



A review of Singular and Hyphenated Analytical techniques in Trace Element analysis

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Abstract At micro trace levels there is a critical need for preconcentration, selective separation, and determine the various metal ions from complex matrices. Trace analysis and various analytical techniques which are used to determine the concentration of a chemical element is described. It can be broadly classified in to singular and hyphenated techniques. For analysis there are different types of singular techniques like Flame Atomic absorption spectroscopy (AAS), Neutron activation analysis (NAA), Graphite furnace atomic absorption spectrometry (GFAAS), inductively coupled plasma mass spectrometry (ICP-MS), X-ray fluorescence (XRF) and inductively coupled plasma atomic emission spectroscopy (ICP-AES). Hyphenated technique provides an opportunity to avoid contamination and large reagent consumption with good selectivity and high sensitivity.

Keywords Trace elements, Singular and Hyphenated Techniques

1. Introduction

Contamination of metal ions in the environment has become an imperative and critical health hazards issues with respect to global environmental problems. The trace metals ions are tested for the presence of analytes even at low levels in the sample. At present, trace elements even at ng per g (10^{-9} g per g) and pg per g (10^{-12} g per g) levels can be determined with satisfactory accuracy and precision (termed as ultra-trace analysis) by using sophisticated analytical techniques [1]. IUPAC system derived the term 'trace component'. In which trace analysis is the concentration of 100 ppm ($100 \mu\text{g g}^{-1}$). Determination of components at a concentration level of 100 ppm, does not create major problems even in the samples with different complex matrices which is generally carried in most of the laboratories. It mainly occurs due to the use of monitoring and measuring devices and also the rapid development of instrumentation or the science of the construction.

Therefore, the atmosphere, drinking water, soil, plants, animal and human diets, urine and tissues are frequently analyzed for trace elements in biological, agricultural and medical sciences as well, in view of environmental concerns [2].

Due to the rapid growth in instrumentation different types of powerful analytical tools were developed for determine trace metal elements. It is impossible to happen, applying directly the various techniques to determine different trace metal analysis of the sample even after decomposition or dissolution because of (i) too low concentration of the desired element i.e. detection limit and (ii) determination of particular analyte in presence of extraneous species, i.e. selectivity. The general scheme of inorganic trace analysis is shown in Fig. 1.

The increased use of metals and chemicals in many industries has resulted in the generation of large amounts of polluted aqueous effluents which contain high concentration of metals and pose environmental disposal problems for plants, animals and human beings.



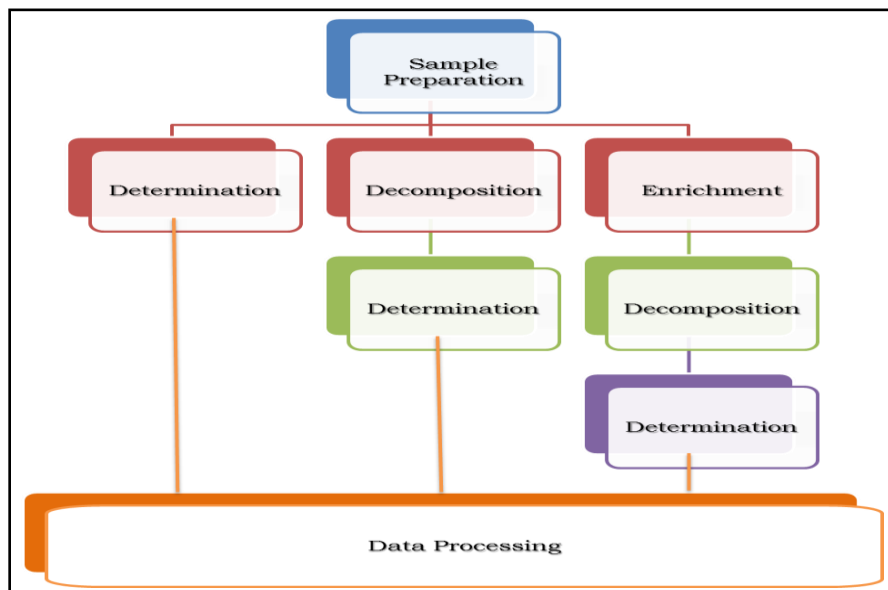


Figure: 1 General scheme of Inorganic Trace Analysis

Metals like copper and zinc contaminate the environment, specially the aqueous environment. This poses a severe problem worldwide as these metals find their place in the food chain.

Copper is an essential trace element for humans, plants and other animals but also causes contamination in water. The deficiency of metals in the human body causes many diseases. For example, lower levels of copper may cause gastrointestinal diseases, such as diarrhea, vomiting, stomach aches and dizziness. On the other hands, high levels of copper causes kidney or liver damages and sometimes certain mental disorders, such as alzheimer's disease, wilson's disease and gastroenteritis. Consequently, high concentration levels of Cu (II) ions contamination in the industrial waste waters must be reduced to acceptable levels before discharging them into the environment [3-4].

Zinc is an essential trace element of great importance for humans, plants, and animals. It is of prime importance in diverse fields, such as environmental, waste water control, metallurgy, agriculture and alloy manufacturing, pharmaceutical and clinical fields [5]. Zinc is a co-factor in many enzymes and it is necessary for production of insulin in humans. However, high concentrations of this metal can cause several damages to human body, such as disturbances in energy metabolism or increase in oxidative stress. The World Health Organization (WHO) has the authority to decide the maximum permissible limits of these metals like copper and zinc in drinking water. The current permissible limits for copper and zinc are 0.05mgL and 5mgL respectively [6].

One of the most toxic elements is Mercury high concentrations of which is becoming a great concern especially for marine system. The increasing amount of mercury in the effluents ends up into water system like rivers and oceans. This contaminates the water hence is responsible for high rate of extinction of several marine species. Mercury acts as a neurotoxicant on mature neuron as well as adult neuronal stem cell. This show its severe effects on humans [7-8].

2. Analytical Techniques

These techniques are suitable for the analysis of various elements and compounds by chemical means. These techniques are used for both qualitative as well as quantitative analysis. The techniques used widely for analysis, from simple weighing to titrations to very advanced techniques using highly specialized instrumentation. The requirements of an ideal analytical technique for trace or ultra-trace analysis are:

- (i) Applicable to all the elements.
- (ii) Able to analyse very small sized samples (μg).
- (iii) Acceptable precision and accuracy.



- (iv) Simultaneous or sequential multi-element detection capability at the major, minor, trace or ultra-trace concentration level.
- (v) No inter-element effects.
- (vi) Capable of providing rapid analysis.

Hence, the selection of analytical technique depends on primarily criteria such as sensitivity, selectivity, precision and accuracy and auxiliary criteria like cost of equipment, time of analysis, scope, sampling and standard requirements. The analytical techniques most commonly employed for trace and ultra-trace analysis can be broadly classified into singular and hyphenated techniques.

2.1. Singular Techniques

Singular analytical technique is defined as one that facilitates qualitative and / or quantitative determination of the composition of any material and chemical state in which it is located. There are a wide variety of techniques used for analysis. A number of singular analytical techniques have been employed in trace or ultra-trace element analysis. The suitability of the technique used depends upon the detection limits. Every technique has different analytical working range which is actually the concentration range over which results can be obtained quantitatively. The chances of errors can be reduced by minimizing sample handling requirements, which is further proportional to the working range. In general, broader analytical working range is desired. Sample throughput is the most important parameter in analysis of any element. Sample throughput gives idea about the time, i.e. the number of samples that can be tested in unit time. The instruments used for analysis in commercial laboratories must have higher throughput. The precision of analysis is also proportional to the time taken, which means better results require higher times.

The different singular techniques are described below:

(i) Flame Atomic Absorption Spectroscopy (FAAS) is a type of UV-Vis spectroscopy because it uses a hollow cathode lamp having bandwidth in the range of UV and visible radiations. This cathode lamp acts as a source of light. In 1962, the first Flame Atomic Absorption instrument was made available for commercial use. FAAS utilizes generally three types of radiation sources. First one is the line sources, where the lamp emits radiations having a narrow range of wavelength. Hollow cathode lamp is an example of line source and is the most widely used source for AAS (Atomic Absorption spectroscopy). Second type of sources is the continuous or the Broadband sources. Examples of continuous sources are xenon, deuterium, tungsten and mercury lamps. The instrument can not give a precise result when this lamp is used as a sources lamp and hence used as a background correction lamps. Third type of source is the laser lamps. They produce the purest form of radiations, but are of no use in AAS because it is untenable within the required range. The latest design of this technology comes as double-beam FAAS, where a chopper is used to split the beam of light. Both the beams are used for separate purpose, one is associated with the measurement of intensity of radiation, while the other beam performs the function of measurement of the radiations interacting with the analyte [9].

(ii) Graphite Furnace Atomic Absorption Spectrometry (GFAAS) also known as Electrothermal Atomic Absorption Spectrometry (ETAAS) is a highly sensitive spectroscopic technique that issues great limits of detection for measuring concentrations of metals in aqueous and solid samples. It is a technique which deals only with one elements at a time. GFAAS has been used primarily in the field for the analysis of metals in water. GFAAS could be used to determine metals in soil, but the sample preparation for metals in soil is extensive and is not practical for field applications. This technique uses a graphite-coated furnace to vaporize the sample and free atoms of most elements can be produced from samples by the application of high temperatures. The light gets absorbed by the analyte present but within certain limits. The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels. The operation time per sample in GFAAS is longer than FAAS. The list of elements detected through this is relatively very small. GFAAS has a relatively low sample throughput and limited analytical working range. A typical graphite furnace determination normally requires 2-3 minutes per element for each sample [10].



(iii) Cold Vapor Atomic Absorption Spectrometer (CVAAS): CVAAS serves mainly as a technique for the detection of mercury in water samples. It is famous by the name “FLAMELESS ATOMIC ABSORPTION SPECTROMETER”. It works with the same procedure which uses absorption of radiation by mercury vapors at 253.7 nm.

Firstly, strong reducing agents such as Sodium borohydride or SnCl_2 are used to reduce mercury to elemental state. It has a detection limit of 30-50 seconds per sample. The process requires a high amount of chemicals and hence large variants are available [11].

(iv) X-ray Fluorescence (XRF): The fluorescent X-rays, generated when the sample is excited by primary X-rays are measured and used for both the qualitative as well as quantitative determination. It is a non-destructive emission technique. Just like fingerprints, each element produces a unique set of characteristic secondary X-rays, hence making the technique excellent for analysis. XRF is of two types, one is Energy dispersive X-ray fluorescence (EDXRF) and the other is Wavelength dispersive X-ray fluorescence (WDXRF). EDXRF is simpler in design, have fewer engineered components and lower accuracy and resolution than WDXRF [12].

(v) Neutron Activation Analysis (NAA): NAA is a technique for the quantitative as well as the qualitative analysis which works when neutrons are excited to emit gamma rays. It is a multi-elemental detection technique discovered in 1936. This technique allows the detection of 1-35 elements at a time with very low sample input of 5-100 mg. the lower detection limit in this technique is ppb (parts per billion) and generally goes to ppm (parts per million). Values more than that may be inaccurate because of the high sensitivity of this technique. This limits NAA for low concentration elements only. The first and foremost step is that neutrons are irradiated on the sample inside a nuclear reactor, the nucleus absorbs neutron and thus become radioactive on verge of its stability.

Presence of particular elements in the sample is detected by the gamma rays emitted by the radioactive nuclei. The detector can be made using suitable semi-conductor. Detection of the decayed nuclei can be done to measure the concentration of suitable elements. Finally, the concentration of various components in the sample is obtained by the X-ray spectra.

This method is widely used as reference for other analytical methods [13-14].

2.2. Hyphenated Techniques

There are a plenty of techniques available for the detection of quality as well as the quantity of various element (such as trace metals) present within a sample. Earlier, no single technique could guarantee the results and hence a series of tests and experiments are required. But now, there are several instruments which can perform both the identification as well as the quantification with a very high accuracy. These techniques are nothing but just a combination of two or more singular techniques and are called hyphenated techniques. The tests are not much expensive and are very accurate. The concept behind developing these techniques was to design such a combination which can separate as well as detect all the analytes. There are three combinations present. First is the separation-detection, which gives an information rich result and is helpful as it gives both the results. Second is the separation-separation, which gives accurate composition with high precision and the third is the identification- identification combo. This coupling of analytical techniques is aimed to provide a detailed report in a single run [15-16].

Hyphenated techniques involving chromatographic or electrochemical separation techniques in conjunction with atomic spectrometric determination techniques are widely employed for speciation by biological and environmental analytical chemists and have been reviewed extensively. These hyphenated techniques can be classified broadly into chromatographic, electrophoresis and flow injection based hyphenated techniques. Hyphenated techniques in the past few years has shown their significance, and an ever-increasing attention has been paid to them. The concept of hyphenation is based on the combination of separation (quantitative) techniques with spectroscopic (identification) techniques. These techniques can be used to analyse complex natural products and fractions. FTIR, UV-Vis, MS, NMR, HPLC, GPC, ICP, AES, etc. results in introduction of various modern hyphenated techniques like CE-MS, LC-MS, GC-MS, etc. HPLC is the most widely used technique for the detection of the natural product extracts. For this, HPLC is either combined with MS and NMR because of the greater value of sensitivity. The past few decades



has changed the process of measuring the total metalloid concentration to analyzing the metalloid species. Metal species formed by arsenic, selenium and mercury can be separated and measured by hyphenated techniques like ICPMS (Inductively Coupled Plasma- Mass Spectroscopy) and combination of GC (Gas Chromatography) and HPLC (High Performance Liquid Chromatography) [17].

Advantages of Hyphenated Techniques

- (i) Complex analytical problems can be solved
- (ii) Fast technique i.e. lesser time consumption
- (iii) Higher degree of automation
- (iv) Higher sample throughput and accurate analysis
- (v) Reproducible results can be obtained
- (vi) Less contamination due to closed system
- (vii) Separation and quantification can be achieved simultaneously

Enhancement in selectivity and thus high degree of information

The types of hyphenated techniques are shown in Fig. 2.

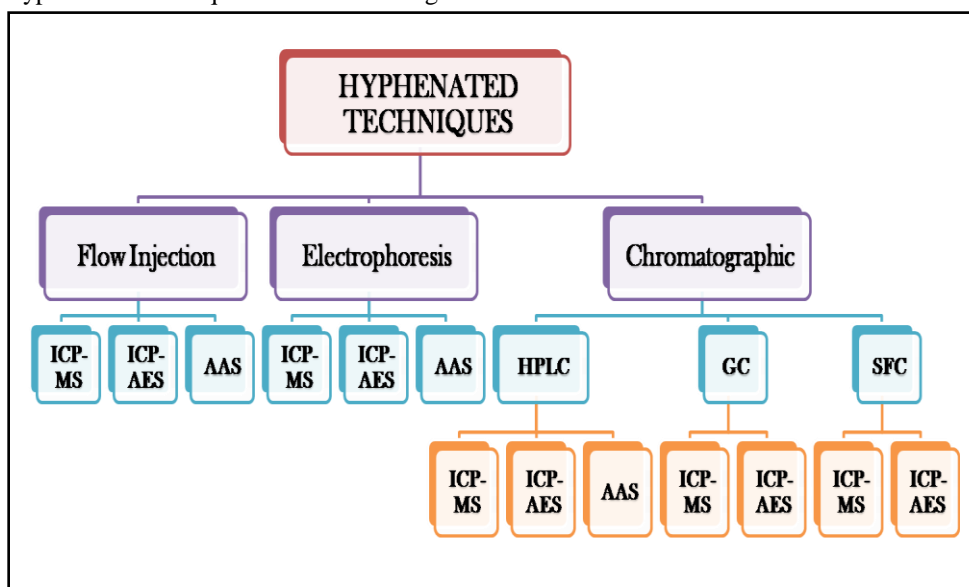


Figure 2: Types of Hyphenated Techniques

(i) Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)

ICP-AES is a powerful hyphenated technique for the rapid multifunctional analysis and detection. It is operated by the use of argon plasma in forward power level of 1 KW or more. The argon gas flows inside the AES carrying the sample at a rate below 1L/min. instead of the rapid growth of ICP-MS, ICP-AES still plays a major role for the detection of elements of geological, biological and environmental materials and systems because of its relatively lower cost than ICP-MS. It costs about \$60,000 to \$120,000 only compared to \$350,000 to \$500,000 of ICP-MS.

There are various modes for the placement of plasma, of which the end-on viewing mode is preferred because it improves the limit of detection, which is further dependent upon three factors:

- Relative standard deviation (RSD)
- Signal-to-background ratio (S/B)
- Conc. at which S/B of analyte line is measured.

For better analyzer, the S/B should be more and RSD should be least. The concentration is generally 100 times the detection limits. Range of the wavelength used is another critical feature which indicates that how many elements could be determined as all elements have a characteristic wavelength. For e.g. Pt (214.423 nm); Pb (220.353 nm) ; to



alkali metals having wavelength above 500nm like Na (589.592 nm) ; K (766.49 nm) ; Li (670.781 nm). Hence the present-day ICP-AES have a wavelength range of 120-770 nm.

The key features which are used for the selection of a good ICP-AES instrument is the warm-up time. It is the time required to reach a steady signal from the point of ignition of the plasma suitable for qualitative analysis where variations in the signal remains within the short term fluctuations. In latest instruments, the warm-up time is about 15 min which is an indication of an outstanding instrument compared to the warm-up time of 90 min in case of conventional instruments.

Several elements like Fe and Ca can not be determined by the conventional ICP-MS. This is due to the mass spectral interferences by argides. It can be viewed within excellent detection limits if the end-on ICP-AES is used [18].

(ii) Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS is the most widely used Hyphenated technique for multiple elemental detection. The sample to be tested is transferred into plasma in the form of aerosol. The process which takes place in the plasma is Desolvation- Vapourisation- Atomization- Excitation- Ionization. ICP-MS has the ability to provide with routine isotopic ratios of elements. The detection limit of ICP-MS is 10-100 times superior to ICP-AES.

It is presently the most powerful technique for the detection of metals and several non-metals. The major resistances for the widespread acceptance of ICP-MS are its high cost and many technical and operational issues. But the cost of instrument has decreased significantly leading to its more widespread applications, also the size has reduced severely. It has a range of eight orders of magnitude.

Sometimes, the ICP-MS is more susceptible to instabilities than ICP-AES. For e.g. in the analysis of high level of TDS, this happens due to direct contact of the sample to the plasma, while the sampler and the skimmer are relatively cooler.

ICP-MS was previously mostly involved in the detection of elements of aqueous solutions, and a greatly simplified spectra compared to ICP-AES was observed with a speed of more than 73 elements per minute. But now, solid samples can also be analyzed but at a relatively lower rate [18-19].

(iii) HPLC/ ICP-MS Combining ICP-MS with High Performance Liquid Chromatography enable separation and speciation analysis. A particular species can cause different effects of living species. A wide list of unknown elements which are significantly responsible for the health risk still exists and hence certain very high speciation hyphenated technique is required.

One of the most widely used techniques for speciation analysis is the HPLC/ICP-MS. A hyphenation set is used to hyphenate HPLC (separation system) ICP (detection system). It is a multielemental analysis and hence the capability to analyses 40 elements simultaneously. Different types of liquid chromatography columns can be used, such as reversed phase, reversed phase ion pair, micellar, ion exchange, size exclusion and chiral Liquid Chromatography [20].

(iv) LC-NMR liquid chromatography (LC) and nuclear magnetic resonance (NMR) technique is used for the structural determination of the samples to be characterized. The technique is similar to LC-MS, but has comparatively lower sensitivity. The sensitivity of this technique could be improved by decreasing the flow rate to less than 1ml/min. Application of this technique is in specialized identification such as in drug metabolism, it identifies even the polar or unstable metabolism, drug impurity, drug discovery and food analysis. It is also applied to amylase plant constituents and marine alkaloids [21].

(v) GC-MS It is a hyphenated technique of elemental analysis which features gas chromatography and mass spectrometry together for the identification of different analytes contained within a sample. The technique has been termed as “Gold Standard” for forensic sciences.

A carrier gas is required to push the material forward from the chromatography chamber to the mass spectroscopy column. Nitrogen gas is generally used as the carrier gas because of its inert nature. GCMS is sometimes equipped with online MS database for reference purpose which also searches the compatibility of the compound [22].



3. Conclusion

Detection of element is the key parameter of research and development. An extensive range of analytical tools already exists for elemental analysis, some of which is presented in this article. To make the detection process a bit easier, hyphenated methods have been introduced which combines both the separation as well as the detection (structural as well as non-structural). Different types of sample and its concentration is responsible for the technology to be adopted. It can also be concluded that Mass Spectrometry has been proved better than NMR. MS is much more sensitive and hence consumes lesser amount of sample compared to NMR.

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