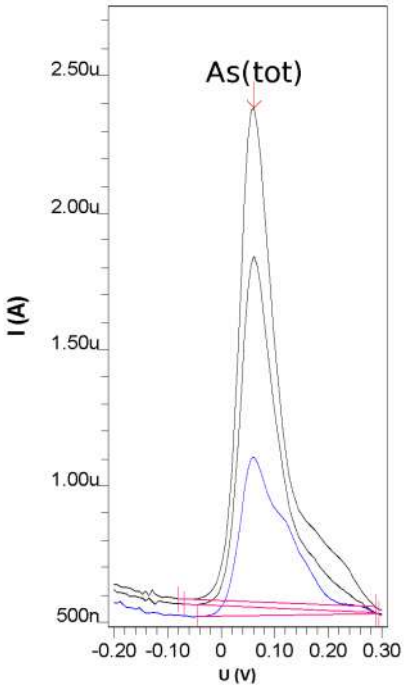



**Fig. 5a:** Voltammogram of urine sample

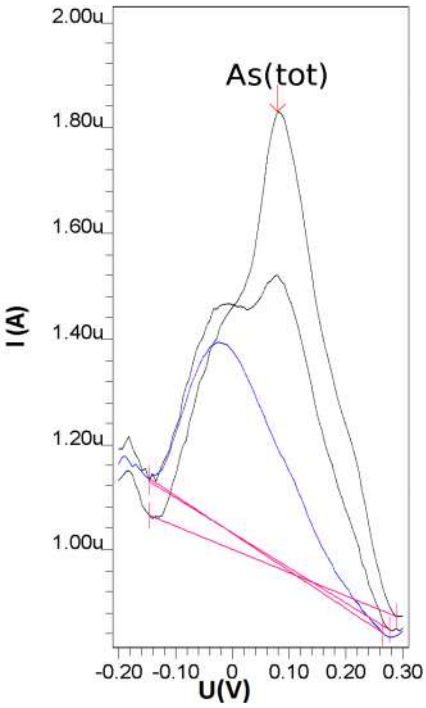



**Fig. 4b:** Extrapolation graph of Arsenic standard

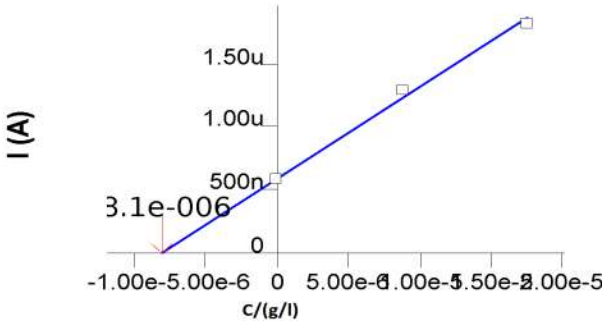
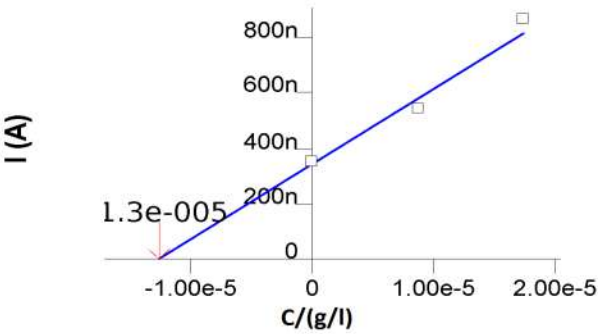



**Fig. 5b:** Extrapolation graph of urine sample



## Conclusion

This article describes the determination of total arsenic by anodic stripping voltammetry (ASV) using gold rotating disc electrode (RDE). A determination limit of 1.0 µg/l was achieved with 10 ml sample solution. The total arsenic content is determined at a gold electrode with the electrode surfaces on its sides. As (III) and As (IV) in HCl were reduced at -1200 mV by nascent hydrogen to As0 and deposited. If the deposition is carried out at -200 mV then only As (III) is reduced which allows the differentiation between total arsenic and As (III). The Trace metal analyzer is advantageous in terms of the range of concentration to which it can measure. It becomes a useful technique as it can be used for the analysis of biological samples such as urine.

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*Original Article*

# Synthesis and Application of 4-Acetyloxy-3-Methoxy Benzaldehyde as Chromogenic Spraying Reagent on Thin Layer Chromatography for Forensic Identification and Detection of Some Food Oils

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**Abstract**

Oils and fats analysis covers a major area in forensic chemistry as these are often adulterated in various ways viz., adulteration of lower grade, different origin (vegetable oil by animal fat or vice-versa) or misbranding or contra banding of products etc., the cases as above may occur frequently as oils and fats are extremely used as cooking media and also in industries like paints, varnishes, pharmaceutical industries. There may be theft cases or illegal possession related to oils and fats. In the above case the samples are frequently sent for their examinations for different purposes under Essential Commodities Act (E.C. Act).

In this study we have synthesized 4-Acetyloxy-3-methoxybenzaldehyde as spray reagent for thin layer chromatography examination of Soyabean oil, Mustard oil, Groundnut oil, Coconut oil, Rapseed oil, Linseed oil, Castor oil, and Almond oil in different solvent system. The spray reagent develops pinkish violet color.

**Keywords:** Oil; Fat; Fatty Acids; Thin Layer Chromatography.

## Introduction

Vegetable oils are mainly constituted by triacylglycerol (95-98%) and complex mixtures of minor compounds (2-5%) of a wide range of chemical nature. These minor constituents show a broad qualitative and quantitative depending on the vegetable species from which they are obtained.

The minor components include mono- and diglycerides, free fatty acids, phosphatides (or phospholipids), sterols, protein fragments, various resinous and mucilaginous materials and oxidative products a triglyceride is a chemical compound formed from one molecule of glycerol and three fatty acids. Fatty acid chains may contain one or more double bonds at specific positions (unsaturated and polyunsaturated), or they may be fully saturated.

The physical and chemical properties of a fat depend on the composition of the fatty acid mixture. Fats from plant sources contain a higher proportion of unsaturated acids and are often liquids at room temperature due to hydrogen bonding. Fats are used in cooking because they are compounds with very high boiling points. Their high boiling points therefore make these classes of compounds ill suited for analysis by the gas chromatography. However, the glycerol ester can be chemically decomposed into partition methyl esters of each individual fatty acid. Oils and fats are important parts of human diet and more than 90 % of the world production from vegetable, animal and marine sources is used as food or as an ingredient in food products. Oils and fats are equivalent amount of sugar. Their functional and textural characteristics contribute to the flavor and palatability of natural and prepared foods.

They contain certain fatty acids, which play an important role in nutrition and are also carriers of fat-soluble vitamins. Vegetable oils has become more attractive recently because of its economic benefits as they are used as components in many manufactured products and the fact that it is made from renewable

resources. The determination of the minor components is of great importance in establishing the oil quality and their genuineness. This paper shows the applications of TLC for detection and identification of various food oils using 4-Acetyloxy-3 methoxybenzaldehyde as chromogenic detector. Fatty acids are used in cosmetics, medicines, paint industries, food industries, in the manufacture of soap as lubricants, as a fragrant material i.e. It covers almost all arena of society. Therefore they frequently encountered for forensic identification. Various oils detected previously using different locating reagents on thin layer chromatography viz. mainly sulphuric acid, anisaldehyde etc., in the present study we have synthesized new aldehyde 4-Acetyloxy-3-methoxy benzaldehyde via Acetylation of 3-Methoxy-4-hydroxybenzaldehyde, which is a white crystalline substance and forms pinkish violet color with different oils. In the present study we have used Soya bean oil, Mustard oil, Groundnut oil, Coconut oil, Rapseed oil, Linseed oil, Castor oil and Almond oil for detection and identification using this aldehyde as locating reagent on TLC plate.

## Materials and Methods

Pure food oils, samples of ISI / agmark grade procured from local market of Gwalior. Methanol, ethanol, chloroform, Petroleum ether, n-butanol, glacial acetic acid, benzene, diethyl ether used were of A. R. grade, double distilled water used throughout the study.

### *Synthesis of 4-Acetoxy-3-methoxybenzaldehyde*

Dissolve 1.5 g of 3-Methoxy-4-hydroxybenzaldehyde in 25 ml of 10% sodium hydroxide in 250 ml conical flask add 30 gm of crushed ice and 4 ml of acetic anhydride place the stop cork and shake for several times over 20 minutes of time a cloudy, milky white precipitate is formed which is filtered through Buchnar funnel and washed with ice cold water and recrystallized it from 95% ethanol which gives white crystalline needles, molecular formula C10H10O4, molecular weight 194.18

**Table 1:** Rf values of different oils under different solvent system

| S.No | Solvent System | Rf value of Food oils | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Soyabean Oil | Mustard Oil | Groundnut Oil | Coconut Oil | Rapseed Oil | Linseed Oil | Castor Oil | Almond Oil |
| 1. | Petroleum: Diethylether: Acetic acid (9:10:1) | 0.1, 0.25, 0.3, 0.5 ,0.7 | 0.2,0.35, 0.5,0.65 | 0.35,0.5,0.7, 0.8, 0.9 | 0.15, 0.3,0.65,0.7 | 0.1, 0.35 0.5,0.7, 0.9 | 0.35, 0.6, 0.8 | 0.2,0.3 5,0.5,0 .6,0.7, 0.9 | 0.2,0.25, 0.3,0.5,0 .65,0.7, 0.9 |
| 2. | Hexane: Ether(1:1) | 0.1, 0.3, 0.5, 0.65, 0.7, 0.85 | 0.15,0.2, 0.4,0.5,0 .55,0.6, 0.8 | 0.15,0.2,0.3, 0.4,0.55,0.6, 0.75,0.9 | 0.3,0.45, 0.5,0.7 | 0.2,0.3,0.35 ,0.45,0.6,0. 75, 0.8,0.9 | 0.4,0.55,0.6 ,0.7,0.9 | 0.2,0.2 5,0.4, 0.5,0.5 5,0.6, 0.7,0.9 | 0.25,0.3, 0.5,0.6,0 .75,0.8, 0.9 |
| 3. | n-Butanol: Acetone:Ethanol:Water (60:20:20:1.5) | 0.65,0. 8,0.9 | Streak b/w 0.6-0.9 | 0.85 | 0.9 | Streak b/w 0.7-1 | streak at 0.7 | 0.65, 0.8 | 0.5,0.6, 0.8 |
| 4. | Benzene: Acetic acid(100:1) | 0.6,0.7 5,0.8 | 0.7,0.85, 0.9 | 0.8 | 0.5,0.7, 0.8 | 0.75,0.8 | 0.65,0.80.9 | 0.7,0.8 5,0.9 | 0.5,0.75, 0.9 |
| 5. | Hexane:Acetone(6:4) | 0.8,09 | 0.9 | 0.9 | 0.85,0.9 | 0.9 | 0.7,0.85 | 0.8 | 0.75,0.8 5,0.9 |
| 6. | Toluene:Chloroform (3:1) | 0.65,0. 8,0.9 | 0.2,0.5,0 .8,0.9 | 0.5,0.7,0.85, 0.9 | 0.6,0.75,0.9 | 0.2,0.65,0.8 ,0.9 | 0.7,0.85,0.9 | 0.2,0.6 ,0.75,0 .8,0.9 | 0.4,0.7, 0.8 |
| 7. | Heptane (100%) | 0.5,0.6 ,0.9 | 0.4,0.5, 0.8 | 0.3,0.4,0.6 | 0.5,0.6 | 0.3,0.4, 0.5,0.8, 0.9 | 0.6,0.9 | 0.2,0.4 ,0.5,0. 6,0.8, 0.9 | 0.7 |
| 8. | n-Butanol: Acetic acid: Water (60:15:25) | ND* | ND* | ND* | ND* | ND* | ND* | ND* | ND* |
| 9. | Methylene chloride:Ether :Methanol: Water (77:15:8:1.2) | 0.5,0.9 | 0.9 | 0.7,0.75, 0.9 | 0.8,0.9 | 0.7 | 0.5,0.9 | 0.9 | 0.6,0.8 |
| 10. | Dichloro ethane: Methanol:Water (95:5:0.2) | 0.7,0.9 | 0.6,0.9 | 0.4,0.5,0.7 | 0.9 | 0.8 | 0.5,0.7, 0.8 | 0.7,0.9 | 0.6,0.75 |

*No Development

*4-Acetoxy -3-methoxybenzaldehyde Preparation of reagents*

(A) 0.328 gm of 4-Acetoxy -3-methoxybenzaldehyde in 100ml of absolute alcohol

(B) 50% aqueous sulphuric acid.

*Thin Layer Chromatography Analysis*

A Standard glass TLC plates was coated with slurry of silica gel G water to a uniform thickness of 0.25 mm. The plate was activated by heating an oven at 110$^0$C for about one hour. Aliquots of different oils were spotted on to the plate, using fine capillary tubes TLC plate and allowed to dry for a few minutes. A TLC developing chamber containing the solvent system was properly saturated using filter paper strips, and the spotted TLC plate was placed vertically in it, and the chamber was covered with a lid. Separation of the samples was achieved after running the solvent system petroleum ether: diethyl ether: acetic acid (90:20:1ml) for a distance of 10 cm from the point of spotting.

The TLC plate was then removed from the chamber and dried at room temperature. The plate was removed from the chamber dried in air sprayed by reagent (a) followed by reagent (b) and heated in oven for one hour then colored spots appeared against the white background. The Rf values of different oils under different solvent systems are given in the Table1.

## Results & Discussion

TLC analysis was carried out to study the difference in the constituent profiles of food oils. A marginal difference in constituent profiles of these samples was observed, which is because plants have their own distinctive chemical component profiles. TLC analysis method for the analysis of above mentioned test oils revealed several spots, which could be separated by using various solvent systems *(Table1),* and visualization was done by spraying 4-Acetoxy-3-methoxybenzaldehyde reagent. Some solvent systems used for the TLC analysis of test oils have revealed several spots after separating in solvent systems. In the present work, ten solvent systems were tried (Table 1), out of which eight solvent systems Hexane: ether (1:1), n-butanol: acetone: ethanol: water (60:20:20:1.5), Benzene: acetic acid (100:1), Hexane: acetone (6:4), Toluene: cholorform (3:1), Heptane (10%), Methylenechloride: ether: methanol: water (77:15:8:1.2), Dichloroethane: methanol: water (95:5:0.2) gave useful results, but n-butanol: aceticacid: water (60:15:25) solvent system shows no development and *Petroleum: diethyl ether: acetic acid (9:10:1)* was the only solvent system that provided satisfactory separation.

In this study the used locating reagent develops bright pinkish violet color which distinctively identifiable. This reagent is quiet sensitive and develops color without hindrance and is highly cost effective and identifies samples in microgram quantities. In the study various solvent systems gave distinctive separations of constituents of food oils.

## Conclusions

In this study, *Petroleum: diethyl ether: acetic acid (9:10:1)* have been found to be the best solvent system for the proper extraction of the selected samples. Ten solvent systems were tried, and only this could produce fruitful and reproducible results. The constituents of the oils of the different plant species undertaken in the present study can be separated and differentiated for the purpose of identification by this method. Spots were visualized best by using 4-Acetoxy -3-methoxy benzaldehyde as spray reagent. The use of 4-Acetoxy-3-methoxybenzaldehyde in detection and identification of food oil is quiet useful, less time consuming, sensitive and gives accurate results. Therefore it can be used in routine forensic examination of oil and fats.

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*Original Article*

# Seperation and Detection of Nitrazepam and Clonazepam by Thin-Layer Chromatography

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**Abstract**

The benzodiazepines are most frequently encountered drugs in emergency toxicology screening, drugs of abuse testing and forensic medicine examinations. The identification of nitrazepam and clonazepam, the most frequently used benzodiazepines is described by simple, rapid and sensitive thin-layer chromatographic method. The structures of these benzodiazepines contain nitro group which is reduced by acidified stannous chloride to give amino group. This couples with diazotized sulphanilic acid to give a yellow brown coloured compound. The detection limit was found to be 3µg and 5µ per spot for nitrazepam and clonazepam respectively. The reaction was not given by other benzodiazepines commonly encountered in toxicological screening.

**Keywords:** Benzodiazepines; Thin-Layer Chromatography; Spray Reagent; Stannous Chloride; Sulphanilic Acid.

## Introduction

Benzodiazepines are the most commonly pre scribed medication worldwide. They are known for their hypnotic, tranquilizing and anticonvulsant properties [1]. Due to their wide spread availability they are chronically abused or as seen more commonly in hospital emergency departments, intentionally or accidently taken in over dose. Such cases cannot be decided by mere medical examination. To have a proper treatment the concern medical officer collects the stomach wash, gastric lavage, blood, urine etc., of the poisoned patient, and sent to forensic toxicology division. The samples are analysed on top priority and on the basis of report of analysis the line of treatment is decided by the concerned medical officer and thus life of the person can be saved.

Several analytical techniques for the isolation and quantitation of benzodiazepines in biological samples have been published [2-11]. Though instrumental methods are sensitive they require elaborate instrumental assay. Therefore they are not suited to emergency room determination. Therefore thin-layer chromatography (TLC) is preferred for screening the drugs, due to its simplicity and rapidity. Chromogenic reagents such as Dragendorff [12], UV detection [13-15], Griess reagent [16], Bratton-Marshall reagent [3], chlorine -o-tolidine [8], etc., are reported in literature for detection of benzodiazepines. The objective of this work is to search alternative and sensitive regent for nitro group containing benzodiazepines. We report stannous chloride-hydrochloric acid followed by diazotised sulfanilic acid reagent for selective detection of nitrazepam and clonazepam.

## Materials and Methods

All chemicals used were of analytical reagent grade

and benzodiazepines were pharmaceutical grade. Distilled water was used throughout. Standard solutions of alprazolam (Cipla Ltd. Solan,H.P.) 1mg/ml in chloroform, clonazepam (Piramal Health Care, Solan, H.P.), 1mg/ml in acetone, diazepam (Ranbaxy, India), lorazepam(Wyeth, India and Nitrazepam (Anglo French Drugs, Mumbai) mg/ml each were prepared in ethanol. These solutions were diluted appropriately before use.

*Spray reagents:* (i) Stannous chloride-hydrochloric acid - 5.6 gm stannous chloride uniformly dissolved in 10ml of 20% hydrochloric acid. (ii) Diazotized sulphanilic acid reagent was prepared by dissolving 0.5gm sulphanilic acid and 1 gm solid sodium nitrite in 100 ml of 10% hydrochloric acid.

## Thin-layer Chromatography

Standard glass TLC plates (10X15 cm) were coated with slurry of silica gel G (Sisco Research Laboratories, Mumbai) in water (1:2) to produce uniform 0.25 mm layers. These were left to dry at room temperature. Plates were activated by heating in oven at 110°C for ca. 1 hour. Before use the plates were stored in desiccators. Standard solutions of 10µl each of alprazolam, clonazepam, diazepam, lorazepam and nitrazepam were spotted 1.5cm from the bottom of the plate by means of a micropipette and spots were left to dry in air. Plates were developed by ascending technique, in pre-saturated TLC chamber using two solvent systems chloroform: acetic acid (9+1) and chloroform: acetone (8+2) at 25°C temperature. The mobile phase was allowed to migrate to a distance of about 10 cm. Approximately 20 ml solvent was required for run (development time ca.20 min). The plate was removed from the chamber, dried in air, and sprayed uniformly with stannous chloride –hydrochloric acid reagent and was air dried. It was kept in oven for about 10 min at 100°C temperature. The plate was removed from oven and cooled to room temperature. It was sprayed uniformly with diazotized sulphanilic acid.

## Results and Discussion

After detection only nitrazepam and clonazepam appeared as yellow brown coloured spots; however other benzodiazepines such as alprazolam, diazepam and lorazepam do not show any colour reaction with the reagent (Fig 1). The structure of these benzodiazepines reveals that only nitro group containing benzodiazepines (nitrazepam and clonazepam) show colour reaction. A representative reaction for nitrazepam is presented in Fig 2. Nitrazepam (I) on reduction with stannous chloride gives amine (II) which couples with diazotized sulphanilic acid (III) reagent to give yellow brown coloured compound (IV). The colour of spots remains stable for couple of days. Both the mobile phases give compact spots. The $R_F$ values, detection limit and spot concentration/$cm^2$ for nitrazepam and clonazepam is listed in Table 1. Both the mobile phases give compact spots.

The method described in this paper permit simple and rapid identification of nitrazepam and clonazepam. Other benzodiazepines such as alprazolam, diazepam and lorazepam do not give colour reaction. The reagent described is very sensitive and selectively used for screening two benzodiazepines in single run.

**Table 1:** $R_F$ values and detection limits of benzodiazepines

| Benzodiazepines | $R_F$ in solvent system I | $R_F$ in solvent system II | Detection limit µg | Spot conc./$cm^2$ |
|---|---|---|---|---|
| Nitrazepam | 0.63 | 0.40 | 3 | 6µg |
| Clonazepam | 0.68 | 0.30 | 5 | 8µg |

Solvent system I Chloroform: Acetic acid (9+1)
II Chloroform: Acetone (8+2)

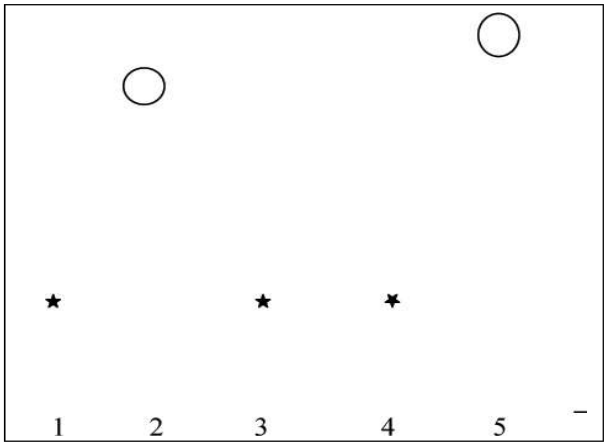
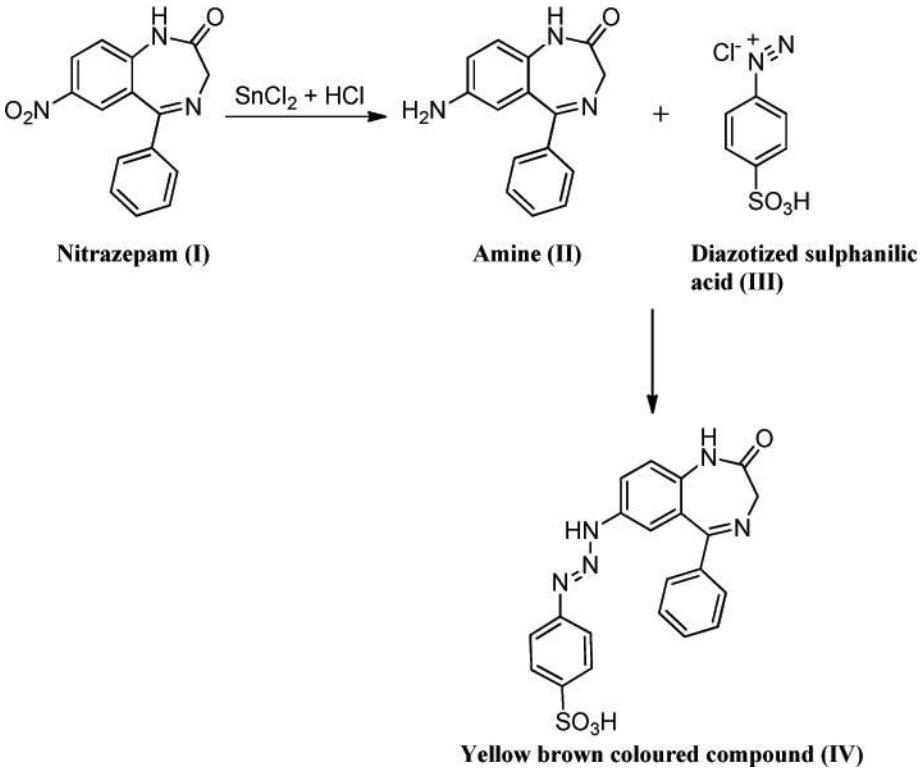



**Fig.1:** Thin-layer chromatogram obtained from: 1, alprazolam; 2, clonazepam; 3, diazepam; 4, lorazepam; 5, nitrazepam. Asterisks indicate no spots were visualized.

**Fig. 2:** Proposed mechanism of Nitrazepam with diazotized sulphanilic acid

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# Removal of Lead from Drinking Water using Banana Peels

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**Abstract**

Presence of heavy metals in drinking water has lead to studies for developing methods to make water safe. Among other methods, biosorption of heavy metals by agricultural waste has gained popularity. Our study is inspired by the work of Gustavo Castro and colleagues who have successfully established the use of banana peel for removal of lead from waste water. In our finding at neutral pH also lead was biosorbed by fresh banana peel and water could be purified upto 83 % without any chemical treatment.

This method may be applied household drinking water especially supplied to rural household where use of modern water purifying systems is yet not common. The method may be explored to develop more sensitive techniques.

Keywords: Not Provided

## Introduction

Unsafe drinking water is a major cause for con cern especially in developing and under developed nations. Drinking water is usually obtained from either fresh water sources such as rivers, lakes and ponds, or quite commonly, ground water, especially in areas that are not served by rivers. The Central Ground Water Board, a sub-office under the Ministry of Water Resources in India, aims to scientifically establish and fully utilize ground water facilities throughout the country, to provide for the needs of the people [1].

While this is a noble effort, the unfortunate truth is that very often ground water may be highly contaminated, by harmful pathogens, heavy metals, pesticide run off and other chemicals [2]. This results in long and short term physical ailments, as well as developmental disorders in both adults and especially children [3]. In India, where in a number of villages, towns and cities bore wells and hand pumps are the only source of drinking water, this is a major cause for concern. In this paper, an attempt has been made to formulate a simple household method, based on scientific fact, which can be used to significantly decrease the concentration of heavy metals, particularly lead, in water.

Lead is a heavy metal with many industrial uses and many more toxicological implications. Although it is a valuable metal, lead is highly toxic to the human body, even in minute quantities of 5 micrograms/dL of blood [4]. In particular, lead causes severe effects on children, old people and pregnant women, due to its tendency to accumulate within the body, replacing calcium in bones [5].

Biosorption [6] of heavy metals from aqueous

solutions has been experimented earlier using paper mill sludge [7] and agricultural wastes like peach and apricot stones [8], jamun seeds [9], rice husks, spent grain, sawdust, sugarcane bagasse, fruit wastes, weeds [10], corn, durian, pummelo [11], pumpkin [12], spinach and papaya [13], tubers [14], peach shell and aquatic plant waste [15], mangostene fruit shell [16], orange and lemon peels [17] soybean oil cake [18] etc.

Bananas are a highly popular, easily available and extremely versatile fruit. Apart from the obvious nutritive value, they are used as fertilizers; their leaves are used as plates, in beauty products, as anti depressants, and many others. The peels of bananas have been particularly useful in the treatment of skin ailments. The newest use of banana peels, however, is as a biosorbent for removing phenolic compounds [19] and also heavy metals [20]. Reported by Brazilian researchers Gustavo Castro and colleagues, minced banana peel can quickly remove lead and copper from river water as well as, or better than, many other materials. Banana peel treatment is low in cost and the peels do not need any chemical modification. The nature and composition of the surface of banana peels has been studied, which due to the high fibre content, have an excellent capacity for biosorption of water as well as heavy metals.

In this paper, we look at the biosorption abilities of banana peels with respect to lead, and aim to formulate a method that may be modified further to be used in households to produce pure and safe drinking water.

## Materials and Methods

### *Apparatus and Chemicals*

*Instrument*

Atomic absorption spectrophotometer (EC Electronics Corporation of India Limited AAS4129) was used for determination of lead (Pb) with deuterium lamp for background correction. The hollow-cathode lamp for Pb which modulates the light of 285 Hz for the metal was employed as radiation source. The flame used was air/acetylene. Nitrogen was used as carrier gas.

*Chemicals*

Water used in all experiment as reagent was ultrapure and obtained from Milli-Q-water purification system (Ranken Rion Ltd, India).

All apparatus were thoroughly cleaned before use. All glassware was soaked for a minimum of 16 hours in 50% nitric acid and then rinsed in reverse osmosis deionised water.

### Preparation of standard solutions

Working lead standard solutions were prepared from 1000ppm stock solution by dilution in water.

Lead standard solution (1000ppm) was used as stock solution. Six lead solutions of different concentrations (1.0, 2.0ppm, 4.0ppm, 6.0ppm, 8.0ppm and 10.0ppm) were prepared by serially diluting the stock solution immediately before use.

The solutions thus prepared were divided into two batches each. The first batch solutions were used as calibrators to calibrate the instrument with every batch and the second batch solutions were used for sample preparation.

### Sample Preparation and Analysis

The second batch solutions were again divided into five batches A, B, C, D and E for analyzing them at 0, 0.5, 1, 1.5 and 24 hours respectively. 100ml each of samples were taken in glass beakers and labelled accordingly.

Fresh banana peel was taken and minced manually with the help of a mortar and pestle to form a paste and then washed several times with distilled water, then in 0.001M HCl so as to remove surface impurities and then in again with distilled water until free from chloride ions. No modification was done by any chemical agents to increase the biosorption capacity of the banana peel.

0.02 g of minced banana peel paste was added to each sample in batches B, C, D and E at time T=0 hours and the sample suspensions were stirred mechanically. Batch A samples were analyzed for lead concentration without adding minced banana peel paste and the readings were considered to be taken at time T=0 hours. Batch B, C, D and E samples were analyzed after 0.5, 1, 1.5 and 24 hours respectively. Manual stirring of the samples was done at intervals of 15 minutes. All biosorption experiments were carried out at neutral pH (=7.0) after adjustment with 0.001M NaOH at temperature $18\pm1^{0}$C.

### Quality Assurance

Appropriate quality assurance procedures and

precautions were taken to ensure reliability of the results. All the measurements were made in triplicate for the samples and calibrator solutions. The instrumental conditions during the analysis lead are given in Table 1.

**Table 1:** Instrumental Conditions for Analysis of Samples

| Element | Current (mA) | Slit Width (nm) | Amax (nm) | Flame Color | Flame Type | AAS Technique |
|---|---|---|---|---|---|---|
| Pb | 5 mA | 1.0 | 217.6 | Blue | Air/$C_2H_2$ | Flame |

The accuracy of the method was determined by measuring the recovery of lead in the matrix containing known concentrations of lead solutions using method of standard addition. The recovery and reproducibility of the method was carried out by spiking and homogenizing several already analyzed samples with varied quantities of standard solutions of lead (Pb) and processed as previously described. The analytical recovery for 'spiked' samples with lead is given in Table 2.

**Table 2:** Recovery studies for lead

| Metal | Base Value (ppm) | Quantity Added (ppm) | Quantity Detected[a] (ppm) | Recovery(%)[b] |
|---|---|---|---|---|
| Pb | 2.0 | 5.0 | 6.96 | 99.2 |

Recovery test, a=Mean value (n = 3), b=100× [(found-base)/added]

## Results and Discussion

*Regression data for the standard calibration plots*

There was a good linear relation between absorbance and standard concentration of lead and copper. The regression equations and coefficient of determinations obtained for each calibration plot are summarised in Table 3**.** Linearity was evaluated for three runs of samples for lead.

**Table 3:** Regression data for analysis of standard solutions

| S. No. | AAS calibration for Pb | Regression equation | Coefficient of determination ($R^2$) |
|---|---|---|---|
| **1.** | 1st run | y = 0.0178x - 0.0018 | 0.9915 |
| **2.** | 2nd run | y = 0.0249x - 0.0008 | 0.9926 |
| **3.** | 3rd run | y = 0.0232x - 0.0024 | 0.9908 |

*Pre and Post-treatment analysis results*

The spectrophotometer automatically determined concentration of lead present in the samples by extrapolating the calibration curve recorded earlier in parts per million. All samples were run in triplicate and the mean values were used along with the respective standard deviation values for statistical analysis. Table 4 gives the mean concentration values of lead in pre-treated at 0 hours and post-treated samples recorded at 0.5,1, 1.5 and 24 hours.

**Table 4:** Concentration of Lead in Pre-Treated and Post-Treated Samples

| S N | Sample Name | Pre-Treatment Conc. (ppm) At Time T= 0 hrs | Post- Treatment Mean Concentration (ppm) At Time T=0.5 hrs | At Time T=1 hr | At Time T=1.5 hrs | At Time T=24 hrs |
|---|---|---|---|---|---|---|
| 1 | S1 | 1.0 | 0.7 ± 0.1474 | 0.5 ± 0.0351 | BDL | BDL |
| 2 | S2 | 2.0 | 1.1 ± 0.1069 | 1.0 ± 0.1015 | 0.95 ± 0.1069 | 0.6 ± 0.0462 |
| 3 | S3 | 4.0 | 1.8 ± 0.0351 | 1.7 ± 0.0551 | 1.6 ± 0.0153 | 1.2 ± 0.0529 |
| 4 | S4 | 6.0 | 3.1 ± 0.1253 | 2.2 ± 0.0603 | 2.0 ± 0.0889 | 1.3 ± 0.0153 |
| 5 | S5 | 8.0 | 4.3 ± 0.0557 | 3.4 ± 0.0153 | 3.2 ± 0.0458 | 1.5 ± 0.0030 |
| 6 | S6 | 10.0 | 5.3 ± 0.1387 | 4.2 ± 0.1015 | 3.9 ± 0.0200 | 1.7 ± 0.1137 |

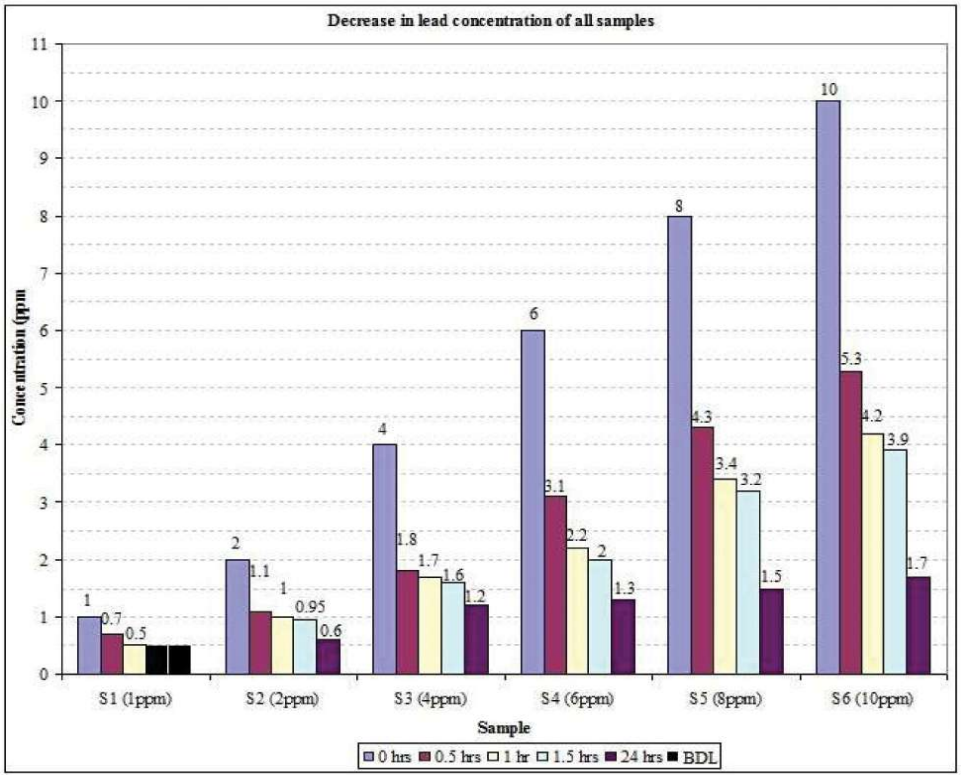
Where, BDL stands for "Below Detection Limit" as Detectable range of Lead by the AAS instrument used is 0.4 – 30.0ppm

*Effect of contact time on biosorption of lead*

Time-dependent study of biosorption of lead from 100ml neutral aqueous solutions of different concentrations (1.0 to 10.0ppm) by 0.02% minced banana peel was done up to 24 hours at room temperature 18±1$^0$C and manual stirring. Sample was tested at different time intervals i.e. 0.5, 1.0, 1.5 and 24 hours for residual lead concentration.

Figure 1 shows the comparative graph of decrease in concentration of lead of in all samples by treatment with banana peel. Figures 2(a), (b), (c), (d), (e) and (f) show decrease in lead concentration of individual sample at concentrations 1, 2, 4, 6, 8 and 10ppm respectively with time. On plotting a the values of lead concentration (in ppm) against time (in hours), a straight line graph is obtained between 0 and 0.5 hours and a hyperbolic graph between 0.5 and 24 hours in all the samples. This shows that there is a sharp linear decrease in concentration of lead in the first 30 minutes of treatment with banana peel.

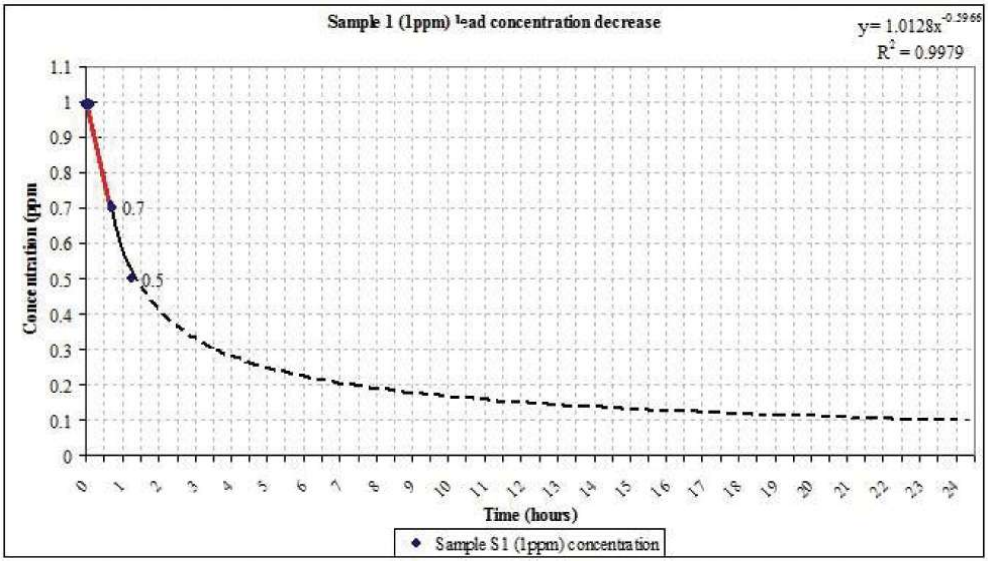
**Fig. 1:** Graph showing Decrease in Concentration of Lead of all Samples by Treatment with Banana Peel




Where, BDL stands for "Below Detection Limit" as Detectable range of Lead by the AAS instrument used is 0.4 – 30.0ppm

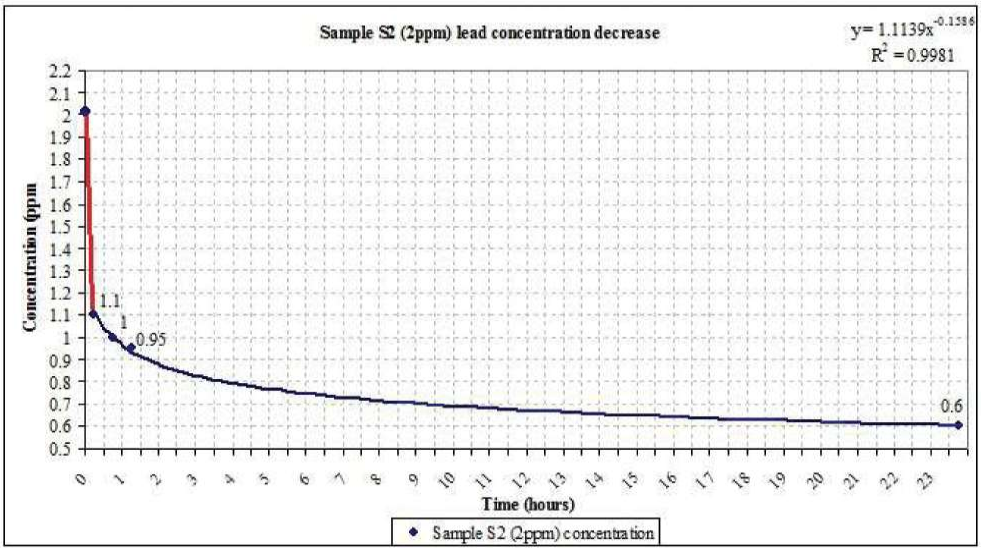
**Fig. 2:** Decrease in Lead Levels of Different Lead Solutions with Time

**(a)**

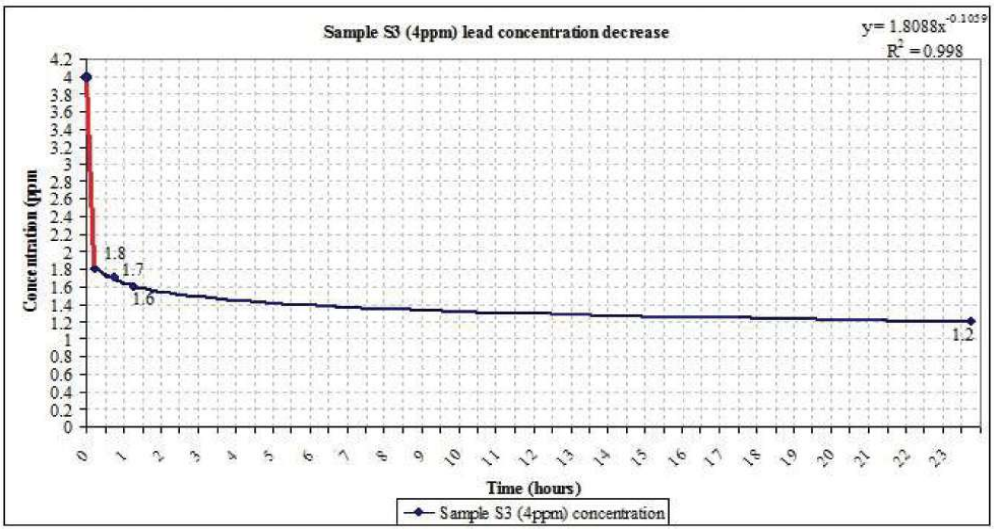



Since, lead concentration beyond 0.5 hours was below detectable limit; therefore the graph was extrapolated and thus shown in dotted lines.

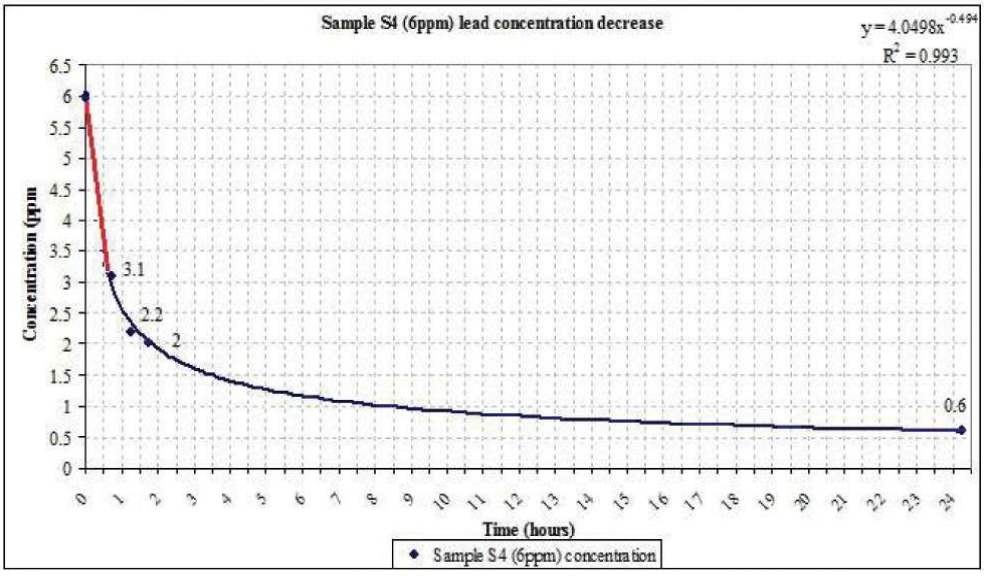
**(b)**


Sample S2 (2ppm) lead concentration decrease
y= 1.1139x^-0.1586
R² = 0.9981
Concentration (ppm
Time (hours)
Sample S2 (2ppm) concentration
1.1
1
0.95
0.6

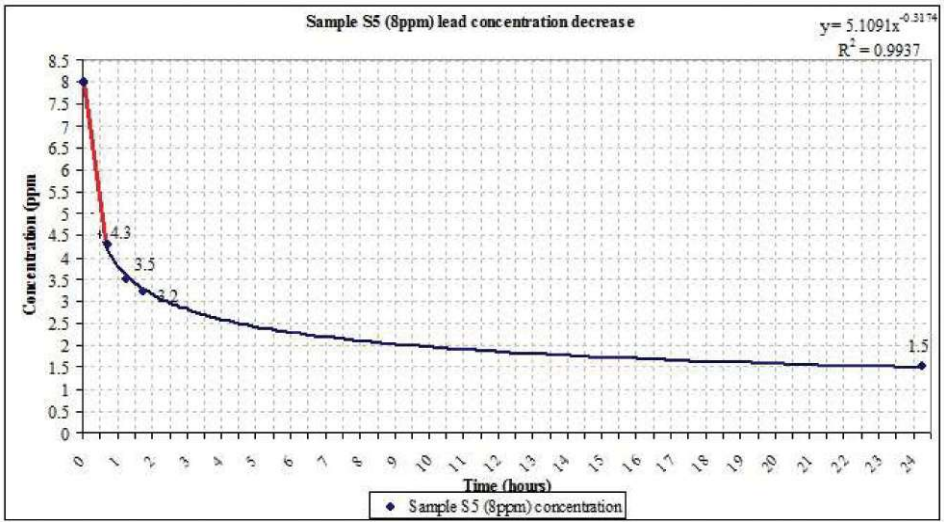
**(c)**


Sample S3 (4ppm) lead concentration decrease
y= 1.8088x^-0.1059
R² = 0.998
Concentration (ppm
Time (hours)
Sample S3 (4ppm) concentration
1.8
1.7
1.6
1.2

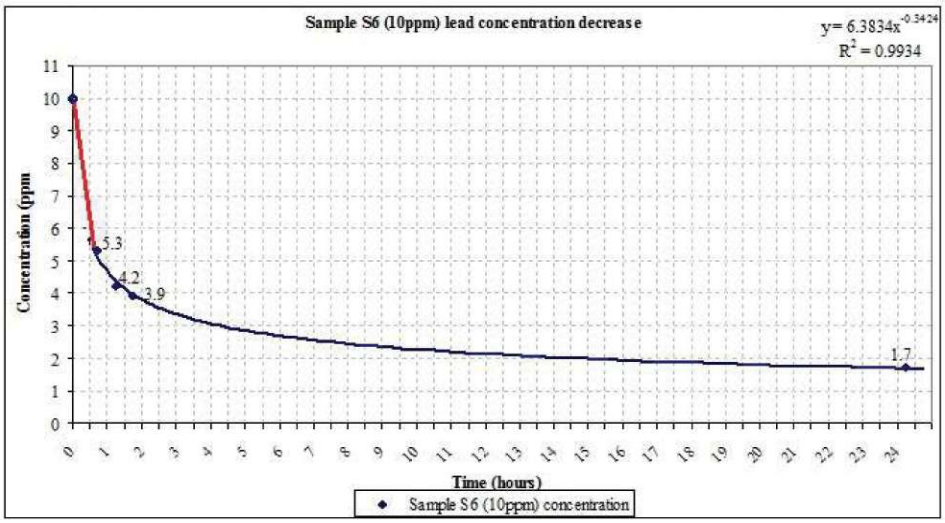
**(d)**


Sample S4 (6ppm) lead concentration decrease
y= 4.0498x^-0.494
R² = 0.993
Concentration (ppm
Time (hours)
Sample S4 (6ppm) concentration
3.1
2.2
2
0.6

**(e)**




**(f)**




**Note**: Red straight line in the graph (between values at 0 and 0.5 hours) shows a **linear decrease** whereas blue curve (between values at 0.5 and 24 hours) shows a **non-linear decrease** in lead concentration in all the samples.

Biosorption rate was very fast and contact time of 0.5 hours was enough to reach equilibrium. Post treatment of solutions, the concentration of lead was between 45 and 70 % in the first 0.5 hours, 36.7 and 50 % in 1 hour, 33.3 and 46.7 % in 1.5 hours and in 24 hours it was between 17 and 30 %. Table 5 gives the result of graph analysis.

Results also showed that biosorption was better for higher lead concentration samples than for lower ones. Table 6 gives the percentage decrease in concentration with time.

**Table 5:** Graph Analysis

| S. No. | Sample Name | Original Concentration (ppm) | Coefficient of determination ($R^2$) |
|---|---|---|---|
| **1.** | S1 | 1.0 | 0.9979 |
| **2.** | S2 | 2.0 | 0.9981 |
| **3.** | S3 | 4.0 | 0.9980 |
| **4.** | S4 | 6.0 | 0.9930 |
| **5.** | S5 | 8.0 | 0.9937 |
| **6.** | S6 | 10.0 | 0.9934 |

**Table 6:** Percentage Decrease in Lead Concentration with Time

| S No | Sample Name | Concentration (ppm) | Pre-treatment | After 0.5 hrs | After 1 hr | After 1.5 hrs | After 24 hrs |
|---|---|---|---|---|---|---|---|
| 1. | S1 | 1.0 | 100 % | 70 % | 50 % | BDL | BDL |
| 2. | S2 | 2.0 | 100 % | 55 % | 50 % | 47.5 % | 30 % |
| 3. | S3 | 4.0 | 100 % | 45 % | 42.5 % | 40 % | 30 % |
| 4. | S4 | 6.0 | 100 % | 51.7 % | 36.7 % | 33.3 % | 21.7 % |
| 5. | S5 | 8.0 | 100 % | 53.8 % | 42.5 % | 40 % | 18.75 % |
| 6. | S6 | 10.0 | 100 % | 53 % | 42 % | 39 % | 17 % |

Where, BDL stands for "Below Detection Limit"

**Equilibrium studies**

Biosorption studies were made in 100ml (0.1 litres) solution of lead at pH 7.0 in different Erlenmeyer flasks (500ml) with 0.02 g of minced banana peel (i.e. 0.2% w/v) by manual stirring at room temperature. Initial concentration of lead ($C_i$) was measured for different concentration solutions after calibrating the atomic absorption spectrometer. Equilibrium was attained at 1 hr, and the amount of lead taken up by banana peel at equilibrium $q_{max}$ was calculated using following equation:

$$Q_{max} = (C_i - C_e)\ (V/w)$$

Where $C_i$ and $C_e$ are the initial and equilibrium concentration of lead in solution respectively, **V** is the volume of the solution in litres and **w** is the weight of biosorbent in milligrams (Table 7**).**

**Table 7:** Amount of lead taken up by banana peel at equilibrium

| S No | Sample Name | Concentration (ppm) | $q_{max}$ (mg/g) |
|---|---|---|---|
| 1. | S1 | 1.0 | 1.5 |
| 2. | S2 | 2.0 | 4.5 |
| 3. | S3 | 4.0 | 11.0 |
| 4. | S4 | 6.0 | 14.5 |
| 5. | S5 | 8.0 | 18.5 |
| 6. | S6 | 10.0 | 23.5 |

Figure 3 shows the graph of effect of concentration on the biosorption of lead.

**Fig. 3:** Effect of concentration on the biosorption of lead onto banana peel

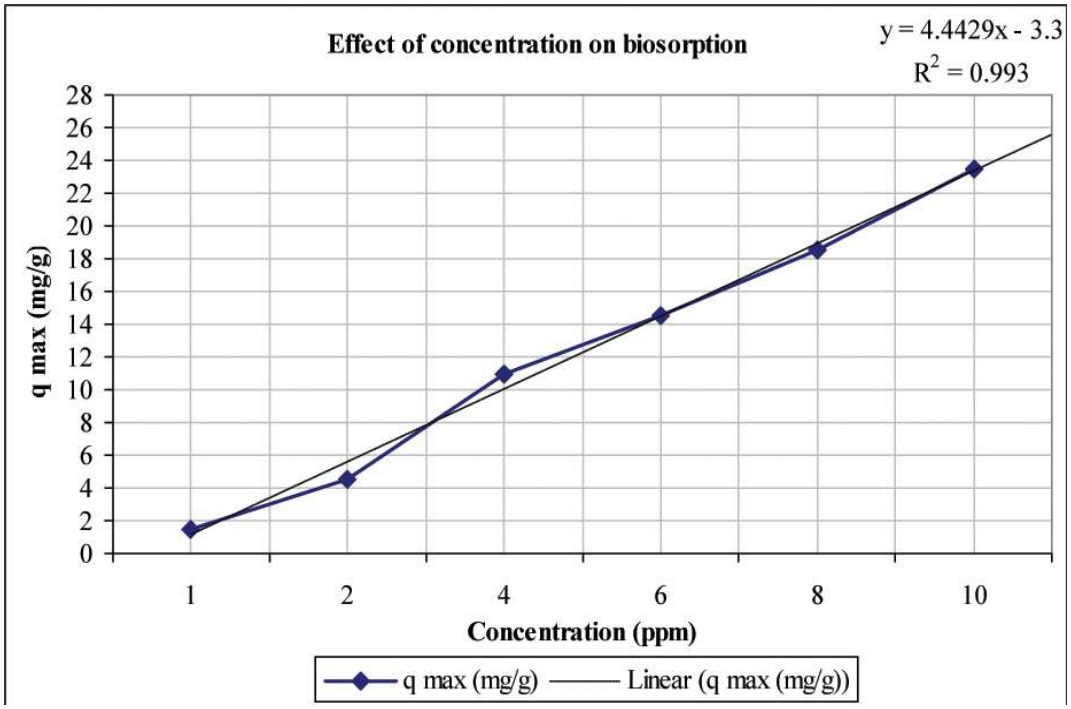


**Table 8:** Summary of statistics of lead analysis

| | |
|---|---|
| Number of samples analysed | 30 |
| Number of samples with detectable metal | 28 |
| % of samples with detectable metal | 93.33 % |
| Minimum conc. of metal ion detected (ppm) | 0.6 ± 0.0462 |
| Maximum conc. of metal ion detected (ppm) | 10.0 |

## Conclusion

Banana peels seem to be a good contender to be an ideal biosorption technology for treatment of water contaminated by heavy metals such as lead. Their ease of availability, minimal pre-treatment procedures and inexpensive use are factors that create a possibility for the development of an efficient system for the removal of lead. It may also be explored further as a technique for the purification of drinking water, especially in rural settings.

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*Original Article*

# Study of Qualitative Analysis of Phosphine in Postmortem Blood

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**Abstract**

Aluminium phosphide is widely used as fumigant and pesticide. Its widespread use is associated with increased incidence of poisoning. Its poisoning occurs directly due to ingestion and indirectly due to inhalation. After coming into contact with gastric acid on ingestion, it produces phosphine, the main active component for poisoning. In medicolegal cases, aluminium phosphide poisoning is diagnosed by the presence of phosphine in the blood and tissue samples using silver nitrate test. An issue has been raised about the postmortem production of phosphine and hence, false positive result for phosphine with the test. In this study, postmortem blood was kept stored at room temperature for varying duration without any preservative. The blood samples were analysed for presence of phosphine using silver nitrate test. Aluminium phosphide poisoning cases gave positive results. No aluminium phosphide negative case showed positive result in this study. It was concluded that silver nitrate test doesn't give false positive reaction with stored postmortem blood.

**Keywords:** Aluminium Phosphide Poisoning; Ammonium Molybdate Test; Silver Nitrate Test; False Positive Test; Hydrogen Sulphide.

## Introduction

Aluminium phosphide is a pesticide for indoor fumigation of agricultural commodities as well as pest control [1]. It is a solid fumigant and widely used to protect grain in storage [2, 3]. It is considered to be an ideal grain fumigant due to its toxic properties to all stages of insect, high potency, no effect on seed viability and no residue on food grain. Upon contact with the moisture in the environment, it releases phosphine gas which is the active pesticide component [1, 3].

Due to its easy availability and widespread use in the farming areas of India, there is a rise in the incidence of its poisoning and this is one of the most common cause of acute poisoning in India [2], especially in rural areas [3]. Acute poisoning occurs in two forms: direct ingestion or indirect inhalation. Its toxicity is mainly due to liberation of phosphine, when the ingested phosphide comes into contact with the gastric acid, and the gas is absorbed through GI mucosa and distributed to the tissues. In blood, it interacts irreversibly with free haemoglobin and RBC haemoglobin [3]. Phosphine acts as a strong nucleophilic reducing agents and inhibits cellular enzymes, especially cytochrome C oxidase [1, 3]. It also produces various superoxide radicals and cellular peroxides and leads to cellular injury [1].

Diagnosis of aluminium phosphide poisoning is done by positive history of ingestion and confirmation is done by Silver Nitrate Test performed with breath of the patient bedside and on blood, gastric content and viscera samples [2, 4].

In medicolegal cases, the viscera samples are

analyzed using Silver Nitrate Test for phosphine as it is highly specific and highly sensitive [5]. However, an issue has been raised about the false positive test result for aluminium phosphide using the Silver Nitrate test. The study has explained that false positive result may be due to postmortem production of phosphine in the stored samples [5].

This study has been performed to know whether there is any postmortem production of phosphine and consequently, false positive result of Silver Nitrate test with postmortem blood, stored at room temperature for various durations.

**Principle of the Experiment**

Aluminium phosphide reacts with hydrogen chloride gives phosphine gas which precipitates as black silver phosphide with silver nitrate.

$$AlP + 3HCl \rightarrow AlCl_3 + PH_3\uparrow$$

$$PH_3\uparrow + 3A_gNO_3 \rightarrow A_{g3}P\downarrow + 3HNO_3$$

Hydrogen sulphide also reacts with silver nitrate and gives black precipitates of Silver sulphide, a false positive result.

$$2A_gNO_3 + H_2S\uparrow \rightarrow A_{g2}S\downarrow + 2HNO_3$$

To avoid hydrogen sulphide to react with silver nitrate, seal the mouth of flask with cotton soaked with concentrated solution of lead acetate to trap hydrogen sulphide. Lead acetate reacts with $H_2S$ and produces black lead sulphide which deposits on the soaked cotton.

$$Pb(CH_3COO)_2 + H_2S\uparrow \rightarrow PbS\downarrow + 2CH_3COOH$$

In this way, hydrogen sulphide cannot react with Silver Nitrate and false positive reaction can be avoided.

To confirm the blackening is due to presence of phosphine, Ammonium Molybdate test is done with blackened Whattman's paper, producing canary yellow colour of Ammonium phosphomolybdate $(NH_4)_3PO_4.12MoO_3$.

**Material and Methods**

In the present study, femoral blood samples from autopsy cases were analyzed for presence of phosphine. For the study, 38 cases with poisoning of Aluminium phosphide and 40 cases without any poisoning were taken. The cases were kept stored at room temperature without any preservative for various duration i.e. 2 days, 1 week, 1 month, 3 month, 6 month, 12 month and 18 month as shown in the table 1. Some samples were analysed on the day of collection. To avoid loss of phosphine present/formed in the samples, the samples were open only once during the study and then discarded. No sample has been used twice for phosphine analysis.

*Chemicals/reagents*

Silver nitrate from Qualigens, India; suprapure water from Rions, India; ammonium heptamolybdate tetrahydrate, lead acetate, hydrochloric acid and nitric acid from Merck, India were used.

*Glasswares*

Conical flask from Borosil, India was used.

*Miscellaneous*

Whattman's filter paper andcotton.

*Preparation of reagents*

- Silver Nitrate solution–1g of silver nitrate salt is dissolved in 10 ml of distilled water.
- Lead Acetate solution–saturated solution of lead acetate is made up in distilled water.
- Ammonium Molybdate solution–10 N sulphuric acid solution is dissolved in 100ml distilled water.2.5 gm ammonium molybdate is dissolved in 30 ml of distilled water. 20 ml of the prepared sulphuric acid solution is added to the ammonium molybdate solution. Total solution is made up to the 100 ml by adding distilled water.

*Steps of the experiment*

1. Lead acetate impregnated cotton was prepared by dipping pieces of cotton in freshly prepared saturated solution of lead acetate and air dried.
2. Silver nitrate impregnated paper was prepared by putting few drops of freshly prepared silver nitrate solution on the Whattman's filter paper and spread it to over enough areas to cover the mouth of flask. Paper was air dried but not in direct sunlight.
3. In a conical flask 5ml blood was taken. 1ml of distilled water and few drops of hydrochloric acid were added to it. Neck of flask was packed with lead acetate impregnated cotton pieces. Mouth of flask was covered with silver nitrate impregnated Whattman's filter paper (figure 1)

and the arrangement was heated for 5-10min at $50^0$C. Colour deposition over the cotton and paper were examined. Positive reaction was shown by blackening of the filter paper (figure 2).

4. Blackened filter paper was cut in small pieces and heated for few minutes with diluted nitric acid. The extract was evaporated to the dryness for 2-3 times. Concentrated nitric acid was added to the residue followed by freshly prepared ammonium molybdate reagent Formation of canary yellow colour confirmed presence of phosphide in blood (figure 3).

**Fig. 1:** Conical flask containing blood and $AgNO_3$ impregnated paper on its mouth

**Fig. 2:** Blackening of $AgNO_3$ impregnated paper showing presence of Phosphine

**Fig. 3:** Canary yellow colour with ammonium Molybdate due to presence of phosphine

**Resultand Discussion**

The tests were done with standard procedure for phosphine with lead acetate impregnated cotton trapping of hydrogen sulphide. Interpretation of the result was done as follows:

(i) Blackening of silver nitrateimpregnated Whattman's paper only is considered positive reaction for presence of phosphine,

(ii) Blackening of both silver nitrateimpregnated Whattman's paper and lead Acetate cotton are considered positive reaction for presence of both phosphine and hydrogen sulphide,

(iii) Blackening of lead acetate cotton only is considered positive reaction for presence of hydrogen sulphide, and

(iv) Positive result is confirmed by ammonium molybdate test showing canary yellow colour with presence of phosphine.

Table 1 shows the result of the study. With all the cases with aluminium phosphide poisoning, silver nitrate test gave positive results which were later confirmed by ammonium molybdate test. The cases without aluminium phosphide poisoning gave negative results with silver nitrate.

We have also repeated the same test with decomposed blood but without lead acetate impregnated cotton trapping forhydrogen sulphidein 2 cases and they gave false positive result (figure 4). However, it gave negative result with ammonium molybdate test which showed that it was due to hydrogen sulphide production in the stored blood and not due to phosphine (figure 5).

**Table 1:** Result of the Silver Nitrate Test for Phosphine

| Duration of preservation | Aluminium Phosphide Positive Cases | | | Aluminium Phosphide Negative Cases | | |
|---|---|---|---|---|---|---|
| | Total case | Spot Test Positive | Spot Test Negative | Total case | Spot Test Positive | Spot Test Negative |
| 0 days | 4 | 4 | 0 | 5 | 0 | 5 |
| 2 days | 5 | 5 | 0 | 4 | 0 | 4 |
| 1 week | 5 | 5 | 0 | 5 | 0 | 5 |
| 1 month | 5 | 5 | 0 | 6 | 0 | 6 |
| 3 month | 5 | 5 | 0 | 6 | 0 | 6 |
| 6 month | 4 | 4 | 0 | 5 | 0 | 5 |
| 12 month | 5 | 5 | 0 | 4 | 0 | 4 |
| 18 month | 5 | 5 | 0 | 5 | 0 | 5 |

**Fig. 4:** spot test with decomposed blood witouth and with lead acetate trapping

**Fig. 5:** negative Molybdate teat with decomposed blood due to $H_2S$

This study was performed to know if there is any postmortem production of phosphine, and subsequently, false positive result with blood using standard Silver Nitrate Test. In this study, we had stored the femoral blood samples from both the aluminium phosphide poisoning cases and from cases without any poisoning. The samples were stored at various duration, upto 18 months at room temperature without any preservative. All the samples were opened only for once and then discarded to avoid any loss of phosphine.

During this study there was no false positive result for phosphine with standard silver nitrate test with lead acetate impregnated cotton trapping of hydrogen sulphide. False positive result is given by hydrogen sulphide produced during the storage but can be confirmed by negative ammonium molybdate test. To avoid this false positive phenomenon, lead acetate impregnated cotton trapping of hydrogen sulphide method has been used.

## Conclusion

This study concludes that silver nitrate test give positive result with blood only if there is antemortem presence of phosphine. Stored and decomposed blood samples don't give false positive result for phosphine. The false positive result is only due to produced hydrogen sulphide during decomposition.

## Limitation

This study was done on small number of samples; a large scale study is needed to confirm the findings.

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*Review Article*

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# Virtopsy: A New Innovation for Forensic Science

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**Abstract**

Modern cross sectional imaging techniques can supplement and may even partially replace traditional autopsy. The century old techniques are still being used, Virtopsy is one step ahead. The aim of the Virtopsy is to validate new approach by systematically comparing the radiologic and surface scanning findings with those obtained at traditional autopsy. Virtopsy consists of body volume documentation and analysis using CT, MR imaging, and micro-radiology; and 3D body surface documentation using forensic photogrammetric and 3D optical scanning. The new method should be able to help determine whether death was the result of natural causes, accident, suicide, or homicide. This paper is aimed to discuss a few points that to what extend Virtopsy is beneficial in the field of Forensic Science.

**Keywords:** CT; MR Imaging; 3D Surface Scanning; Virtopsy; Autopsy.

## Introduction

Virtopsy is a portmanteau of virtual and autopsy. Word virtual is originated from the Latin word virtus, (means "useful, efficient, and good") and Autopsy is combination of the classical Greek terms autos ("self") and opsomei ("I will see"), means "to see with one's own eyes". Thus Virtopsy is stand for the innovative combining of the power of the virtual world in the form of graphics and the usefulness to researchers and forensic science of the autopsy into a powerful technique to help investigators determine the circumstances surrounding an individual's death[14]. Virtopsy, the name of the process registered by the research team in Bern, Switzerland, is a scalpel-free procedure, latest technology to provide a complete three-dimensional view of the inside and outside of the body [7]. Currently manner of death can be determined by Virtopsy whereas cause and mechanism are potential possibilities as the technology improves. Vitropsy which combines very powerful scanning and radiographic technology with the power and resolution of modern computing, is a promising tool that complements the information and discoveries of investigators, doctors, and forensic pathologists when used in combination with traditional autopsies to help identify the manner and cause of death in individuals, more quickly and effectively discover important clues without the need to physically dissect the cadaver.

The aim of Virtopsy is to establish an observer independent, objective and reproducible method using modern imaging technology, leading to minimally invasive "Virtual" autopsy analogous to "Keyhole Surgery" in clinical medicine.

Digital autopsy is a software based procedure utilising the power of imaging and visualization to conduct autopsy on a digital body generated using raw data from whole body scanning by Multislice Computerised Tomography (MSCT) and high performance computing system. Virtopsy has a

scientific and technological background. It is scientific because of autopsy and related forensic medicines and technological due to virtual or digital characteristic.

**The Virtopsy laboratory consists**

- Photography and 3D Optical Scanner Scanning
- Multi-slice CT and MRI
- Dentistry and fingerprinting
- Heart lung machine and CT Angiography
- Image-guided Dissection and Robotic Image-guided biopsy
- Histopathology and Cytology
- Toxicology, Biochemical and Molecular Studies

**Virtopsy Process**

The first step in performing a Virtopsy is to prepare the body for imaging. The markers are used by the computer processors to calibrate the surface scan of the corpse and match it to the later internal imaging processes. After the markers are placed the Virtibot takes a 3D colour model to the body. This scan uses stereoscopic cameras to capture the colour image and a projector to cast a mesh pattern on the body. The resolution of the cameras is 0.02 millimetres [10]. Once this image is created the picture can be manipulated on a computer screen so the researchers or investigators can turn it and identify tattoos or other surface marks. Virtibot has replaced the need of having human operators place tripods and cameras at various points around the body. This is because the robot glides over the body creating a 3d image. This process, using Virtibot, takes as little as 10 seconds.

After the surface scan the body is brought to the Computed Tomography and MRI labs usually double-wrapped inside a blue bag through which X-rays can easily pass and laid on the sliding table of the CAT, MRI and MRS equipment. The bag will remain closed while the body is scanned both to respect privacy of the dead, maintain cleanliness of the surroundings and not to disturb any non-forensic personnel in the room [11]. The body then undergoes a computed tomography (CT) scan, a process which takes about 20 seconds and creates up to 25,000 images, each one a slice through the body. After this it is also subjected to MRI and MRS scans. The information from all the internal and external scans is fed to powerful desktop computers where the data is combined and rendered using CAD-style programs and ultra-powerful graphics processors.

The Virtibot consist of:

"Heart Lung machine used for post-mortem angiography with an artificial circulation"

"MSCT scanner with Fluoroscopy, MRI machine and two external network connected workstations"

"3D surface scanner – an optical measuring machine based on the principle of triangulation"

"CT-image guided biopsy system–the radiological imaging has to be supplemented by careful tissue sampling to facilitate further examination"

"The anonymity of the deceased is preserved by wrapping the corpses in artefact free body bag"

**Advantages and Disadvantages of Virtopsy**

There are powerful positive and significant negative elements to discuss. Among the positive aspects of Virtopsy are the process allows a pathologist to detect internal bleeding, bullet paths, and hidden fractures that are currently difficult to isolate. The CT and MRI scans Highlight emboli- air bubbles that obstruct blood vessels. This evidence can vanish as soon as the pathologist slices open a vein or organ and have had pathologist call for underwater autopsies so they can see the air escaping. Magnetic Resonance Spectroscopy (MRS) combined with MRI might be helpful in determining the time of death (5,8,12). The Virtopsy scan makes it easier to detect as pirated or inhaled water and blood in the lungs. These sings tell a pathologist that a victim was alive when he entered the water or sustained the injury. The scan also allows the pathologist to efficiently find bullet or other fragments in the body as the scans show exactly where they are located without having to cut open the body and search for them (4). Uniform documentation of findings will increase the quality of the evidence presented in court by experts. Increased understandability- the availability of 2D and 3D reconstructions will impressively improve the clarity and will play an important role in the acceptance of the evidence. Virtopsy reduces the stress of the deceased person's family members and friends. Alternatives for cultures where conventional autopsy is forbidden virtual autopsy will allow medical legal examinations in cultural circles. The method could be useful in the examination of highly infectious bodies. No scalpel method, no hazard of infections from the blood and other tissue fluids. No mutilation of the body, so, can be examined again without any autopsy artefacts.

*Use of imaging data in forensic autopsies*

MSCT and MRI useful for; severe crushing, decomposition, bullet paths, vascular injuries, drowning, gas embolus, foreign bodies, lung and brain, trauma documentation, dissection planning, limited autopsy.

*Post mortem imaging (hospital death)*

The Virtopsy makes sense from a forensic standpoint, identification of victims/ remains, firearm deaths(location and retrieval of projectiles), child abuse/ non accidental injuries(skeletal surveys), barotraumas or suspected air embolism, traumatic subarachnoid haemorrhage and other complex cases where the examination and interpretation are compromised by destruction of the body. Studies of child abuse victim confirm the sensitivity of post mortem MRI for contusion shearing injuries and subdural hematoma. There is a great future for non-destructive analysis of visceral pathology, such as cardiac (including Coronary), pulmonary and hepatic disease.

On the other side it has a number of troubling limitations. The Virtopsy is inadequate to the task of diagnosing natural causes of death, poisonings, infections or heart failure and difficult to differentiate ante-mortem or the post mortem wounds. Small tissue injuries may not be seen. The equipment is prohibitively expensive. The scans cannot determine infectious agents or tumour types. We cannot establish colour of organs and detect discharge from the vascular system. Another problem is more complicated, specific software for MRI and MSCT.

## Conclusion

The present day subjective protocoling of autopsy findings can be replaced by a uniform and observer-independent radiological documentation. This will increase the Forensic medicine reconstruction of an event is only possible by means of the exact determination of findings, head to toe, accompanied by precise geometric ordering that is understandable to lay people. Moreover, and very importantly, Virtopsy is useful in situations of religion, the law or cultural mores that prohibits invasion of the body without upsetting or angering a family. One very important use of the technology will be in the quality of evidences presented in courts by experts as the expert witnesses discuss evidence found on bodies of crime victim bloodless and likely less graphic way [11].

Nevertheless it is a new development in the field of investigation of death, but still it has a long way to go to establish itself as an alternative to the conventional autopsy. In near future, we all will be accustomed to some kind of virtual autopsy which will be beneficial for the court as well as the autopsy surgeons and the relatives of the deceased.

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# Forensic Importance of Cosmetics and Cosmeceuticals

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**Abstract**

The branch of science which deals with the study of cosmetics is known as *cosmetology*. Women love wearing makeup, as they feel it can help them enhance their beauty and make them feel and look more attractive and gorgeous. Further, according to Locard's Principle of Exchange, "every contact leaves a trace". Many of the violent crimes such as assaults, robberies, rapes and murders involve direct contact between the assailant and a female victim. Well known examples of some trace or associative evidences are hair, fibers, paint chips, broken glass fragments, soil particles, etc. Thus, a transfer of some type of cosmetic product is possible and, consequently, the clothing or body of the suspect may bear smeared traces of a cosmetic. The analysis of these smudges can provide circumstantial evidence connecting a suspect and victim or placing a suspect at the crime scene. This review is an effort for enlisting of various cosmetics and cosmeceuticals which may helpful in solving crimes against women.

**Keywords:** cosmetics, cosmeceuticals, forensic investigation, chemical evidences

## Introduction

The use of cosmetics is worldwide and dates from the remotest antiquity. People of all ages are aware of their appearance and give a lot of attention on grooming their looks. Cosmetics is a general term applied to all preparations which are intended to be applied externally to beautify and condition the body by cleaning, coloring, softening or protecting the skin, nail, hair, lips or eyes. "*The branch of science which deals with the study of cosmetics is known as cosmetology.*" *As per Oxford Dictionary,* a cosmetic is defined as "a preparation applied to the body, especially the face, to improve its application. The range covers everything from the latest cosmetics to skin and hair care".

According to *Drugs and Cosmetic Act, 1940* cosmetic means "any article intended to be rubbed, poured, sprinkled, or sprayed on, or introduced into, or otherwise applicable to, the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance, and includes any article intended for use as a component of cosmetic".

## Different Kinds of Cosmetics

Women love wearing makeup, as they feel it can help them enhance their beauty and make them feel and look more attractive and gorgeous. While some cosmetics are useful for making the skin feel soft, healthy and flawless, others assist in adding color to the skin, thereby giving the appearance of good health and youth. Some different types of cosmetics are given below:

### *Solutions*

The simplest kind of cosmetics, solution cosmetic

is a homogeneous mixture of soluble ingredients. These are prepared by simply filling the containers with main diluent (usually water) and mixing with the other ingredients to create the resultant cosmetic product. Examples: shampoos, body wash, hand cleansers, mascaras, eye liners, colognes, and so on.

*Creams/Emulsions*

Most of the cosmetics contain raw materials that are not compatible. Hence, creams or emulsions are preferred. These are pseudo stable mixtures of immiscible liquids dispersed in another liquid. Creams and emulsions are prepared by combining three formula components, such as oil phase, aqueous phase and an emulsifier. Examples: cosmetics like hand moisturizers, make up, hair conditioners, sunscreens, etc.

*Lotions*

Lotions are less greasy and lighter counterparts of creams that come in handy for applications, wherein creams cannot be used. Lotions are basically thin creams that undergo the same production procedure as that of creams. Moreover, they can easily be applied without worrying about them getting thick as opposed to emulsions that get thick on cooling down. Examples: facial moisturizers, leave-in hair conditioners and moisturizing cleansers.

*Suspensions*

Suspensions are cosmetics that are used for overcoming incompatible ingredients. Similar to creams, suspensions are clear solutions containing visible particles, such as gelatin beads or inorganic minerals, spread throughout. Examples: sunscreens, hand washes and shampoos are some such examples.

*Ointments/Pastes*

Ointments or pastes are extremely thick products used for dressing hair and cleaning skin. They are, generally, anhydrous (contain no water), sticky and greasy.

*Powders*

Powders are the most common and popular form of cosmetic products. They are an amalgamation of solid raw materials which are ground together to get a fine powder. Examples: products like baby powder, eye powder, foot powder, talcum powder, etc.

*Gels*

Gels are thick, clear products characterized by a property known as "shear thinning". Examples: hair products, body washes, shaving products and toothpastes.

*Sticks*

Sticks enter the cosmetic product list when consumers look out for cosmetics that they would not want to touch, say, lipstick or underarm deodorant.

*Tablets & Capsules*

Color cosmetics are generally found in the form of cakes, tablets or capsules. The solid ingredients are blended well with one another and pressed to get the desired shape. Examples: compact powder, eye shadow, cheek shadow cakes, etc.

**Generally Available Cosmetics in Market**

Cosmetic products have a history covering thousands of years with the use of many ingredients from plants, animals and mineral sources. Generally available cosmetic products in market are:

(a) Oral care products include toothpaste, toothpowder and mouthwash.

(b) Hair care products include shampoos, conditioners, serums, hair oils and sprays.

(c) Skin care products include lotions, moisturizers, sunscreens, cold creams, cleansers, face wash.

(d) Lip care products include lipsticks, lip gloss, lip balms, lip liners.

(e) Other miscellaneous products include nail lacquers, nail removers, kohl, anti-perspirants, etc.

But, once the product claims venture into diagnosis, treatment, prevention of any disease, the product is considered to be a '*DRUG*'. Therefore, products can be both cosmetic as well as drug if it fulfills the intended use.

Now-a-days a new term is gaining much more importance in the field of cosmetology i.e. '*COSMECEUTICALS*'**.** These are cosmetic products with biologically active ingredients purporting to have medical or drug-like benefits.

Cosmeceutical is a pragmatic term that enables us to state without pretense the benefits of a product. The FD & C Act, 1938 defines cosmetic by its intended use, meaning cleansing, beautifying, promoting attractiveness or altering appearance. Products in this

category include lipsticks, perfumes, skin moisturizers, nail polish, shampoos, deodorants, and other beautifying products.

Although there is no legal class called cosmeceuticals and the designated products stand at the border line of cosmetics and drugs. The cosmeceutical label applies only to products which are used topically, such as creams, lotions, and ointments.

### Types of Cosmeceuticals

On the basis of active ingredients present, cosmeceuticals are:

A. *Antioxidants*: prevent risk of sun damage to skin.

B. *Peptide*: stimulates the production of collagen and thickens the skin.

Growth Factors–chemical messengers between cells playing a key role in cell division, blood vessel growth as well as in production and distribution of collagen and elastin.

C. *Combination Product:* includes multiple antioxidants, retinol plus antioxidants, growth factors plus vitamin C or other unique combinations.

### An Industrial Overview of Cosmetics in India

The cosmetic and personal care industry has been growing at an average rate of about 15% for the last few years. This is mainly accounted from low and medium priced category products, comprises of about 90% of cosmetic market, in terms of volume.

The rising demands for cosmetic products, particularly in urban population owes to numerous reasons like mall culture, better purchasing power, awareness towards looking better, by both men and women, nature of occupation, development in advertisement technology (thanks to celebrities and people of glamour world !!) and in whole, availability of wide range of cosmetic products- from head to toe. In general, India is a very price sensitive market (Nanda *et al,* 2005).

### Indian Standards

According to BIS (Bureau of Indian Standards), all the products shall meet the requirements pertaining to safety, quality and performance. These standards can be utilized in their forensic detection and identification. Table 1 describes the list of products with their IS code number.

**Table 1:** List of Products with their IS Code Number

| S.No. | Items | Is Code No. |
|---|---|---|
| 1. | Skin Powder | IS 3959:1978 |
| 2. | Skin Powder For Infant | IS 5399:1978 |
| 3. | Tooth Powder | IS 5383:1978 |
| 4. | Tooth Paste | IS 6356:1993 |
| 5. | Skin Cream | IS 6608:1978 |
| 6. | Hair Oil | IS 7123:1978 |
| 7. | Shampoo Soap Based | IS 7669:1990 |
| 8. | Shampoo Synthetic Detergent Based | IS 7884:1992 |
| 9. | Hair Cream | IS 7679:1978 |
| 10. | Oxidation Hair Dye Liquid | IS 8481:1993 |
| 11. | Cologne | IS 8482:1997 |
| 12. | Nail Polish (Nail Enamel) | IS 9245:1993 |
| 13. | After Shave Lotion | IS 9255:1979 |
| 14. | Pomades And Brilliantines | IS 9339:1988 |
| 15. | Depliatories Chemicals | IS 9636:1988 |
| 16. | Shaving Cream | IS 9740:1981 |
| 17. | Cosmetic Pencil | IS 9832:1981 |
| 18. | Lip Stick | IS 9875:1990 |

### Chemicals Commonly Used in Cosmetics

Now days, cosmetics are made from a range of ingredients which are deemed to be industrial chemicals. Industrial chemicals may be either synthetic chemicals or naturally occurring chemicals.

*Alcohol – isopropyl alcohol*

Isopropyl alcohol, also known as isopropanol, is an alcohol that evaporates quickly. Isopropyl alcohol is a widely used ingredient in cosmetics and personal care products and can be found in products such as aftershave lotions, bath products, eye makeup, other makeup products, cleansing products, as well as nail, hair and skin care products.

*Cetyl alcohol*

Extracted from coconut oil, it is an emollient that is included in skin care products to stabilize the formulations or to alter their consistencies, or to increase their foaming capacity. It is often included in baby lotions, hand creams, foundation, lipsticks, shampoos, mascara, deodorants, nail polish removers etc.

*Stearyl alcohol*

Stearyl alcohol is also derived from coconut oil. Because it is an emollient as well as an emulsifier, it can be substituted for cetyl alcohol to firm skin care formulations. It is mostly found in creams, lubricants, depilatories and conditioners.

*Cetearyl alcohol*

Cetearyl alcohol is an emulsifying wax that is used to soften thick formulas like skin ointments. Derived from natural oils and fats, it is very efficient in stabilizing skin care formulations because it imparts an emollient feel to the skin. It can be used in water-in-oil emulsions, oil-in-water emulsions and anhydrous formulations. Cetearyl alcohol is widely used in cleansers, permanent hair color, face creams, eye make-up and sunblocks.

*Ammonium lauryl sulfate*

Ammonium lauryl sulfate (ALS) is an anionic surfactant used in cosmetics such as cleansing agents, hair shampoos, bubble baths and hair bleaches.

*Butylated hydroxytoluene*

Butylated hydroxytoluene (BHT) is used as an antioxidant in food, animal feed and cosmetics. Primarily acts as an antioxidant food additive because of its ability to preserve fats. In cosmetics, it is also used as a preservative. As an antioxidant, it helps fight against the deterioration of cosmetic products caused by chemical reactions with oxygen. It is mostly used in makeup products such as eyeliner, lipstick, blush and foundation, but you can also find it in various other cosmetic products like moisturizer, cleanser and perfume.

*Triethanolamine, Diethanolamine & Monoethanolamine*

Triethanolamine, diethanolamine and ethanolamine are clear, colorless, viscous liquids with ammonia-like odors. In cosmetics and personal care products, triethanolamine is used in makeup products such as eyeliners, mascara, eye shadows, blushers, make-up bases and foundations, as well as in fragrances, hair care products, hair dyes, wave sets, shaving products, sunscreens, and skin care and skin cleansing products. Diethanolamine and ethanolamine are used mostly in permanent waves and hair dyes and colors.

*Ethoxylated Surfactants–PEG*

1, 4-dioxane is produced in trace amounts as an unwanted by-product in the manufacture of ethoxylated substances (for example, as impurity in PEG). Polyethylene glycols (PEGs) and their anionic or nonionic derivatives are widely used in cosmetics as surfactants, cleansing agents, emulsifiers, skin conditioners, and humectants. Exposure to trace amounts of 1,4-dioxane can potentially occur when using products that contain ethoxylated substances such as detergents, cosmetics, toiletries, etc.

*Formaldehyde*

Formaldehyde is a colorless, flammable gas often used in cosmetics. It is most commonly used as a water solution called formalin, rather than in its pure form. With the help of preservatives, formaldehyde is released in small amounts over time to help protect cosmetic products against contamination by bacteria during storage and during continued use. Formaldehyde can be found in nail polishes, nail hardeners, eyelash glues, hair gels, soaps, makeup, shampoos, lotions, and deodorants, among other products.

*Imidazolidinyl urea*

Imidazolidinyl urea is an odorless white powder. In cosmetics and personal care products, Imidazolidinyl urea can be found in many product types including lotions, creams, hair conditioners, shampoos and deodorants.

*Lanolin*

Lanolin is an ointment-like material isolated from

wool that is sheared from sheep. Lanolin can be separated into lanolin oil, a liquid phase, and lanolin wax a solid phase. Heating Lanolin with water produces a mixture of organic acids (lanolin acid) and a mixture of organic alcohols (lanolin alcohol). Lanolin and its related ingredients are widely used in the formulation of cosmetics and personal care products. These ingredients can be found in baby products, skin care, shaving, manicuring, hair care, suntan and sunscreen products, as well as eye, lip and facial makeup.

*Lead*

Lead is a bluish-gray, heavy metal that occurs naturally in the Earth's crust and is present in trace amount in the environment, in numerous foods and in some natural products. Lead can be present in nearly all things we use and consume on a daily basis, including food and cosmetics. The toxicity of lead compounds is mostly related to the lead portion in the compound.

*Mercury*

Mercury is a common ingredient found in skin lightening soaps and creams. It is also found in other cosmetics, such as eye makeup cleansing products and mascara. Mercury salts inhibit the formation of melanin, resulting in a lighter skin tone. Mercury in cosmetics exists in two forms: inorganic and organic. Inorganic mercury (e.g. ammoniated mercury) is used in skin lightening soaps and creams. Organic mercury compounds (thiomersal [ethyl mercury] and phenyl mercuric salts) are used as cosmetic preservatives in eye makeup cleansing products and mascara.

*Mineral oil/waxes – including liquidum paraffinum, paraffin oil, paraffin wax*

White mineral oil is a mixture of liquid hydrocarbons, essentially paraffinic and naphthenic in nature. It is obtained from petroleum, and is intensively refined following several steps including atmospheric and vacuum distillation, removal of aromatic and unsaturated compounds, de waxing and further processing. Some petroleum oil derivatives may contain polycyclic aromatic hydrocarbons, some of which are known to be carcinogens. Only the highest purity medicinal-grade white mineral oil, with extremely low levels of harmful hydrocarbons, is used in cosmetics.

*Oxybenzone*

Oxybenzone (also called "benzophenone-33 or "BP-33 ) is an organic compound used as an ingredient in sunscreens because it absorbs UVB and UVA rays. The ingredient easily dissolves into lotions and creams. Oxybenzone is also used in lip balms, lipstick, moisturizers, anti-aging creams, conditioners, and fragrances.

*Paraben preservatives: methyl, propyl, butyl, and ethyl*

Paraben preservatives are widely used in makeup, moisturizers, shampoos and conditioners, and shaving products but they have hormone-like activities. Parabens are chemicals that slow down the growth of mold in personal care products, pharmaceuticals, and foods.

*Phthalates: diethylphthalate (DEP) and dimethylphthalate (DMP)*

Phthalates are a group of chemicals that are used predominantly as solvents and plasticisers (plastic softeners) in both industrial and consumer products. There are many phthalate chemicals in use worldwide.

*Propylene/butylene glycol*

Propylene glycol is widely used as a food additive and in pharmaceutical preparations.

*Sodium lauryl sulfate*

Sodium lauryl sulfate (SLS) is a widely used surfactant. In cosmetics and personal care products, sodium lauryl sulfate is used primarily in shampoos, bath products, hair colorings, facial makeup, deodorants, perfumes, and shaving preparations, but can also be found in other product formulations. Sodium lauryl sulfate cleans the skin and hair by helping water to mix with oil and dirt so that they can be rinsed away.

*Toluene*

Toluene is a clear liquid with an aromatic odor. In cosmetics and personal care products, the use of toluene is limited to nail products. Toluene is used as a solvent to dissolve other substances, such as resins and plasticizers, used in the formulation of nail products.

Apart from the above mentioned chemicals, some more chemicals and their approximate concentrations are given in Table 2. These chemicals can be chemically analyzed in cosmetic products for evidential value.

**Table 2:** List of Chemicals Commonly Used in Cosmetic Products

| S/No. | Substance | Max. Concentration |
|---|---|---|
| 1 | Chlorates of Alkali Metal | 5% |
| 2 | $H_2O_2$ | 0.1% $H_2O_2$ Present or Release |
| 3 | Ammonium Monofluorophosphate | 0.15% Calculated as "F" when mixed with other Fluorine compound, Total F Conc. must not exceed 0.15% |
| 4 | Sodium Monofluorophosphate | 0.15% |
| 5 | Potassium Monofluorophosphate | 0.15% |
| 6 | Calcium Monofluorophosphate | 0.15% |
| 7 | Calcium Fluoride | 0.15% |
| 8 | Potassium Fluoride | 0.15% |
| 9 | Sodium Fluoride | 0.15% |
| 10 | Ammonium Fluoride | 0.15% |
| 11 | Magnissium Afluoride | 0.15% |
| 12 | Aluminium Fluoride | 0.15% |
| 13 | Stannous Fluoride | 0.15% |
| 14 | Hexadecyl Ammonium Fluorode | 0.15% |
| 15 | 3-(N-Hexadecyl-N-2hydroxyethyammonia) Propybis (2-Hydroxyethyl) Ammonia Dihydrofluoride | 0.15% |
| 16 | Nn'n'-Tris(Polyethylene)-N-Hexadepropylene Diamine Dihydrofluoride | 0.15% |
| 17 | Octadecenyl-Ammonium Fluoride | 0.15% |
| 18 | Sodium Fluorosilicate | 0.15% |
| 19 | Potassium Fluorosilicate | 0.15% |
| 20 | Ammonium Fluorosilicate | 0.15% |
| 21 | Magnesium Fluorosilicate | 0.15% |
| 22 | 6 Methylcoumarin | 0.03% |
| 23 | Nicomethanol Hydrofluoride | 0.15% |
| 24 | Strontium Chloride Hexahydrate | 3.5% |
| 25 | Strontium Acetate Hemihydrate | 3.5% |

**Importance of Cosmetics and Cosmeceuticals in Forensic Investigations**

With a population of over 1 billion people in India, the cosmetic and personal care industry has been growing at an average rate of about 15% for the last few years. The present article is a brief overview of cosmetics, differences between cosmetics and cosmeceuticals, and they can be utilized in forensic detection and identification.

Further, according to Locard's Principle of Exchange, "every contact leaves a trace", many of the violent crimes such as assaults, robberies, rapes and murders involve direct contact between the assailant and a female victim. Well known examples of some trace or associative evidences are hair, fibers, paint chips, broken glass fragments, soil particles, etc. Thus, a transfer of some type of cosmetic product is possible and, consequently, the clothing or body of the suspect may bear smeared traces of a cosmetic. The analysis of these smudges could provide circumstantial evidence connecting a suspect and victim or placing a suspect at the crime scene.

Forensic scientists are assigned the task to examine the physical evidence with a range of analytical techniques to potentially identify trace amounts of evidence. Analysis of cosmetic traces from crime scenes can be used to establish physical contact between two individuals, such as a victim and a suspect, or to place an individual at a crime scene. The majority of techniques which are employed in forensic investigations of cosmetics are Gas Chromatography and Fourier Transform Infrared

Spectroscopy. Current cosmetics are mass produced by a range of manufacturers, and each manufacturer lists generic ingredients on the packaging, which are common amongst all their competitors. Some of these generic ingredients include organic dyes, inorganic pigments, oils, minerals, waxes and emollients. However the quantitative composition which each manufacturer uses, usually vary. Analysis of cosmetic products, therefore require a multivariate approach. Forensic scientists use cosmetics as evidence in solving crimes. Here are some examples:

**Lipstick prints**

By comparing the composition of a lipstick smear with that of a victim, forensic scientists can demonstrate indirect proof of contact or a relationship between victim and suspect. Also, it is sometimes possible to extract saliva DNA from the print.

**Foundation smears**

Like lipstick smears, make-up foundation can easily be transferred to clothing or other surfaces just through contact. Forensic scientists can discriminate between different types by using FTIR, SEM-EDX and GC-FID analysis.

**Shampoo identifications**

Although it is thought to be a myth that your hair gets "used to" a certain shampoo, there is some evidence that components from your shampoo will accumulate in your hair. Using HPLC, forensic scientists have found that they can determine the type of shampoo that may have been used on a hair sample.

**Hair bleach interference**

Forensic scientists can use hair to determine whether a person has been taking illicit drugs. They analyze samples using GC-MS and can detect codeine, morphine, cocaine, and opiates.

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*Case Report*

# Identification of Rhodamine Dye in Rape Assault: A Case Study

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**Abstract**

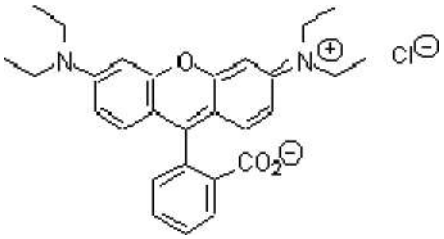
Due to rise in crowds naturally there is fall in cultural values and escalation in crimes. Crime against women is on the rise especially sexual crimes. Though crime is prevalent in every country and society, most of them .either goes undetected or unreported. Forensic chemistry plays an important role in helping early detection, providing expert scientific reports at earliest, which will result in quick trial and instant administration of justice to victims.

In the present communication a case of rape assault is reported in which Rhodamine B a cheap red dye used as a coloring agent in Holi festival identified by thin layer chromatographic analysis, ultraviolet spectroscopy and FT-IR analysis.

**Keywords:** Dye; Color; Rhodamine; Rape Assault.

## Introduction

The examination of physical evidences by a forensic scientist usually undertaken will be for comparison or identification. Identification has as its purpose the determination of the physical or chemical identity of a substance with as absolute certainty as existing analytical technique will permit. A comparative analysis subjects a suspects and a control specimen to the same tests and examination for the ultimate purpose of determining whether or not they have a comman origin. Rhodamin B dye is a cheap easily available in local markets can be a vital clue in criminal cases as happened in this case. Rhodamin B is red/brown or green crystalline compound with chemical name is Ammonium, (o-carboxyphenyl)-6-(diethylamino)-3H-xanthen-3-ylidene) diethyl-chloride having melting point: 165ÚC, molecular formula: $C_{28}H_{31}N_2O_3Cl$**,** molecular Weight**:** 479.02 the chemical structure of Rhodamine B is shown below.



Chemical structure of Rhodamine B

## Brief study of case

At the time of Holi festival parents of a thirteen year old aged girl lodged a complaint in police station that an unidentified intruder who painted his face with some dye attacked on girl with an intention to molest and rape the girl. According to girl the boy was of age of eighteen year wearing blue coloured jeans and white lined shirt having height five feet eight inches. A suspects subsequently arrested on the same day after one hour searching by I.O. was found

to have similar characteristics along with red coloured dye material sticked on the face of girl and similar type of greenish red particles was also found on the face and cloths of accused. These articles were seized and sent to the forensic science laboratory for the identification and comparison of these stains and dye material.

After through analysis the presence of Rhodamine B (a cheap dye) mostly used at the time of Holi was confirmed by FT-IR analysis on the sent articles and the contact of culprit to the victim was confirmed.

*Experimental*

All the reagents were of analytical grade distilled water were used as and when required. A standard Rhodamine B procured from local market for comparison study. A dry crystalline material scrapped from victims / accused clothing's was directly used for study.

**Thin layer Chromatography**

A standard TLC plated was coated with slurry of silica gel G in water to a uniform thickness of 0.25 mm the plate was activated by heating in an oven at 100ËºC for about 1 hour an aliquots of Rhodamine B 0.01-0.5mg in ethanol along with scrapped material were spotted on to the plate which was developed with butanol, acetic acid and water 40:10:50 (upper organic layer were used for development) in a presaturated TLC chamber to a height of 10cm. The plate was removed from the chamber dried in air in which red self-locating spot appeared at Rf 0.88 in white background.

**UV Spectroscopy**

The UV spectra were taken as Shimadzu UV spectrophotometer model 2550 the scrapped material shown ë max at 542.75 nm with strictly accordance to ë max of standard Rhodamine B dye sample.

**F.T.I.R. Spectroscopy**

F.T.I.R. spectra were recorded on Perkin Elmer one F.T.I.R. spectrophotometer instrument in the range 400 $cm^{-1}$-4000 $cm^{-1}$ of electromagnetic radiation using KBr pallet method prepared by handset die about 100 mg of spectroscopic grade dried KBr stored in decicator was used every time KBr was pulverized

**Fig. 1:** FT-IR Spectra of dye from exhibit

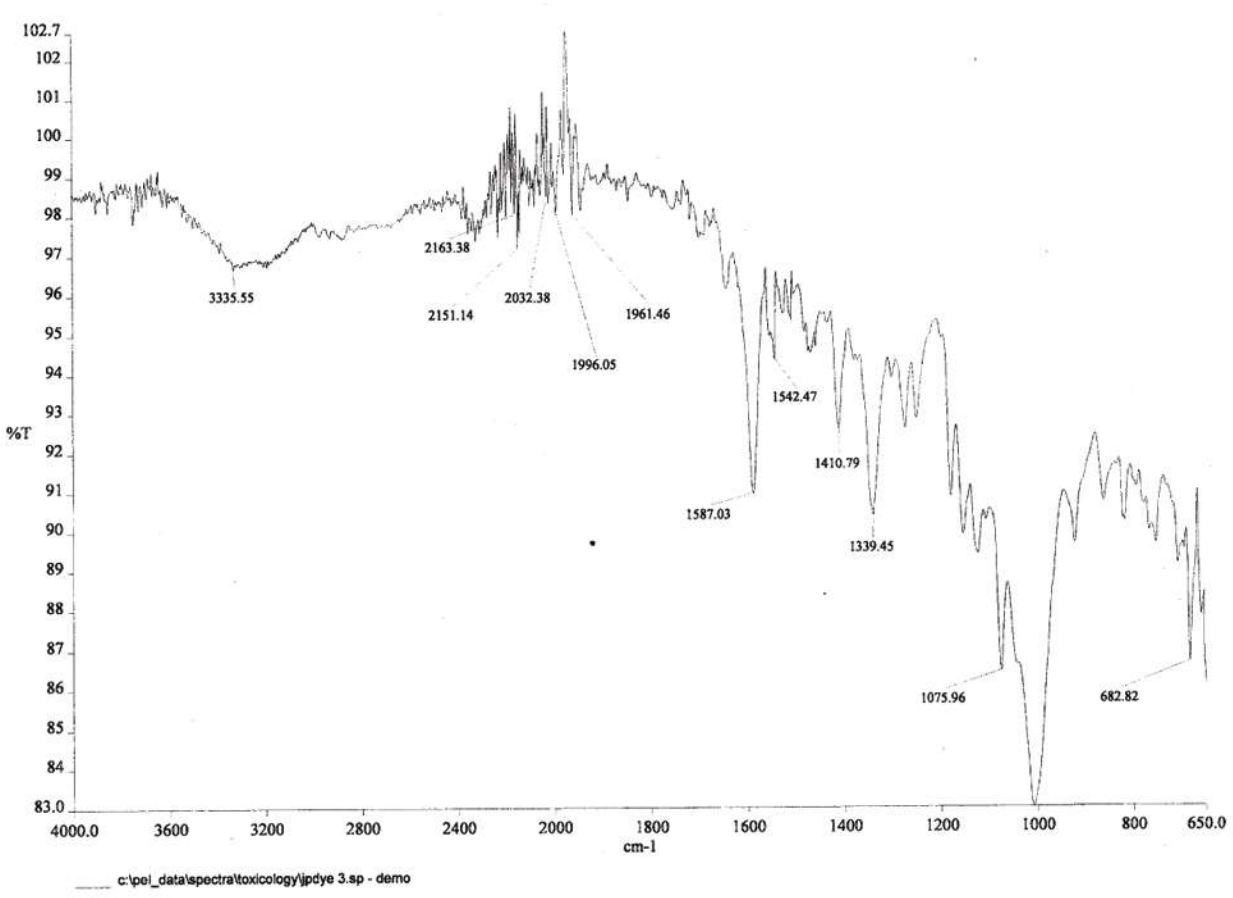
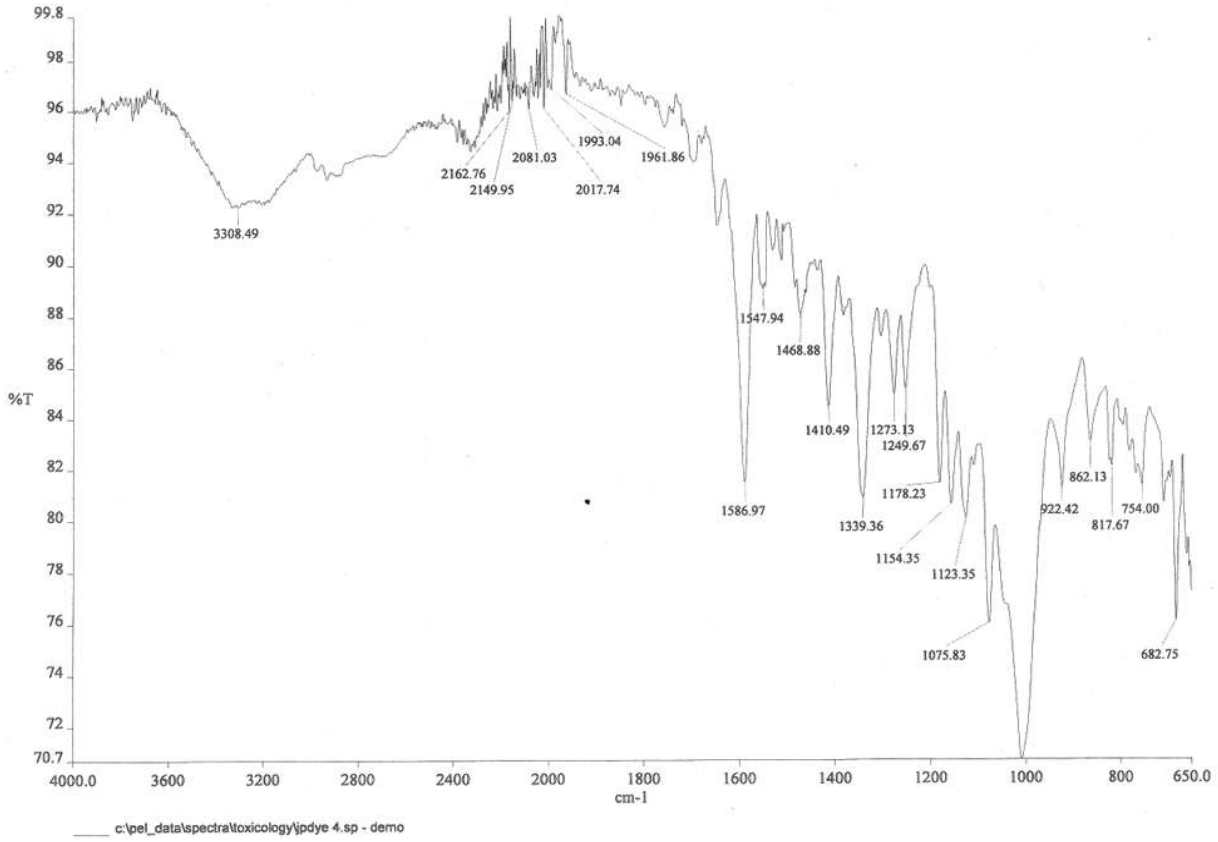


**Fig. 2:** FT-IR Spectra of standard Rhodamine B dye




using agate pastel mortar about 1mg of the dye was also mixed and ground thoroughly infrared spectra of such mixture were recorded and the obtained peaks at 3335.55, 2163.38, 2151.14, 2032.38, 1996.05, 1961.46, 1587.03, 1542.47, 1410.79, 1339.45, 1075.96, 1000 and 682.82 with strict accordance of structure of Rhodamine B.

The similar results were also obtained with scrapped sample of dye.

## Result and Discussions

The above method is rapid, sensitive and simple in day-to-day forensic analysis of chemical dyes since little information is available on analysis of this type of dye so it will add new avenue in analysis of dye samples in day-to-day analysis work.

## Acknowledgement

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