CHAPTER 2

MATERIALS AND METHODS:

n-Alkanes:

An alkane is a hydrocarbon of single bonds with general formula \( C_nH_{2n+2} \). Depending on the number of carbon atoms, they are named as, nonane (C9), decane(C10), undecane(C11), dodecane(C12), tridecane(C13), tetradecane(C14), pentadecane(C15) and hexadecane(C16). As kerosene is known to contain C9 to C16, in the present work, these straight chain hydrocarbons were used for establishing their presence and make comparisons with standards.

Gold(III) chloride (HAuCl₄·3H₂O)

Gold(III) chloride is also called auric chloride is completely soluble in water and with molecular weight 393.83 g/mol. It was a Sigma Aldrich sample.

Catechol:

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\text{Catechol} = \text{1,2-dihydroxy benzene with molecular weight 110.1 g/mol and freely soluble in water with light brown feathery, shining crystal structure.}
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Ortho Dianisidine:

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\text{Ortho Dianisidine (3,3'-dimethoxy benzidine) is also called Fast Blue B, with molecular weight 244.3 g/mol and freely soluble in ethanol.}
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Aniline sulphate:

Aniline sulphate is white colour crystal with molecular weight 284.33 g/mol and freely soluble in water.

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\text{Aniline sulphate: } \text{C}_6\text{H}_5\text{NH}_2 \cdot \frac{1}{2} \text{H}_2\text{SO}_4
\]

In the present work, o-dianisidine (3,3’-dimethoxy benzidine) reacts with gold(III) ion to form a red coloured dye having \( \lambda_{\text{max}} \) at 446 nm with the formation bisazodiphenyl [Wei Sun et al., 2005, Flavio Luiz Benedito et al., 2003, A.V.Kireyko et al., 2006],

Catechol oxidizes to form 1,2-benzoquinone [Shyla.B et al., 2012].

This benzoquinone couples with ortho dianisidine or aniline to form dyes with improved stability and sensitivity [Shyla.B et al., 2012, Simon P.Fricker, 1996] with light violet colour.
Lansoprazole:

Lansoprazole is chemically 2-((3-methyl-4-(2,2,2-trifluoroethoxy)pyridine-2-yl)methylsulfinyl)-1H-benzimidazole with molecular weight 369.4 g/mol solubly methanol and dimethyl formamide and practically insoluble in water.

Domperidone:

Domperidone is chemically 5-chloro-1-(1-((2-oxo-2,3-dihydro-1H-benzimidazole-1-yl)propyl)piperidine-4-yl)-1H benzimidazol-2-one with molecular weight 425.9, freely soluble in methanol and insoluble in water.
Diazepam

Diazepam is chemically 7-chloro-1-methyl-5-phenyl-3H-1,4-benzodiazepine-2-one, freely soluble in methanol and chloroform and its molecular weight is 284.7 g/mol.

Arsenic trisulphide (As$_2$S$_3$):

Arsenic trisulphide is bright yellow to orange colour powder with molecular weight 246.04 g/mol soluble either in hot concentrated nitric acid or sodium hydroxide solution. It was a Sigma Aldrich sample.

Spectroscopy:

The interaction of electromagnetic radiation with matter produces absorption, transmission, scattering, fluorescence, phosphorescence or Raman scattering of the incident light. Study of this phenomenon is spectroscopy. If the atoms interact with incident radiation, then the phenomenon is termed as atomic absorption or emission spectroscopy and X-ray spectroscopy. If the interaction is between the molecules and the incident radiation, then it is termed as UV-visible and infrared spectroscopy. The electrons present in the atom after absorbing energy of the incident light, gets promoted to unstable higher energy levels. Eventually, from that unstable state, electrons jump back to their original position by emitting the radiation of lower energy. The emitted radiation is always with lesser energy than the incident as some of energy is lost during the process. Depending on the energy which otherwise related to wavelength of radiation (light) different spectroscopies exists. Beer Lambert’s law which explains that with
increase in concentration of the species, amount of radiation absorbed also increases is the basis for all spectroscopic techniques and helpful in quantification of those species.

\[ A = \varepsilon c l \]

Where \( A \) is the absorance defined as \( A = \log_{10} \left( \frac{I_0}{I} \right) \), if \( I_0 \) is the intensity of incident radiation and \( I \) is the transmitted intensity after passing through the species

\( l \) is the path legth
\( c \) is concentration of the spieces or solution
\( \varepsilon \) is absorptivity or molar extinction coefficient

**Spectrophotometry:**

Study of transmission property of material as a function of concentration of species i.e. chromophones in visible or ultraviolet region of electromagnetic spectrum is spectrophotometry which deals with electron transition within molecules. Using the Beer Lambert’s law, the intensity of transmitted light or absorbance of incident light is correlated to the concentration and concentration of solutions are measured using the instrument with a photometer.

**Fourier Trnsform Infra Red spectroscopy:**

The absorption or transmission properties of either solid, liquid or gas which appears in infra red region of electromagnetic spectrum due to vibrational transition occuring within the molecule are used in FTIR spectroscopy. FTIR spectroscopy uses mathematical concept to convert the IR spectrum in a wide range of wavelength. Conventional IR spectroscopy deals with recording radiant power as a function of frequency whereas FTIR spectroscopy records radiant power as a function of time. The interferrograms of FTIR converts time to frequency [S.D.Sawant et al., 2011].

**Energy Dispersive X-Ray Fluorescence Spectroscopy (EDXRF):**
It is possible to characterize a sample by knowing the elemental profile obtained with the help of EDXRF spectrometer. When high energy electron beam (X-ray) falls on the specimen, the atoms of the element not only get excited, but rather, high energy radiation pulls out the electron from inner shell creating a hole in the shell. Since pulled out electron in higher energy shells are always unstable, then jump back to the lower shell and in doing so emit characteristic radiations unique to each element with energy different from that of incident x-ray and hence the name fluorescent. The characteristic radiation is calculated as its binding energy and number of such emissions will give the intensity. So this technique can be used to characterize and confirm the element and also enables to make quantitative estimations.

**Scanning Electron Microscope-Energy Dispersive X-Ray spectroscopy (SEM EDX):**

In this technique, electron beam under vacuum is focused on to the surface of the sample and the beam of electron scans the top layer of the sample. The electrons of the atoms of top layer will be excited and while coming back to the stable original position will emit x-rays which give high resolution images of magnification of 2,00,000x which helps to get morphological features on the surface in microns. In the present study, we used 10kX magnification for arsenic particle identification. The energy dispersive analyzer measures the back scattered x-rays, those which are reflected back from the surface of the specimen reaching the detector gives characteristic profiles of the element present in the sample.

**Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP AES):**

Electromagnetic induction produces high values of current and temperature which in turn makes the samples to go to plasma state with formation of ions. In the plasma
state the ions start emitting characteristic wavelength rays which helps in identifying the element [G. Mahesh Kumar et al., 2014]. The intensity of the emitted rays will be proportional to the concentration of the element which is the basis for quantification of particular element. This technique has been successfully used for quantification of gold and arsenic in the present work.

**Gas Chromatography (GC):**

Gas Chromatography is a very important technique for the analysis of volatile organic compounds using inert gas as mobile phases. The samples are made to pass through a tubular stationary phase known as column. Temperature inside the instrument is programmed according to the requirement so that the sample will always move in the form of a gas inside the column. Depending on the physical property of the components of the sample and its interaction with column (packed columns), separation of individual components takes place as they reach the detector. Proportional to the concentration of the compound reaching the detector, an electronic signal will be produced. Each separated component of the sample will travel inside the column and reach the detector at different time intervals which is called its retention time which is characteristic of the component at defined set of programmed conditions. Many types of detectors are available and in this work, a highly sensitive and universal [Forsyth DS, 2004, W.E.Wentworth et al., 1992] Pulse Discharge Helium Ionization Detector was used for separation of kerosene into individual components like n-alkanes.

**High Performance Thin Layer Chromatography (HPTLC):**

In Thin Layer Chromatography the samples to be separated are spotted on plates normally coated with silica gel as a thin layer are used as stationary phase and hence the
name TLC. After evaporating the solvent, the plates are dipped inside a solvent chamber in slanting position, taking care not to immerse the applied spots. Due to capillary action, the solvent starts moving over the silica and while doing so, it takes away the samples with it. Interaction between the solvent and sample takes place and depending on the polarity of the solvent, polar solvent moves faster than non polar which brings about the separation. High Performance Thin Layer Chromatography (HPTLC) is an enhancement over TLC as the developed plates can be scanned under UV light and the corresponding spectrum of each separated spot can be obtained. In HPTLC, simultaneous multisampling analysis on a single plate allows a real in system calibration in contrast to other chromatographic techniques [P.D.Sethi, 1996]. HPTLC was preferred over HPLC in some studies due to simplicity, ease and low cost [M.Bakavoli et al., 2003]. HPTLC is proven to be a very useful instrument for analysis of many types of samples in many areas [Naveen Bimal and Bupinder Singh Sekhon., 2013]. HPTLC is used here to develop a method for determination of diazepam in pure blood and pharmaceutical samples.
References:


