Chapter II

Experimentation
2.1 RESEARCH IN ANALYTICAL CHEMISTRY

Analytical chemistry is a key component of chemistry education and research. It is also essential for developing new methods and determination of various qualitative and quantitative aspects of the environmental, Pharmaceutical, clinical, applied sciences and material sciences at trace and ultra trace level compositions of analytes to ensure the quality products and services.

The research work in analytical chemistry focus on trace metals analysis in ground water quality using the various techniques such as ICP-OES and ICP-MS. Most of the universities and research centers in throughout the world are focused on research studies on measurement of trace metals in ground water studies due to increase of industrial activities and urbanization.

Analytical chemistry has been playing an important role since the early days of chemistry, for providing methods to determine composition elements, molecular structure, and other chemicals present in the samples. The first instrumental analysis was flame emission spectrometry developed by Robert Bunsen and Gustav Kirchhoff who discovered Rubidium (Rb) and Cesium (Cs) in 1860. Most of the major developments in analytical chemistry take place after 1900. During this period instrumental analysis becomes progressively dominant in the field. Many of the basic spectroscopic and spectrometric techniques were discovered in the early 20th century and refined in the late 20th century.

Analytical Chemistry has developed at a fast phase due to several reasons. First is the rapid development and advancement made in instrumentation and analytical techniques worldwide. Second is that analytical chemistry is needed to support rapid socio-economic development. In present time, there is a strong demand for goods and services that are energy-efficient, environmentally friendly and emitting less greenhouse gases. There is also a parallel development in health and medical care for raising the standard of living, better health care, quality of life, and disease cure and prevention. As such, there is an excellent prospect for the development of Analytical Chemistry not only India but also throughout the world.
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Electro analytical techniques also used for determination of trace metals. Speciation studies for some trace pollutants such as Arsenic, Mercury and Chromium etc., in water are being studied with the ICP-MS technique. Environmental Pollution, Separation Methods, application of different instruments in Environmental chemistry, Electrochemistry, Natural products, Environmental studies, Materials study, Polymers and Catalysis are some of the important areas of Analytical Chemistry.

Analytical research studies are caring both in basic and advanced levels. There are three large research groups, are there in analytical chemists. First group, Chromatography and Separation Research Group focuses on chromatographic separation techniques involving fundamental studies on separation techniques, sample preparation techniques, Gas Chromatography (GC), Liquid Chromatography (LC), Ion Chromatography techniques, Capillary Electrophoresis (CE) and hyphenated techniques with mass spectrometry: GC-MS and LC-MS. They work on various fields of interest including environmental analysis (Which includes Water, Air, Waste water, Soil & Solid waste etc), agricultural residue analysis, food analysis, enantiomeric separation, biochemical analysis, pharmaceutical and medicinal analysis, as well as analysis of natural products, medicinal plants, petrochemical and polymer. These studies cover theory, development of instrumentation and techniques, validation of analytical methods as well as applications.

The second group is the Environmental Analysis Research Group, which aims to develop analytical methods for determination of trace metals in ground water by using different sophisticated instruments like ICP-MS, ICP-OES, AAS, GC, GC-MS, IC, HPLC, LC-MS/MS, GC-MS/MS, TOF-LCMS and TOF-GCMS etc. The research also focuses on the development of controlling / treatment technologies for minimization of trace levels in ground water quality. The research scope consists of the following items: development of new technique for water analysis, development of sample preparation method for food analysis, treatment technology of chemical hazardous wastes and environmental assessment of treated hazardous wastes in landfill scenarios.
The last group is Electrochemical Research Group. The research interests include electroanalytical detection of dissolved trace metal analysis in ground water, electrochemical catalysis, fuel cell technology, flow-based system and lab-on-a-chip devices coupled with electrochemical detection for various applications. Future prospect for the Analytical Chemistry education expect to help each student get a strong foundation in chemical analysis, learn something new about analytical chemistry, and understand modern analytical techniques.

ENVIRONMENTAL RESEARCH:

A number of research studies are there to study the trace metals in ground water, air, waste water, soil, solid waste etc. Ground water quality is the one of the major concern in the environmental studies. Throughout the world all regulatory authorities and research institutes are focusing on ground water and also to determine trace level concentrations of metals in ground water quality.

Now a days a number of sophisticated analytical techniques are developed for the determination of water Quality in trace level concentrations of inorganic and organic constituents. The Spectroscopy, spectrometric and chromatography studies are widely using for environmental applications. Advanced analytical techniques are developed by coupling of chromatography, spectroscopy and spectrometric techniques to get the better sensitivity and lower detection limits.

The most widely used techniques for determination of trace metals in ground water quality are ICP-MS & ICP-OES and the most widely used for different techniques are GC-MS/MS, LC-MS/MS, GC, HPLC, GC-MS, LC-MS, etc.

The present study has focused on estimation of 22 metals in ground water in rural villages of coastal area of SPSR Nellore district, Andhra Pradesh. For this present study 35 rural areas selected for estimation of 22 metals named by Beryllium (Be), Boron (B), Aluminium (Al), Antimony (Sb), Manganese (Mn), Molybdenum (Mo), Iron (Fe), Copper (Cu), Zinc (Zn), Indium (In), Tin (Sn), Barium (Ba), Thallium (Tl), Silver (Ag), Arsenic (As), Nickel (Ni), Chromium (Cr), Lead (Pb), Cobalt (Co), Selenium (Se), Mercury (Hg)
and Cadmium (Cd) by using different analytical techniques named by Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) and Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES).

**Analytical Techniques:**

- Inductively Coupled Plasma – Mass Spectrometer (ICP-MS)
- Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES)

This chapter summarizes the basic introduction of research in analytical chemistry and the basic principles and components of the techniques (ICP-MS, ICP-OES) used for this study.

**2.2 BRIEF INTRODUCTION OF INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETER (ICP-MS):**

In Inductively Coupled Plasma - Mass Spectrometer (ICP-MS), thermal energy is transferred to the analyte elements by Inductively Coupled Plasma at even higher temperatures in order preferentially to produce electrically charged particles, which can be analyzed in a mass spectrometer. These ions are separated as a function of their electrical charge and mass, enabling analytical information to be obtained for each isotope of an element.

This technique is therefore in some cases more precise than merely element-specific methods and allows investigation of samples in accordance with the principle of isotope dilution analysis. High pure quality (shall be traceable to NIST) to be used for the estimation of trace metals, available from Merck and other reputed manufacturers. All metals and transition metals as well as some non-metals can be determined quasi-simultaneously by ICP-MS. The schematic diagram of Functionality in ICP-MS system is given in Figure 2.1 and schematic diagram of ICP-MS system is given in Figure -2.4.
Figure 2.1 Schematic Diagram of Functionality in ICP-MS system

Function of Components in ICP-MS:

The following are the basic functions of ICP-MS system:-

- Sample Introduction
- Inductively Coupled Plasma (ICP) source
- Interface / Vacuum
- Ion lenses
- Analyzer
- Detector
- Vacuum System
- RF Generators

Sample Introduction:

The sample introduction system will play major role in the introduction of samples to the plasma source. In the sample introduction system analytes in the sample solution will be introduced through peristaltic pump and the sample mixed with carrier gas at nebulizer and sprayed into the spray chamber. A sample solution mist forms and passes through a double-pass spray chamber where the larger sample droplets are removed by collision
with the spray chamber wall. To prevent the spray chamber from filling up with liquid, the peristaltic pump drain channel constantly removes excess sample solution.

A Peltier cooler system, a thermoelectric devices are covered the spray chamber to maintain the stable temperature, which gives a very stable ion signal. In addition, cooling the sample aerosol also removes some of the water from the sample, reducing the level of polyatomic oxide species formed, which in turn reduces the interference on certain analytes. The stable aerosols will be introduced into the ICP plasma source.

**Inductively Coupled Plasma (ICP) Source:**

A tangential flow of a argon gas is directed between the outer and middle tube of quartz torch. A load coil surrounds the top end of the torch and is connected to an RF generator. When RF power (typically 1500W) is applied to the coil at the rate of corresponding frequency (typically 27 MHz) of the generator. This RF oscillation of the current in this coil causes an intensive electromagnetic field to be created in the area at the top of the torch. The stable aerosols formed in the spray chamber will be introduced directly into the injector tube of the horizontally mounted inductively coupled plasma torch. The ICP torch is comprised of three concentric quartz tubes through which streams of argon pass. These three gas streams are referred to as the plasma gas, auxiliary gas and nebulizer (carrier) gas. The schematic diagram of formation of positively charged ions in plasma has shown in Figure – 2.2.
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Drying  Vaporization  Atomization  Ionization

Figure-2.2 Formation of Positively Charged Ions in Plasma

Interface / Vacuum Region:

The main role of the interface region is to transport the ions efficiently, constantly and with electrical integrity from the plasma, which is at atmospheric pressure (760 torr), to the mass spectrometric region at approximately $10^{-6}$ torr.

After the analyte ions are formed, they are extracted into the first vacuum stage through a hole in the front plate of the vacuum chamber referred to as the sampling cone. Ions pass through the sampling cone into the interface, which is an expansion region evacuated by a foreline pump. The ions then pass through a second orifice called the skimmer cone, which acts as a differential aperture between the interface and intermediate vacuum stage. Ions are extracted from the interface stage and collimated by two conical extraction lenses prior to focusing by the ion optics. The intermediate stage contains the ion optic system and is evacuated by a turbo molecular pump.

Ion Lenses:

Ion lenses will help to achieve high signal sensitivity, the ion beam must be focused before entering the quadrupole mass analyzer. The ion lenses perform the dual role of focusing the ion beam and preventing photons and neutral species (arising from the plasma) from reaching the detector, giving the very low random background. Although electron multipliers are very sensitive ion detectors, they are also sensitive to photons. To eliminate photon noise, the quadrupole and detector are mounted off-axis to the ion beam entering the system from the interface.
Octopole Reaction System (ORS):

The octopole system is one of the best techniques which will eliminate the polyatomic interferences in the sample matrix that are not argon based. The ORS is an octopole ion guide contained within a stainless steel vessel and pressurized with a gas, most often He or H₂. The ORS is positioned between the ion lens assembly and the quadrupole mass filter. The ions from the sample enter this cell, they interact with the gas, resulting in the reduction of the molecular interference, normally below the level of the blank solution. The use of 8 rods in the octopole has greater ion transmission efficiency compared to 6 rod (hexapole) or 4 rod (quadrupole) systems.

Analyzer (Quadrupole):

The quadrupole mass analyzer consists of four long metal rods with a hyperbolic cross section which are arranged parallel to each other, and have RF and DC voltages applied to them. By varying these voltages, the rods act as a mass filter allowing only ions of a specific mass-to-charge ratio to pass through the center of the quadrupole at any given combination of applied voltages.

All other masses are unstable and collide with the rods. These voltages are ramped very rapidly so the quadrupole can scan the entire mass range (2-260 amu) in 100 milliseconds. As a result, spectra of mass versus intensity can be obtained for all elements virtually simultaneously. Quadrupoles are limited effectively to unit mass resolution so they can't resolve polyatomic and isobaric interferences. However, of all of the elements detectable by ICP-MS, only indium does not have an isotope that is free from overlap by another element. Quadrupoles used in ICP-MS are typically 15–25 cm in length, about 1 cm in diameter, and operate at a frequency of 2–3 MHz.

Detector:

After passing through the quadrupole, ion signals are measured by the electron multiplier detector. Both the quadrupole and detector are located in the analyzer stage, which is evacuated by a second turbo-molecular pump. The electron multiplier (EM) has many dynodes. When an ion enters the EM, the ion hits the first dynode and a shower of
electrons is generated. The electrons then hit the next dynode generating more electrons. The schematic diagram of electron multiplier has shown in Figure-2.3.

Detection Capability:

The detection capacity here is very good, meaning that this technique has become the standard method for ultra trace analysis of elements. However, high matrix proportions or salt contents in samples may cause a variety of interferences.

Interferences:

Several types of interferences might be observed during the estimation of trace level impurities, to overcome such interference effects the following factors have to be taken care before carrying out routine analysis.

1. Selection of alternate isotope.
2. Use of high pure acid and water, while preparing solutions.
3. Employing proper dissolution procedure.
5. Reducing total dissolved solids by sufficiently diluting the samples.
6. Reducing oxide and doubly charged ions by adjusting gas flows and other parameters.
7. Internal standardization.
8. Use of matrix matching standards for calibration.
9. Periodical cleaning of cones, mobilization – spray chamber system etc.,
Applications of ICP-MS:

Inductively Coupled plasma-mass spectrometry (ICP-MS) is a multi-element technique that uses plasma to disassociate the ions contained in a sample. In a few minutes, the ICP-MS can produce high quality data for elements with wide range of atomic masses, from 6Li to 238U. The best results are obtained for elements that have ionization potentials lower than those of the carrying gas (Ar, 15.8 eV) and that are free of isobaric interferences. Ions are extracted from the plasma and passed through a mass spectrometer for detection based on mass to charge ratio. ICP-MS is a highly sophisticated analytical technique that is capable of sub-ppt (parts per trillion) detection limits. The lowest detection levels can only be achieved in a clean room environment.

ICP-MS is an important analytical technique for the pharmaceutical, food and beverage and environmental industries. The technique has a high productivity rate and allows for multi-element detection from a single sample. ICP-MS is susceptible to spectral interference from ions having the same mass to charge ratio. For example, nitrogen in the form of N\textsubscript{2} can interfere with silicon analyses due to the same mass to charge ratio of 28. This is called an isobaric interference and should be considered relative to type of sample and sample preparation chemistries.
By using ICP-MS we can determine multiple elements at single aspiration. By using ICP-MS technique we can able to determine around 75 elemental concentration levels upto parts per trillion levels in various environmental matrices like Water, source emission, air, waste water, soil, petroleum products, hazardous waste etc, food and agricultural products and drug and pharma matrices.

Hyphenated techniques involving ICP-MS are among the fastest growing research and application areas in atomic spectroscopy. This is because, by itself, ICP-MS does not give information on the chemical or structural form of the analytes present (since all forms of the analytes are converted to positively charged atomic ions in the plasma).

However, as an excellent elemental analyzer, it also performs as a superb detector for chromatography. Hyphenated ICP-MS is achieved through the coupling of the ICP-MS to a separation technique – normally a chromatographic separation. In this way, target analytes are separated into their constituent chemical forms or oxidation states before elemental analysis. The most common separation techniques are gas chromatography (GC) and high-performance liquid chromatography (HPLC), which includes ion chromatography (IC), but, other separation techniques, such as capillary electrophoresis (CE) and field flow fractionation (FFF), are also used.
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The applications of hyphenated ICP-MS fall into the general category termed speciation analysis. In the speciation studies, the fractionation device (chromatograph or other) is used to separate the species from each other and the matrix, and the ICP-MS is used to detect the species of interest. The analyte species may be as simple as elemental ions of various oxidation states in solution, or as complex as mixtures of pesticides or biomolecules. In all cases though, the ICP-MS is simply acting as an elemental detector.

The fractionation device serves to separate the various components in the sample before detection as well as providing additional information in the form of retention time. Often this combination is sufficient to identify and quantify the target analytes. However where accurate retention time data is not available, analysis of standards or the use of additional mass spectrometric techniques can provide further confirmation of identification.

The simultaneous determination of inorganic and organic mercury is difficult because the typical concentration of Me-Hg is much lower than that of inorganic mercury. The most common methods for mercury speciation are gas chromatography (GC) or high performance liquid chromatography (HPLC) coupled with a mercury-specific detector (fluorescence, photometry, or other elemental detector). The low concentration of mercury in natural waters leads to the need for very large sample volumes to be processed. A pre-concentration step is usually necessary because the reporting limit required is often below the sensitivity of the detector used.

2.3 BRIEF INTRODUCTION OF INDUCTIVELY COUPLED PLASMA - OPTICAL EMISSION SPECTROMETER (ICP-OES):

The ICP is the most simple and direct method of chemical analysis. The sample to be analysed is introduced into argon based high temperature plasma by a nebulizer spray chamber system. The sample stream causes desolvation, vaporization, atomization and ionization of target elements. Ions thus generated are extracted from the plasma into a low-pressure region through a sampler and skimmer cones and are allowed to pass through an electrostatic lens system, which extracts positively charged ions. The schematic diagram of ICP-OES basic components and excitation process in ICP are shown in Figure – 2.5 and Figure-2.6.
The high temperature plasma leads to:

- Increased response from emitting species
- More emission lines in the UV
- More possible spectral interferences
- Fewer chemical interferences

Excitation Process in ICP:

- **Ionization**
  
  \[
  M_{(g)}^{+} \xrightarrow{\text{Excitation}} M_{(g)}^{+} \xrightarrow{\text{Emission}} M_{(g)}^{+} + h\nu_1
  \]

- **Atomization**
  
  \[
  M_{(g)} \xrightarrow{\text{Excitation}} M_{(g)}^{+} \xrightarrow{\text{Emission}} M_{(g)}^{+} + h\nu_2
  \]

- **Vaporization**

- **Desolvation**

**Figure-2.5** ICP schematic diagram

**Figure-2.6** Excitation process in ICP
ICP Instrumentation:
The following are the instrument parts of the ICP

- Sample Introduction
- Torches
- RF Generators
- Optics
- Detectors

Convert the Analyte Solution into Free Atoms and/or Ions in the Plasma

Accomplished Using

- Nebulizer
- Spray Chamber
- Torch

Transport sample to plasma

- Generate an Aerosol
- Introduce Aerosol into Plasma
  - Prevent Nebulizer Blockage
  - Prevent Injector Tube Blockage

**Step one:** create aerosol

- Nebulizer and spray chamber

**Step two:** aerosol penetration into plasma

- Nebulizer gas pressure through torch injector tube

**Step three:** conversion of analyte into free atoms and/or ions in the plasma

Glass Expansion Nebulizers

- Conical - glass concentric - standard all purpose
- Sea Spray - glass concentric - designed for up to 20% salt solutions and 75 µm particulates
- Micro Mist - glass concentric - available with uptake rates of 50-600 µL/min
• Slurry - glass concentric -designed to tolerate high particle loads (150 µm) with excellent stability
• Poly Con - polyimide concentric - can nebulizer 10% HF - uptake rates from 50-5000 µL/min
• Poly Con - polyimide concentric - can nebulizer 10% HF - uptake rates from 50-5000 µL/min

Sorts aerosol droplets created in the nebulization process by size

Removes larger droplets

Types of spray chamber
• Double pass
  – Sturman Masters
  – Twister
  – Scott
• Single pass
  – Cyclonic

Confines Argon gases to form plasma

Insulates the induction coil from the plasma

Directs the sample into the plasma

Types of torches
• One piece quartz
• Semi-demountable
  – various sizes and types of injector tubes
• Fully demountable
  – various sizes and types of injector tubes
  – allows replacement of outer and/or inner tube

Provides radio frequency power to the induction coil
• Two isolated frequencies used
  – 40.68MHz and 27.12MHz
Types of RF generator

- Free running
  - power is fixed
  - frequency varies
- Crystal controlled
  - frequency is fixed
  - power varies

40.68MHz free running generator will be used for ICP

- The higher the frequency the thinner the plasma
- Thinner plasmas give:
  - greater linearity (less self absorption)
  - lower backgrounds
  - higher signal for some elements
- Higher frequency is better for organic solvents

Gathers light energy from an emission source and separates it into component wavelengths

Optical designs

- Sequential
  - collects data from all emission wavelengths in sequence
- Simultaneous
  - collects data from all emission wavelengths at the same time

Interferences:

Like any other technique ICP-OES is also subjected to several types of interferences and to overcome such interference effects the following factors have to be taken care before carrying out routine analysis.

- Use of high pure acid and water, while preparing solutions.
- Employing proper dissolution procedure.
- Using multi-variant correction methods for inter-elemental effects.
- Reducing total dissolved solids by sufficiently diluting the samples.
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- Internal standardization.
- Use of matrix matching standards for calibration.
- Periodical cleaning of torch, nebulizer, tube connections, mobilization – spray chamber system etc.,

Applications of ICP-OES:

- By using ICP-OES we can determine multiple elements at single aspiration. Determination of element levels in wide range of samples little higher concentration levels than compared to ICP-MS technique.
- By using ICP-OES we can able to determine the elemental concentration levels little higher levels than compared to ICP-MS. By using ICP-OES we can able to detect the minimum 10 ppb of elemental concentration levels.

Agricultural and food applications:

The ICP-OES is used for determination of heavy metals, nutrients and toxic metals in food and agricultural products like Animal tissues, beverages, feeds, fertilizers, garlic, nutrients, pesticides, plant materials, rice flour, soils, vegetables, wheat flour.

Biological and clinical applications:

The ICP-OES is used for determination of heavy metals, nutrients and toxic metals in Biological and clinical applications like Brain tissue, blood, bone, bovine liver, feces, fishes, milk powder, orchard leaves, pharmaceuticals, pollen, serum, urine.

Geological applications:

The ICP-OES is used for determination of heavy metals, nutrients and toxic metals in geological samples like Coal, minerals, fossils, fossil fuel, ore, rocks, sediments, soils, water.

Environmental and water applications:

The ICP-OES is used for determination of heavy metals, nutrients and toxic metals in environmental samples like Brines, coal fly ash, drinking water, dust, mineral water,
municipal wastewater, plating bath, sewage sludge, slags, seawater, soil, air, stack emissions.

**Metals and Alloys applications:**

The ICP-OES is used for determination of heavy metals, nutrients and toxic metals in Alloys, aluminum, high-purity metals, iron, precious metals, solders, steel, tin, etc.

**Organic and Pharmaceutical Product applications:**

The ICP-OES is used for determination of heavy metals, nutrients and toxic metals in Adhesives, amino acids, antifreeze, combustion materials, cosmetics, cotton cellulose, dried wood, dyes, elastomers, epoxy, lubricant, organ metallic, organophosphates, oils, organic solvent, polymers, sugars, etc.

**Other materials:**

The ICP-OES is used for determination of heavy metals, nutrients and toxic metals in Acids, carbon, catalytic materials, electronics, fiber, film, packaging materials, paints and coatings, phosphates, semiconductors, superconducting materials.

Figure 2.7 Schematic Diagrams of Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES)
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The characteristics of the ICP as an analytical atomic emission source are so impressive that virtually all other emission sources [such as the flame, microwave induced plasma (MIP), direct current plasma (DCP), laser-induced plasma (LIP), and electrical discharge] have been relegated to specific, narrowly defined application niches. Indeed, even much of the application field originally assigned to atomic absorption spectrometry (AAS), using both the flame and graphite furnace atomic absorption spectrometry (GFAAS), has been relinquished to the ICP. Compared to these other techniques, ICP/OES enjoys a higher atomization temperature, a more inert environment, and the natural ability to provide simultaneous determinations for up to 70 elements.

This makes the ICP less susceptible to matrix interferences, and better able to correct for them when they occur. In cases where sample volume is not limited, ICP/OES provides detection limits as low as, or lower than its best competitor, GFAAS, for all but a few elements. Even for these elements, the simplicity with which the ICP/OES instrument is operated often outweighs the loss in sensitivity.