CHAPTER-I

INTRODUCTORY
Section (i): Introduction

Water and soil are essential components of environment. Water is an essential commodity for human survival and industrial development. Effective management of water resources and control of pollution are becoming increasingly important for sustainable development and human welfare. Water is elixir of life. But polluted / and contaminated water is the culprit for occurrence of deadful diseases in humans and animals. Inorganic pollutants especially selenium, arsenic, lead and mercury discharged from industries get into, human food chain, enter the human body and disturb biochemical processes leading to fatal diseases such as Mina-Mata, Ouch-Ouch, cirrhosis of liver, skin cancer etc.

The determination of toxic metal ions in water and edible (food) materials is an interesting research activity of inorganic-analytical chemist. The results obtained from such research activities are very useful to decide the degree of water pollution and food contamination. When the pollutants are found above permissible levels in water, measures may be taken to control water pollution. Thus analytical data has immense value in the control of water pollution, detection of diseases and in providing public awareness.

Inorganic analytical chemistry has made a spectacular progress mainly due to the reasons. The first was due to advances in the design and development of sophisticated analytical instruments permitting analysis at microgram concentration. While second reason was that the synthesis of novel organic ligands permitting the quantitative analysis by complexation of metals with these ligands at trace concentration. Although a large number of organic reagents were synthesized and characterized, unfortunately very few of them were used for the quantitative analysis of heavy metal ions, especially mercury, lead. Several methods involve extraction step.

Determination of microgram amounts of metals in biological and environmental samples using sensitive organic reagents has been an important and interesting research activity in University colleges having minimum facilities. UV-Visible double beam spectrophotometer is one of the analytical instruments available in the laboratories of university colleges. The success of spectrophotometric method largely depends in the selection of new reagent synthesized in a research programme. Chromogenic reagents containing aromatic groups, however give intense colour with metal ions possibly due to complex formation. In general, metal complexes of organic ligands show intense colour due to metal to ligand charge transfer transition (MLCT).
The use of organic reagents in the inorganic analytical chemistry was known during the last three decades. The colour forming reagents are called chromogenic reagents. This field of work took great strides towards identification and determination of metals at extremely low concentration. As a matter of fact entire qualitative analysis schemes were based on the use of chromogenic ligands for the detection of cations. A large number of metal ions were determined with different organic ligands such as \( \beta \)-diketone, oxines, oximes, naphthols, azonaphthols, dithizones, diethyldithiocarbamates, dithiols, semicarbazones, thioacetamides, thiosemicarbazones and hydrazones which contain donor atoms like oxygen, nitrogen or sulphur. These reagents are not only used in qualitative analysis but also in quantitative methods. Thus the quantitative analysis of metals has been predominated by spectrophotometric methods using chromogenic regents.

**Sources of Inorganic pollutants and their toxicity**

A wide variety of inorganic pollutants have been identified in the environment consequent to urbanization, industrialization and new technological developments. The sources of heavy metals (As, Se, Pb and Hg) are mainly aquatic releases from industrial operations, atmospheric releases from fossil fuel burning, domestic sewage discharges and land runoff. These elements exhibit varying environmental behavior and toxicity to aquatic organisms and man.

Heavy metals in general cause only local pollution problems. Environmental important of these metals should be assessed in terms of the degree of toxicity and the extent of exploitation of the metal, its applications and the ease of mobilization of metal into the air, water and soil.

**A. Mercury**

Mercury occurs in the environment as metallic Hg and as HgS. Annual production of the world is estimated to be about 10,000 tons. About 50 percent of mercury is estimated to enter into the environment\(^1\).
The principal sources of Hg pollution/contamination are mentioned here.

1. **Chlor-alkali plants:** Hg cells are used in the manufacture of chlorine and caustic soda. A plant producing 100 tons of chlorine per day may release 4000 - 8000 Kgs of Hg per year in the waste effluents. The products of chlor-alkali industry are bleaching powder and sodium hydroxide. These are also found to be sources of long term Hg Pollution.

2. **Mercury Catalysts:** HgCl₂ is used as catalyst in the manufacture of vinyl chloride plastics and acetaldehyde. Effluents from such plants contribute Hg to aquatic environment.

3. **Electrical Industry:** Hg is used in the production of batteries, street lamps fluorescent tubes, circuit breakers etc., all which are finally discarded as waste.

4. **Paints:** Hg compound are used in antifouling paints.

5. **Pulp and Paper Industry:** Hg is used to prevent formation of slime and is lost to the waste water effluents.

6. **Fungicides:** Phenyl mercury acetate and ethyl mercuric chloride have been used as fungicides - finally they enter water due to agricultural run-off.

7. The use of mercury in research, jewellery, moulding processes, pharmaceuticals cause water pollution.

8. Fossil fuel burning and cement manufacture cause emission of mercury into atmosphere.

Aquatic food appears to be major source of human intake of Hg. The first major incident involving Hg poisoning from this source was reported from Minamata Bay, Japan. Malfunctioning of central nervous systems, now known as Minamata disease was caused by eating of contaminated fish²⁻⁴ that accumulated methyl mercury as a result of water discharges from an acetaldehyde plant.
Mercury combines with sulfahydryl groups of enzymes and is toxic to all cells. Even trace quantity (0.001 ppm) of organomercurials reduces photosynthesis in marine phytoplankton.

Sources and toxicity of other metal ions are summarized below

The present work deals with the spectrophotometric determination of heavy metal ions using thiophenol, nicotinic acid and ethylenediamine. Hence a review on thiophenol, nicotinic acid and ethylenediamine as spectrophotometric reagents presented in section. ii.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Metal ion</th>
<th>Sources</th>
<th>Toxicity/Diseases</th>
</tr>
</thead>
</table>
| 1.   | Arsenic   | a. Insecticides  
b. Fossil fuel burning 
c. Smelting of ore  
d. Fertilizers plants | a. Loss of appetite (weight)  
b. Diarrhoea  
c. Constipation  
d. Skin cancer |
| 2.   | Selenium  | a. Industrial, agriculture discharge  
b. Keshan disease Kaschin black disease |
| 3.   | Cobalt    | a. nature ore  
b. liver and plants | a. vitamin B12, weakness  
b. high levels, it may harm the lungs and heart |
| 4.   | Zinc      | a. industrial discharge  
b. meat, liver, fish | a. stomach pain, diarrhoea  
b. growth failure in children and colds |
| 5.   | Lead      | a. industrial discharges  
b. Anti-knocking property of petrol  
c. Forest fuel burning  
d. Sewage sludge | a. Lead replaces calcium in bones  
b. Brain damage in children  
c. Malfunction of kidney |
References


Section (ii): Review of past work and objectives

A vast number of organic reagents were reported for the spectrophotometric determination of the arsenic, selenium, lead and mercury. The author has therefore not ventured to describe all these reagents in view of the fact that good reference books¹ Two are available. The author has used 2,4-dihydroxy benzophenone-2-amino thiophenol (BPBT), vanillin-2-amino nicotinic acid (VANA) and 5-methylthiophene–2-carboxaldehyde ethylenediamine (MTCED) for the determination of As (III), Se (IV), Pb (II) and Hg (II) spectrophotometrically.

Hence, those thiophenol, nicotinic acid and ethylenediamine are used in the spectrophotometric determination of arsenic (III), selenium (IV), lead (II) and mercury (II) are reviewed in this chapter.

Thiosemicarbazones, thiophenol and ethylenediamine are important organic analytical reagents. These reagents act as good chelating agents and form complexes with various metal ions by bonding through azomethine nitrogen atom and thioketo sulphur atom. The active grouping for chelation is

\[
\begin{align*}
\text{C} & \quad \text{N}=\text{N} & \quad \text{C} & \quad \text{N} & \quad \text{R}_1 \\
\text{R}_2 & \quad \text{R}_3 & \quad \text{M} & \quad \text{S}
\end{align*}
\]

In a few cases they behave as a unidentate ligands by bonding only through the sulphur atom. In certain cases these also act as multidentate ligands if donor groups are also present in the aldehyde or ketone moiety. For example, used 2,4-dihydroxy benzophenone-2-amino thiophenol (BPBT) is found to act as a monoanionic tri dentate ligand.

Thiosemicarbazones and thiophenol in general are obtained by refluxing the stoichiometric amounts of thiosemicarbazide/thiophenol and carbonyl compound (aldehyde or ketone) dissolved in suitable solvent (ethanol, methanol etc.) or base (pyridine) mono derivatives could be easily prepared where as preparation of di derivatives is not only difficult but the yields obtained are poor.
Thiosemicarbazones and thiophenol have been widely used as analytical reagents. Analytical applications of these reagents have been reviewed\textsuperscript{3-7}. These reagents have been used as analytical reagents in spectrophotometry, fluorometry, atomic absorption spectrophotometry and as indicators.

**Scope of the Review**

A vast number of organic reagents are reported\textsuperscript{1, 2} for the spectrophotometry determinate of As (III), Se (IV), Pb (II) and Hg (II) using thiophenol, nicotinic acid and ethylenediamine. Therefore, it is considered pertinent to review spectrophotometric methods for the determination of As (III), Se (IV), Pb (II) and Hg (II) using reagents.

**A. Spectrophotometric determination of arsenic (III)**

Arsenic compounds are widely used and have long been recognized as toxicants\textsuperscript{8-10}. Arsenic is widely distributed in the nature. It occurs as inorganic and organic compounds as trivalent. Animals vary in their arsenic accumulation depending upon the type of food they consume (John & Jeanne, 1994)\textsuperscript{12,13}. Acute arsenic exposure can give symptoms with rapid onset of headache, nausea and severe gastrointestinal irritation (Allan et al., 1995)\textsuperscript{14}. Due to increased industrialization more and more industrial waste get accumulated in various regions and make their passage through soil cause severe environmental pollution and wide life toxicity\textsuperscript{11} and also enter into animal body especially in their liver, kidney and lean meat.

Arsenic occurs naturally in the Earth’s crust in its inorganic form, trivalent (arsenite) or pentavalent (arsenate) form. Erosion of arsenic containing surface rocks probably accounts for a significant amount of arsenic in water supplies. It is a ubiquitous element in water, soil and sediments. The occurrence of arsenic in plants and animals generally reflects its accumulation from the environment. The presence of arsenic in drinking water has reached calamitous proportions in many parts of the world. There are numerous reports in the literature based on past and ongoing experience in various countries in Asia and South America concerning the higher risks of skin, bladder, lung, liver and kidney cancer that result from continued consumption of elevated levels of arsenic in drinking water\textsuperscript{15}. Consumption of even low levels of arsenic over a long period can cause a multitude of diseases. The maximum permissible limit for As (III) drinking
water is 0.05 mg/L as recommended by WHO\textsuperscript{16}. In certain areas in India, Bangladesh, China and Mongolia\textsuperscript{17} arsenic levels in groundwater exceed 1 ng/ mL. Regarding inorganic arsenic, As (III) is appreciably more toxic than As (V). Usually these species of arsenic in natural water are found at the trace levels\textsuperscript{17}.

There are only a few analytical techniques available, which have sufficient sensitivity and selectivity to directly determine arsenic at the trace levels in natural water. Therefore, the development of sensitive and accurate methods for speciation and preconcentration of trace amounts of As (III) and As (V) is necessary. Recently many kinds of conventional analytical techniques such as hydride generatively coupled plasma atomic emission spectrometry (HG-ICP-AES)\textsuperscript{12}, capillary electrophoresis inductively coupled plasma mass spectrometry (CE-ICP-MS)\textsuperscript{11}, high performance liquid chromatography–inductively coupled plasma mass spectroscopy\textsuperscript{18}, electro thermal atomic absorption spectrometry (ETAAS)\textsuperscript{19}, hydride generation – atomic absorption spectrometry\textsuperscript{20}, hydride generation – atomic fluorescence spectrometry\textsuperscript{21}, cathodic stripping Voltammetry\textsuperscript{22}, anodic stripping voltammetry\textsuperscript{23}, neutron activation analysis\textsuperscript{24}, photometric analysis\textsuperscript{25}, ion selective electrodes\textsuperscript{26} and energy dispersive X-ray fluorescence spectrometry\textsuperscript{27} have been used for the determination of low concentrations of arsenic. But all these techniques are costly and require trained staff. Recently most of the spectrophotometric methods have been developed as an alternative for the determination of arsenic instead of conventional techniques.

Arsenic is a naturally occurring element found in rocks, soil, water, air and animals. Arsines can penetrate rubber and are well absorbed through the skin which will become vesicated and blistered during the exposure. Arsines combine with haemoglobin in RBCs, cause hemolysis and cell destruction. Chronic exposures to arsines can result in anemia. Myocardial failure due to oxygen deprivation can occur in severe cases.

Arsenic is a naturally occurring dissolved element in ground and surface waters throughout the world. Arsenic is an element classed as a semi-metal or metalloid. This means it has some properties of metal and some properties of nonmetal. Arsenic occurs in two distinct solid forms. One is a brittle gray metal, while the other is a yellow, non-metallic form, rarely seen outside the laboratory. Arsenic and its compounds often have a garlic-like odor when crushed or when scratched with a hard object. Elemental arsenic
has very few uses. Nearly all the applications are as salts or oxides of arsenic. Arsenic compounds can be very toxic and their uses are strictly controlled by health and environmental regulations.

Arsenic has significant medicinal properties and it has been used as a therapeutic agent for more than 2,400 years\(^3\). In the 15\(^\text{th}\) century, William Withering, who discovered digitalis was a strong proponent of arsenic-based therapies. He argued "Poisons in small doses are the best medicines and the best medicines in too large doses are poisonous"\(^3\). In the 18\(^\text{th}\) century, Thomas Fowler compounded a potassium bicarbonate based solution of arsenic trioxide (As\(_2\)O\(_3\)) that would bear his name. Fowler's solution was used empirically to treat a variety of diseases during the 18\(^\text{th}\), 19\(^\text{th}\) and early 20\(^\text{th}\) centuries\(^3\). Pharmacology texts of the 1880’s describe the use of arsenical pastes for cancers of the skin and breast and arsenous acid was used to treat hypertension, bleeding gastric ulcers, heartburn and chronic rheumatism\(^3\). Arsenic's reputation as a therapeutic agent was enhanced in 1910 when Nobel laureate Paul Ehrlich developed salvarsan an organic arsenial for treating syphilis and trypanosomiasis. However, as medicine evolved in the 20\(^\text{th}\) century, enthusiasm for medicinal arsenic waned rapidly\(^3\).

The main sources of arsenic are: ore smelting/refining/processing plants, galvanizing, etching and plating processes, soil erosion and organic forms. Tailings from or river bottoms near gold mining areas (past or present) may contain arsenic. Insecticides, rodenticides and fungicides (Na, K, arsenates, also oxides are commercially available). Commercial arsenic products include: sodium arsenate, calcium arsenate, lead arsenate and "Paris green" (cupric aceto arsenite) a wood preservative. There is concern that this deposits in the meat that humans consume and has become a source of arsenic.

Throughout this article the term contamination means an unusual concentration of something whilst the term pollution means an unusual concentration that leads to environmental harm. Arsenic pollution of groundwater in the Bengal Basin was known about in the late 1980s (Guha Mazumder et al. 1988, PHED1991)\(^3\), however overlooked by a later survey (BGS 1992) is now known to be one of the World’s worst disasters affecting humans. At least 28 million people currently drink water containing more than 50 μ g/l of arsenic (the current Bangladesh drinking water standard), and many more consume water with > 10 μ g/l of arsenic (Dhar et al. 1997; Ullah 1998;
Mandal et al. 1998; DPHE 1999, 2000)\textsuperscript{35-37}. The human consequence of the pollution has been described as the worst mass poisoning of a population in history (Smith et al. 2000)\textsuperscript{38}.

Understanding the source of such severe arsenic pollution has a number of important benefits. Firstly, it helps in locating polluted aquifers and in predicting the future trends of arsenic in groundwater. Secondly, it is of great significance in formulating agricultural policy. For example, a common misconception is that pumping of groundwater for irrigation has caused arsenic pollution with one consequence being a call for a ban on tube well irrigation in Bangladesh. Yet irrigation has been the principal factor behind Bangladesh attaining virtual self-sufficiency in food grain production and hence a great diminution in the incidence of famine. A ban on groundwater irrigation, unjustified by rigorous scientific compulsion, would have severe adverse consequences for food security, rural livelihoods and the economic future of the poor of Bangladesh. We show that irrigation has no causal relation with arsenic pollution.

We also show that to arsenic contamination is not caused by oxidation of sulphide minerals as water tables are lowered by pumping (Mallick and Rajagopal, 1996; Mandal et al. 1998; Roy Chowdhury et al. 1999)\textsuperscript{39,40}. We dismiss giving reasons, the suggestion (Acharyya et al. 2000)\textsuperscript{41} that arsenic pollution is caused by ion-exchange by phosphate from fertilizers (or any other source of phosphorus). We confirm previous suggestions Bhattacharya et al. 1997; Nickson et al. 1998, 2000; McArthur et al. 2001)\textsuperscript{42-44} that arsenic pollution in the Bengal Basin is a natural process whereby arsenic is released during reductive dissolution of iron ox hydroxides a process that also reduces the absorbed arsenate to arsenite (Zobrist et al. 2000)\textsuperscript{45}.

The reduction hypothesis (Nickson et al. 1998, 2000; McArthur et al. 2001)\textsuperscript{42-46} assigns great importance to the role of organic matter in particular, peaty sediments, in generating anoxia in groundwater. This paper gives evidence that peat is the redox driver for reduction of FeOOH and then examines the sedimentological evidence that supports the proposition that the distribution of pollution by arsenic reflects the distribution of buried deposits of peat. In doing the above we make use of our own data, data from a survey of bacterial contamination and ammonium by Hoque (1998)\textsuperscript{47} and data from DPHE (1999, 2000)\textsuperscript{48,49}.
We used an ICP-OES machine which converts all arsenic forms into inorganic arsenic. Inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) are very feasible techniques for quantitative determination of the As (III). The most important advantages of these techniques are high sample throughput, simplicity and good sensitivity in comparison to other techniques\textsuperscript{28-29} used for As (III) determination.

Nowadays, due to the higher sensitivity achieved with axially viewed plasma and better spectral resolution given by high-resolution monochromators, it is expected that low concentrations of all naturally occurring arsenic may be directly quantified by ICP-OES. Furthermore, depending on the nebulizer used to introduce the sample solution in the plasma, sensitivity improves.

Various spectrophotometric methods developed for the determination of arsenic (III) using different benzophenones, thiophenes and ethylenediamine are given in Table 1.1.
<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the reagent</th>
<th>( \lambda_{\text{max}} )</th>
<th>( \varepsilon \times 10^4 )</th>
<th>Medium</th>
<th>pH</th>
<th>Beer's law range (ppm)</th>
<th>Extraction/Extraction (Application)M</th>
<th>M:L</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,4-Dihydroxy Benzophenone Thiosemicarbazones (DAPT)</td>
<td>400</td>
<td>2.10</td>
<td>Buffer</td>
<td>7.9</td>
<td>23.5-2.25</td>
<td>-</td>
<td>1:2</td>
<td>164</td>
</tr>
<tr>
<td>2</td>
<td>2,4-Dihydroxy Benzophenone benzoic acid hydrazone</td>
<td>410</td>
<td>1.55</td>
<td>Buffer</td>
<td>-</td>
<td>0.31-2.20</td>
<td>-</td>
<td>1:1</td>
<td>166</td>
</tr>
<tr>
<td>3</td>
<td>2,2-Dihydroxy Benzophenone benzoic acid hydrazone (2,2-DBPT)</td>
<td>380</td>
<td>0.2</td>
<td>Buffer</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>165</td>
</tr>
<tr>
<td>4</td>
<td>3-methyl thiophene-2-carboxaldehyde Thiosemicarbazones (3-MTAT)</td>
<td>360</td>
<td>4.0</td>
<td>Buffer</td>
<td>6.0</td>
<td>0.2248-2.2482</td>
<td>-</td>
<td>1:2</td>
<td>167</td>
</tr>
<tr>
<td>5</td>
<td>2,4-Dihydroxy Benzophenone-2-amino thiophenol (BPBT)</td>
<td>343</td>
<td>6.01</td>
<td>Buffer</td>
<td>6.0</td>
<td>0.125–2.637</td>
<td>-</td>
<td>1:1</td>
<td>168</td>
</tr>
</tbody>
</table>

* Units: L.mol\(^{-1}\) cm\(^{-1}\)
B. Spectrophotometric determination of selenium (IV)

Selenium is an essential trace nutrient and selenium deficiency diseases are well known in veterinary medicine. Above trace levels ingested selenium is toxic to animals and may be toxic to humans. The selenium concentration of most drinking waters and natural waters is less than 10 $\mu$g/ml. Selenium is widely distributed in nature, in relatively small concentrations in rocks, plants, coal and other fossil fuels. Selenium enters into natural water through seepage from seleniferous soil and industrial waste. Selenium compounds have extensive applications and because of its significance, several analytical techniques have been reported for the determination of selenium, which includes spectro-fluorometry, electro thermal atomic absorption spectrometry, hydride generation, polarography, cathode-stripping voltammetry, radiochemical neutron activation analysis, and flow- injection techniques. There are many reagents available for the spectrophotometric determination of selenium among which the recently used were J-acid, Leuco crystal violet, resazurin, sodium salt of hexamethylenemine carbodithioate, 1- naphthylamine-7-sulphonic acid, variamine blue. When selenium is present in animal feeds at a concentration less than 0.1 mg l$^{-1}$ deficiency symptoms develop but when present at a higher concentration exceeding 5 mg l$^{-1}$ chronic selenosis occurs. Selenium tends to weaken the toxic action of some heavy metals in animal and human organisms.

Selenium is widely distributed in the environment (waters, soil and air) albeit generally in very low concentrations ($\leq 1 \mu$g/g). The selenium content sometimes reaches 0.5 mg/g in limonite rocks and 2.6 mg/g in vanadium-uranium rocks. Selenium is a naturally occurring element found in rocks, soil, water, air and animals. Selenium is a trace mineral that is essential to good health but required only in small amounts. Selenium is incorporated into proteins to make selenoproteins which are important antioxidant enzymes. The antioxidant properties of selenoproteins help to prevent cellular damage from free radicals. Free radicals are natural by-products of oxygen metabolism that may contribute to the development of chronic diseases such as cancer and heart disease. Other selenoproteins help regulate thyroid function and play a role in the immune system. Selenium compound are widely used in paints, dyes, glass electrical, rubber, insecticides and many other industries.
Some industrial and agricultural processes release selenium as a by-product and selenium from such sources has caused environmental disaster\textsuperscript{66}. The threshold limit value for selenium compounds in air is 0.1- 0.2 mg dm\textsuperscript{-3} in water it is 4.0 ppm\textsuperscript{108}. The toxicity, availability and environmental mobility of selenium are very much dependent on its chemical form\textsuperscript{67,109}. Selenium can occur in different oxidation states in organic and inorganic compounds. In many environmental matrices, e.g. natural water and soils, the predominant oxidation states of selenium are Se (IV) and Se (VI). Water drained from such soil cause severe environmental pollution and wide life toxicity. Selenium is also reported to be present in cigarette paper, tobacco\textsuperscript{68} and various cosmetic sample\textsuperscript{69}. Because of its significance several analytical techniques have been reported concerning the determination of selenium\textsuperscript{70,73}.

Plant foods are the major dietary sources of selenium in most countries throughout the world. The content of selenium in food depends on the selenium content of the soil where plants are grown or animals are raised. For example, researchers know that soils in the high plains of northern Nebraska and the Dakotas have very high levels of selenium. People living in those regions generally have the highest selenium intakes in the United States (U.S.). In the U.S., food distribution patterns across the country help prevent people living in low-selenium geographic areas from having low dietary selenium intakes. Soils in some parts of China and Russia have very low amounts of selenium. Selenium deficiency is often reported in those regions because most food in those areas is grown and eaten locally.

Observational studies indicate that death from cancer, including lung, colorectal and prostate cancers is lower among people with higher blood levels or intake of selenium\textsuperscript{74-79}. In addition the incidence of non melanoma skin cancer is significantly higher in areas of the United States with low soil selenium content. The effect of selenium supplementation on the recurrence of different types of skin cancers was studied in seven dermatology clinics in the U.S. since 1983. Taking a daily supplement containing 200 \(\mu\)g of selenium did not affect recurrence of skin cancer, but significantly reduced the occurrence and death from total cancers. The incidence of prostate cancer, colorectal cancer and lung cancer was notably lower in the group given selenium supplements\textsuperscript{80}.
Research suggests that selenium affects cancer risk in two ways. As an antioxidant, selenium can help protect the body from damaging effects of free radicals. Selenium may also prevent or slow tumor growth. Certain breakdown products of selenium are believed to prevent tumor growth by enhancing immune cell activity and suppressing development of blood vessels to the tumor\textsuperscript{81}.

Selenium resistance to the flow of electricity is greatly affected by the amount of light shining on it. The brighter the light, the better selenium conduct electricity. This property has made selenium useful in devices that respond to the intensity of light, such as electric eyes, photocells \textsuperscript{82}, light meters for cameras and copiers. Selenium can also produce electricity directly from sunlight and is used in solar cells. Selenium is also a semiconductor and is used in some types of solid-state electronics as well as in rectifiers\textsuperscript{83}, devices which convert alternating current electricity into direct current electricity. In addition to its use in electrical devices, selenium is also used to make ruby-red colour in glasses and enamels, as a photographic toner and as an additive to stainless steel. Selenium is also used in the preparation of pharmaceuticals; as a nutritional feed additive for poultry and livestock; in pesticide formulations; in rubber production; as an ingredient in antidandruff shampoos; and as a constituent of fungicides. Radioactive selenium is used in diagnostic medicine.

Selenium is one of the trace elements which play an active role in many biological systems\textsuperscript{84} as it has toxicological and physiological effects\textsuperscript{85,86}. It is an essential nutrient at trace level but toxic in excess\textsuperscript{31}. Selenium has both beneficial and harmful effects. Low doses of selenium are needed to maintain good health. However, exposure to high levels can cause adverse health effects. Short-term oral exposure to high concentrations of selenium may cause nausea, vomiting and diarrhea. Chronic oral exposure to high concentrations of selenium compounds can produce a disease called selenosis. The major signs of selenosis are hair loss, nail brittleness and neurological abnormalities (such as numbness and other odd sensations in the extremities). Brief exposures to high levels of elemental selenium or selenium dioxide in air can result in respiratory tract irritation, bronchitis, difficulty breathing and stomach pains. Longer-term exposure to either of these airborne forms can cause respiratory irritation, bronchial spasms and coughing.
The determination of selenium is of considerable interest because of its contrasting biological effects. Selenium is a toxic element as well as a trace element for animals and humans. Because of its significance several analytical methods have been reported for the determination of selenium. These methods include neutron activation analysis, x-ray fluorescence, chromatography, hydride generation inductively coupled plasma atomic emission spectrometry and catalytic kinetic method.

Selenium was discovered by Berzelius and Gahn in 1817. It is widely present in nature in relatively small concentrations in rocks, plants, coal and other fossil fuels. Selenium is comparatively rare and its abundance in the lithosphere is $9 \times 10^{-6}$%. The important minerals containing selenium are clausthalite PbS, crookesite (Cu,Tl,Ag)$_2$Se, eucairite (Cu,Ag)$_2$Se, naumannite (Ag,Pb)Se. Selenium is also present in the soil in certain areas of the U.S.A (the dry plains of Dakota, Wyoming and Kansas) and is taken up by vegetation which then becomes poisonous to animals; their meat is then rendered unfit for human consumption. Selenium is however an essential trace element in some animal diets.

Very pure selenium is obtained by heating the crude material in hydrogen at 650°C to form hydrogen selenide which is then passed through a silica tube at 1000°C to decompose. Hydrogen sulfide is more stable to heat than the selenide and it passes out of the system unchanged. The hydrides of elements which are less stable to heat than hydrogen selenide are not formed at 650°C. It is apparent from vapor density determinations that Se$_8$ molecules are present below 550°C. The vapor is 188 yellow at the boiling (685°C) and it dissociates into Se$_6$, Se$_2$ (above 900°C) molecules and to atomic selenium occurs with the increase in the temperature. A mass spectrometric study of selenium vapor has provided evidence for the existence of Se$_4$ and Se$_7$ molecules in the vapor and the enthalpies of vaporization have been measured for each of these species. Water drained from such soil may cause severe environmental pollution and wild life toxicity. Selenium is also reported to be present in cigarette paper, tobacco and various cosmetic samples.

Selenium is a trace mineral that is essential to good health but required only in small amounts. Because of its ant-oxidant role, selenium has been studied for its potential to protect the body from many degenerative diseases, including Parkinson’s and cancer. Selenium is thought to protect cells against cancer because a form of selenium
from yeast was found to have caused cancer cells in test tubes and in animals to undergo apoptosis or programmed cell death. Selenium is found in some meats and seafood. Selenium is also a semiconductor and is used in some types of solid-state electronics as well as in rectifiers\textsuperscript{106}, it is an essential nutrient at trace level but toxic in excess\textsuperscript{107}.

We used an ICP-OES machine which converts all selenium forms into inorganic selenium. Inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) are very feasible techniques for quantitative determination of the Se (IV). The most important advantages of these techniques are high sample throughput, simplicity and good sensitivity in comparison to other techniques\textsuperscript{28-29} used for Se (IV) determination.

Nowadays, due to the higher sensitivity achieved with axially viewed plasma and better spectral resolution given by high-resolution monochromators, it is expected that low concentrations of all naturally occurring selenium may be directly quantified by ICP-OES. Furthermore, depending on the nebulizer used to introduce the sample solution in the plasma, sensitivity improves.

Various spectrophotometric methods developed for the determination of selenium (IV) using different bezophenones, thiophenes and ethylenediamine are given in Table 1.2.
### Table 1.2

**Spectrophotometric determination of selenium (IV) using reagents**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the reagent</th>
<th>$\lambda_{\text{max}}$</th>
<th>$\varepsilon \times 10^4$</th>
<th>Medium</th>
<th>pH</th>
<th>Beer's law range (ppm)</th>
<th>Extraction/ Application</th>
<th>M:L</th>
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<td>2,4-Dihydroxy Benzophenone Thiosemicarbazones (DAPT)</td>
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<td>380</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>165</td>
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<tr>
<td>4</td>
<td>3-methyl thiophene -2-carboxaldehyde Thiosemicarbazones</td>
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<td>6.01</td>
<td>Buffer</td>
<td>6.0</td>
<td>0.125–2.637</td>
<td>-</td>
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<td>168</td>
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</table>

* Units: L.mol$^{-1}$ cm$^{-1}$

18
C. Spectrophotometric determination of lead (II)

Lead is accumulative poison that enters the body from lead water pipes, lead-based paints and leaded petrol (Renner, R. 1995). Presence of even traces of Pb (II) in environmental samples leads to environmental pollution and many fatal diseases including dysfunction of renal blood and neurological systems. Pb (II) easily deposits in blood, kidney, reproductive system, nervous system and brain and acute lead poisoning can result in colic shock, severe anemia and irreversible brain damage. Lead compounds as antiknocking agents in automobile fuels cause air pollution.


Lead is a metabolic poison and a Neurotoxin that binds to essential enzymes and several other cellular components and inactivates them (Cunningham & Saigo, 1997). Toxic effects of lead are seen on hemopoietic, nervous, gastrointestinal and renal systems (Baykov et al.,) metal fumes or suspended particulates from fuel combustion or smelting and disposal of wastes however, most of the lead poisoning is from leaded gasoline.

Lead belongs to those trace heavy metals that are of major interest in environmental protection due to its high toxicity. The determination of lead in environmental samples plays an important role in the monitoring of environmental pollution. Lead is used in storage batteries, cable sheaths, solders and radiation shields and it is widely distributed in the nature. It is confirmed that most of the lead contamination in humans is from foods and drinks consumed. Lead in the water system has a serious influence on the quality of life especially in developing countries. Even small amounts of lead that enter the environment can result in elevated concentrations that can result in adverse effects. A regular absorption of small quantities of lead may cause serious injuries to health such as encephalopathy, kidney damage and several adverse effects in the body. In natural
water, its typical concentration lies between 2-10 $\mu$ g L$^{-1}$, whereas the upper limit recommended by WHO is less than (10 $\mu$ g L$^{-1}$)\textsuperscript{124}.

Medicinal plants are starting material for any herbal preparation such as herbal medicines, herbal teas, herbal oil etc. These preparations are being used worldwide due to their therapeutic potential and as they are considered to be safe as compared to allopathic medicines. Lead is the most common toxic metal that has become a matter of concern due to the reports of their contamination in various herbal preparations and herbal ingredients\textsuperscript{125-130}. Lead is known to cause neurological disorders, anemia, kidney damage, miscarriage, lower sperm count and hepatotoxicity in higher concentration\textsuperscript{131-132}. Ocimum sanctum, Linn (English: Holy Basil, Sanskrit: tulsi; Family: Labiatae) is a well known traditional plant used in Ayurvedic system of medicine\textsuperscript{133}. Amongst the indigenous herbals of India, several therapeutic properties have been attributed to O. sanctum-a Medhya rasayanas.

The essential oils from Ocimum contain many terpenes (linalool, citral, 1, 8-cineole) and phenylpropanoids (e.g. methyl chavicol, eugenol) produced in specialized glandular trichomes\textsuperscript{134}. Presence of eugenol attributes to its anti-oxidative property and is also thought to be responsible for inhibition of lipid per oxidation\textsuperscript{135}. This property helps in maintaining good health and in preventing the chances occurrence of heart diseases as well as most of the other biochemical diseases because oxidative stress is the hallmark of such diseases\textsuperscript{136}. In addition, available literature data indicates that there is a great deal of diversity in the composition of essential oil of O. sanctum cultivated in different localities\textsuperscript{137}.

We used an ICP-OES machine which converts all lead forms into inorganic lead. Inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) are very feasible techniques for quantitative determination of the Pb (II). The most important advantages of these techniques are high sample throughput, simplicity and good sensitivity in comparison to other techniques\textsuperscript{28-29} used for Pb (II) determination.
Nowadays, due to the higher sensitivity achieved with axially viewed plasma and better spectral resolution given by high-resolution monochromators, it is expected that low concentrations of all naturally occurring lead may be directly quantified by ICP-OES. Furthermore, depending on the nebulizer used to introduce the sample solution in the plasma, sensitivity improves.

In view of the above spectrophotometric studies on the determination of lead in different formulations are investigated. Various benzophenones, thiophenes and ethylene diamine are employed in the spectrophotometric determination of lead (II) are summarized in Table 1.3.
Table 1.3

Spectrophotometric determination of lead (II) using reagents

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the reagent</th>
<th>( \lambda_{\text{max}} )</th>
<th>( \varepsilon \times 10^4 )</th>
<th>Medium</th>
<th>pH</th>
<th>Beer's law range (ppm)</th>
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*Units: L.mol\(^{-1}\) cm\(^{-1}\)
D. Spectrophotometric determination of mercury (II)

Mercury is a naturally occurring metal which has several forms. The metallic mercury is a shiny, silver-white, odorless liquid. If heated, it is a colorless, odorless gas.

Mercury combines with other elements, such as chlorine, sulfur or oxygen, to form inorganic mercury compounds or "salts" which are usually white powders or crystals. Mercury also combines with carbon to make organic mercury compounds. The most common are methyl mercury is produced mainly by microscopic organisms in the water and soil. More mercury in the environment can increase the amounts of methyl mercury that these small organisms make.

Metallic mercury is used to produce chlorine gas and caustic soda and is also used in thermometers, dental fillings and batteries. Mercury salts are sometimes used in skin lightening creams and as antiseptic creams and ointments. Inorganic mercury enters the air from mining ore deposits, burning coal and waste and from manufacturing plants. It enters the water or soil from natural deposits, disposal of wastes and volcanic activity. Methyl mercury may be formed in water and soil by small organisms called bacteria. Methyl mercury builds up in the tissues of fish. Large and older fish tend to have the highest levels of mercury.

Exposure to mercury occurs from breathing contaminated air, ingesting contaminated water and food, and having dental and medical treatments. Mercury at high levels, may damage the brain, kidneys and developing fetus. Mercury has been found in at least in 714 of 1,467 compounds listed by national environmental protection agency.

Mercury is one of the most toxic heavy metal in the earth and it exists in nature at trace and ultra trace amounts in three valence states. Mercury (0, I, II) species and are able to combine with most inorganic and organic ligands to form various complexes, e.g. $\text{HgX}_4^{2-}$ (where $X= \text{Cl, Br and I}$) and methyl mercury. Mercury can accumulate in animals and plants and also enters into human body through the food chain causing damage to central nervous system. Due to the toxicological effects and potential accumulation of mercury onto human bodies and aquatic organisms, the determination of mercury (II) or organo mercury (II) has seen an upsurge of interest in the last few
years\textsuperscript{142}. According to WHO, the allowed limits of mercury in drinking water are less than \((1.0 \text{ ngL}^{-1})\textsuperscript{143}.

Mercury is a serious environmental pollutant because of its toxic effects on all living organisms\textsuperscript{144}. Mercury and its compounds cause serious diseases such as leukemia\textsuperscript{145}. Mercury compounds can be present as a result of anthropogenic activities in various environmental samples\textsuperscript{146}. They are usually present in natural waters at trace levels\textsuperscript{146-147}. The lakes, rivers in vicinity of the industrial areas are the important indicators for mercury pollution. So, it needs to develop new, selective, effective, cheap methods for determination of mercury\textsuperscript{148}.

A serious problem in the determination of mercury is related to low concentrations of target species. The main species of mercury in natural waters are inorganic mercury (\(\text{Hg}^2+, \text{Hg}^2+\)) and methyl mercury (\(\text{CH}_3\text{Hg}^+\)). Recent reports estimate that total mercury concentration is in the range of 0.2-100 ng L\(^{-1}\) and methyl mercury concentrations are lower (ca. 0.05 ngL\(^{-1}\)) in natural waters\textsuperscript{149}.

Mercury compounds have been used as catalysts, fungicides, herbicides, disinfectants, pigments and for other purposes. The world production was about 10,000 t in 1973\textsuperscript{150} and about 6,500 t in 1980\textsuperscript{151}. In addition to the production of pure mercury by industrial processes, mercury is released into the environment by human activities such as the combustion of fossil fuels, waste disposal and by industry. Recent estimates of anthropogenic emissions are in the order of 2000 to 3000 t year\(^{-1}\)\textsuperscript{152-155}.

Mercury is one of the most toxic heavy metals. It enters the environment as metallic, inorganic and organic mercury compounds through various industries like pulp and paper industry, chlor-alkali plants, gold and silver mining, electrical industry, paints, fungicides and pharmaceuticals. Fossil fuel burning and cement manufacture cause emission of mercury in air\textsuperscript{156-158}. The average value of mercury reported in oil is 10 ppm and sludge from sewage treatment plant is reported to contain up to 25 ppm mercury\textsuperscript{159}. WHO has suggested intake of 0.3 mg of mercury of which not more than 0.2 mg should be in the form of methyl mercury and maximum permissible value in drinking water is 0.001 mg\(^{-1}\)\textsuperscript{160}. The toxicity of mercury depends on its chemical species and it is found that organomercurials are more toxic than inorganic mercury compounds\textsuperscript{161,162}. Mercury and its compounds are reported to be mutagenic and teratogenic in nature\textsuperscript{163}.
We used an ICP-OES machine which converts all mercury forms into inorganic mercury. Inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) are very feasible techniques for quantitative determination of the Hg (II). The most important advantages of these techniques are high sample throughput, simplicity and good sensitivity in comparison to other techniques\textsuperscript{28-29} used for Hg (II) determination.

Nowadays, due to the higher sensitivity achieved with axially viewed plasma and better spectral resolution given by high-resolution monochromators, it is expected that low concentrations of all naturally occurring mercury may be directly quantified by ICP-OES. Furthermore, depending on the nebulizer used to introduce the sample solution in the plasma, sensitivity improves.

A few spectrophotometric methods are developed for the determination of mercury (II) using reagents benzophenones, thiophenes and ethylene diamine. These are listed in Table 1.4.
Table 1.4

Spectrophotometric determination of mercury (II) using reagents

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*Units: L.mol$^{-1}$ cm$^{-1}$
Objectives:-

1. To synthesize and to characterize new reagent viz. 2,4-dihydroxy benzophenone-2-amino thiophenol (BPBT), vanillin-2-amino nicotinic acid (VANA) and 5-methylthiophene–2-carboxaldehyde ethylenediamine (MTCED).

2. To investigate Physico-chemical and analytical properties of new reagents viz. 2,4-dihydroxy benzophenone-2-amino thiophenol (BPBT), vanillin-2-amino nicotinic acid (VANA) and 5-methylthiophene–2-carboxaldehyde ethylenediamine (MTCED).

3. To employ above reagents for the spectrophotometric determination of As (III), Se (IV), Pb (II) and Hg (II) in aqueous medium when present alone and in water, soils and medicinal leaf samples.
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