REVIEW ARTICLE

Techniques for Metal Ion Determination in Samples

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ABSTRACT

In human body building as well as in plants Metal ions play a vital role in vital functioning such as in various food chain reactions. Due to increase in industrialization everyday various industrial wastes contaminated with metal ions and metalloids. Several enzymes need them for their catalytic action, and their absence can cause a number of illnesses in humans. When this metal ions concentration level increases above the certain limits, it is harmful to human, animals, plant as well as aqua system. Because various essential biological processes in humans depend on metal ions, their absence or scarcity may result in diseases. In pharmaceutical sectors various transition metals are used as catalyst to improve kinetics of reaction. Therefore, it is necessary to determine concentration of metals ions whether it is present in within the limits, otherwise excess of its concentration may leads to toxicity in body. Also, the advisory body has made some regulatory guidelines to monitor and control levels of metals ions in food, environment and medicines. Hence more precise, accurate, economically acceptable, sensible and selective method are required to detect metal ions concentration in pharmaceutical, environmental, agrochemical and aquatic samples as well as in body tissues/fluids is also essential. Hence in our review we try to sum up various metal ion detection techniques, there mechanism and principle behind detection of heavy metal ion detection and instrumentation. The given methods are more sensitive at very low detection limits of up to pictogram level, accurate and more precise levels detection has been achieved.

Keywords: Metal ion, Speciation of trace metal ions, classes of elements, Techniques for the detection of metal ion.

INTRODUCTION

Heavy metals are present in all over the ecosystems due to anthropogenic sources like industrial and agricultural activities.[1] Some of categorized metal ions essential in human body to frame human body as well as functions as catalyst and co-factors in nucleic acids, blood, bone, blood cells, hairs, teeth, brain, receptors etc., But slight increase in its concentration in human body may be harmful

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or even toxic to human life. In the past few decades, directly or indirectly consumption of heavy metals has substantially increased due to the industrialization. Drainage from Industrial waste reported to be containing with variety of metal pollutant which have ability to affects human, animal as well as aquatic systems. The Industrial waste survey reported that the major sources of metal ions pollution of metalloid waste in the aquatic environment was waste discharge mainly from metal finishing industrial effluents, Glass production industries, electroplating stainless-steel industries, sewage, Water solar treatment plants and waste-water treatment plants discharge and various metal ions from metal-cooling water.[2]

Heavy metal ions acts as toxic chelating agent and can form complexes with oxygen, nitrogen and Sulphur atoms of various bio-ligands in living body, human and in animals also. They act as nucleophilic molecules and intercalate with living organism and human body, thus shows redox processes, hence heavy metals ions concentration have received considerable attention from analysts.[3]

The mechanism of heavy metal toxicity is due to their ability to interact with functional groups of biological macromolecules. The various metal toxicity, serious adverse events was noticed in the humans, aquatics and the environment and thus, this tragedy attract stressful attention, which requires monitoring of their limits/levels in different samples. The metal ions contamination during in any product process, process intermediates and final products is monitored as well as limited to meet regulatory approval as well as it's an important activity in food, drug and pharmaceutical industry.[5]

Metal impurities incorporated by means of various sources such as those e.g., metal catalysts as an impurities that are deliberately added to the reaction system to fasten the reaction process , sometimes metal ions species were leached from pipes and other equipment those incorporated in the manufacturing process, The starting materials or reagents containing impurities directly-indirectly incorporate impurities in a manufacturing process, and naturally derived plant or mineral acts as raw material sources also may contains certain impurity.[6]

The impurities are classified into two parts by pharmacopeia forum of USP are shown in the form of two chapters which are given as follows in Table. 1

Table 1: Elemental impurity classes and elemental impurities examples with their limits

Class	Assessment	Element	Component	Limit	(µg/g)	Oral daily
Class-1	Elements should be essentially absent Known for strongly suspected human toxicants Environmental hazards	Arsenic	1.5	15	0.15	1.5
		Cadmium	0.5	5	0.05	0.5
		Lead	1	10	0.1	1
		Mercury	1.5	15	0.15	1.5
Class-2	Elements should be limited Elements deliberately added to an article	Chromium	25	250	2.5	25
		Copper	250	2500	25	250
		Manganese	250	2500	25	250
		Molybdenum	25	250	2.5	25
		Nickel	25	250	2.5	25
		Palladium	10	100	1,0	10
		Platinum	10	100	1.0	10
		Vanadium	25	250	2.5	25
		Osmium Rhodium, Ruthenium Iridium	10 (combination not to exceed)	100 (combination not to exceed)	1.0 (combination not to exceed)	10 (combination not to exceed)

- Elemental Impurities-Limits which includes Arsenic, Cadmium, Lead and mercury as metal ions and
- Elemental Impurities-Procedures to replace the general test chapter-Heavy Metals which includes Nickel, Osmium, Rhodium, Ruthenium, Iridium, Chromium, Palladium, Platinum, Molybdenum, Copper, Vanadium, Manganese.[7]

The pharmacopoeias recommended various techniques for determination of elemental impurities in food, drug products, pharmaceuticals, excipients or pharmaceutical dosage form, mostly based on limit tests. Some of the general analysis tools and techniques were utilized by various analysts include Acid value, heavy metal test, wet-acid digestion, Residual analysis, sample dissolution, ash or dry ashing and ignition followed by precipitation of metals in sample. The presence of metal is measured by visual comparison of test samples with a standard solution. They are easy to perform but have many disadvantages such as lack of specificity and sensitivity. The interference of other concomitant elements is not possible and will react with reagent and precipitate. In case of ashing method, the various volatile elements/material get volatile during heating and results in loss of volatile elements. Such type of disadvantages of these methods limits their use and requires more accurate, precise, economical method development or technique to estimate metals in environmental and pharmaceutical products. Various ancients' methods of analysis like titrimetric analysis methods which includes electrochemical and voltametric analysis. Nowadays advanced instrumental analytical methods such as atomic absorption spectrometry (AAS); atomic emission/fluorescence spectrometry (AES/AFS); neutron activation analysis (NAA), X-ray fluorescence (XRF); inductively coupled plasma mass spectrometry (ICP-MS); inductively coupled plasma optical emission spectrometry (ICP-OES) and anodic striping voltammetry (AVS), etc. may be employed to determine the trace concentration level of heavy metals in various samples. The widely used and most predominant techniques (enlisted in Table 2).[9]

Speciation Analysis

Speciation of trace metal ions in an environmental analysis is the useful technique to quantify metal ions in aqueous samples and successful use of appropriate methods for monitoring of water quality. Speciation have different subtypes among them, method selection is the most important aspect of speciation analysis. The speciation of chemical compounds having different types

Table 2: Different types of metal ion detection techniques

Method	Examples
Optical methods	Molecular-absorption spectrophotometry
	Molecular-fluorescence spectrophotometry
	Flame-emission photometry, Spark-and-arc emission spectrometry
	Atomic-absorption spectrophotometry
	Atomic-fluorescence spectrophotometry
Electrical methods	Classical polarography
	Special voltametric methods
	Anodic-stripping voltammetry Hanging-drop Hg
	Thin-film Hg or solid electrode
	Specific ion electrodes
Other methods	X-ray emission
	Neutron-activation analysis
	Mass spectrometry
	Gas chromatography (also GC-MS couple

Table 3: Basic types of speciation analysis and its applications

Type of speciation	Characteristics	Area of application	Examples of Application
Physical speciation	Involves the determination of different forms of same chemical species.	Air, water, soil and pollution analysis	a) Trace metals analysis (soluble and suspended fraction). b) Trace metals analysis of different forms present in soil and sediment after sequential extraction.
Chemical speciation	A type of chemical speciation that involves the extraction, detection and determination of a specific chemical species or analyte.	a) Air, water and soil pollution analyses. b) Food contamination studies.	a) Determination of tributyltin (TBT) or triphenyl tin (TPhT) in environmental biota, sediments, seawater, etc. b) Determination of methyl mercury in fish tissue or lead in food products.
Screening/ Distribution speciation	A type of screening speciation that involves the detection and determination selected chemical individual in particular elements of analyzed sample. Usually employed in analyses of biological samples.	a) Air, water and soil pollution analyses. b) Ecotoxicological studies.	a) Trace metals analyses especially in blood serum and cells. b) Determination of trace metals in plants samples.
Group speciation	A type of group speciation that involves the extraction, detection and determination of all chemical species in analyzed sample.	a) Air, water and soil pollution analyses. b) Ecotoxicological studies. c) Food contamination studies.	a) Identification and determination of chemical species defined as to molecular, complex, electronic or nuclear structure.

of structural propertied such as electronic configuration, isotopic composition, oxidation or electronic state, changes in conformation or in the complexity or binding ability of substituent's in terms of covalency leads to a challenging task for an analyst regarded as distinct chemical species separation.

Speciation of elements study may affects various biological factors such as bioavailability; pharmacokinetics, T_{max}, bioaccumulation, toxicity, bioconcentration; plasma protein binding and the other are geochemical factors which include adsorption, distribution and the precipitation of the element of biocomponents.

Types of Speciation and Application in Chemical Analysis

The Speciation analytical investigation includes extraction, detection of ultra-trace quantitative and qualitative determinations of elements. Some basic types of speciation analysis commonly used in chemical analysis, their characteristics, areas of application and examples of where the speciation analysis principles could be applied are given in Table 3.[10]

As compared to chemical components, analysis of matrix type sample elemental analysis is the challenging task as it varies with:

- Different approaches are required for speciation analysis in waters or biological materials, or soils/ sediments.
- There are difficulties associated with separating the compound(s) of interest from complex matrices;
- Most of the speciation techniques available may

disturb the equilibrium existing between the various chemical species present;
- Species present at ultra-trace levels; few analytical procedures possess the degree of sensitivity and selectivity.
- Suitable standard reference materials are often unavailable for comparison.

Most of the methods that are developed for analytical speciation involve multiple reaction steps like extraction, preconcentration, cleaning, derivatization, chromatographic separation and element specific detection. Due to multiple steps involved, may alter the stability of sample, changes ambient conditions of processes, therefore pretreatment of sample preparation is a very important area in pharmaceutical and biological analysis to stabilize the sample environment.

During sample preparation, sample containing trace/ small amount of analyte requires special care for sample preparation and highest purity of ingredients should be used to avoid risk of contamination. When elements estimated in samples, upper and lower limits of the elements also detected and calculated. Pre-concentration of samples can be done by using chromatographic techniques, solvent extraction and decomposition of the sample by using a reagent or heat. With the invention of various high-resolution separation methods coupled on-line with the final analytical measurement like ICP-MS can be employed.[11]

In this review, we have tried to present advances in metal ion detection in the biological and environmental samples containing trace metals. This review represents on the update of advances in trace metal detection and measurements.

Techniques for Metals Detection

Atomic absorption spectrometry (AAS)

Being a quantitative method, the AAS can be detect about seventy elements as metal ions in different samples. The concentration estimation is based on the how much amount of light is absorb by an Atoms in a sample, increased in atoms in sample will results in a reduction in the amount of light intensity reaching towards the detector. Generally, Hollow cathode lamp (HCL) is used as an energy source in order to get narrow light absorption with maximum sensitivity as. Hence the measurement of concentration of a particular element is based on the amount of transmitted light towards the detector. A general instrumentation of AA spectrometer composed of radiation (light) source, sample holder (atomizer- flame, a graphite furnace, or a quartz tube), grating monochromator, detection system and a data analyzer and processing system as shown in (Figure 1).

The lamp contains of a tungsten filaments which acts as anode and a hollowed-out metallic cylindrical which acts as cathode. Both anode and cathode were sealed in a glass tube/cylinder generally it is filled with neutral gasses like an argon or neon under pressure (1 to 5 torr); Hence the media is transparent to emitted light. Monochromator is used to obtain monochromatic light of the desired wavelengths by a number of lenses, gratings, dispersive elements and mirrors which were used to focus the radiation. The monochromators used to get narrow and desired wavelength of light. The detector such as Photomultiplier tube is used to multiply small amount of radiation waves into a multiply a higher concentration so that it will converts the obtained light signal to an electrical signal proportional to the emitted light intensity obtained from sample solution. The conductance across the between the anode and cathode was maintained as of about 100-400voltage (2-30 mA) results in ionization of the fill gas.

$$Ar + e^- \rightarrow Ar+ + 2e^-$$
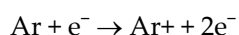
The generated current helps in differentiation of ions depending on positively charged ions attracted towards the cathode the and the charged molecules collides with the with fill-gas molecule ions results in collision between molecules and charged particles. Thus sufficient kinetic energy generated due to the collision that may leads to liberation of metal ions in process called as 'sputtering'.

$$M(s) \rightarrow M(g)$$
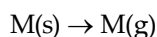
The liberated metal atoms, fill-gas ions and electrons undergo further collision and thus causing excitation of the metal in process.

$$M(g) \rightarrow M^*(g)$$
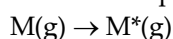
When the excited metal atoms start to come back into its ground state as soon as they emit characteristic
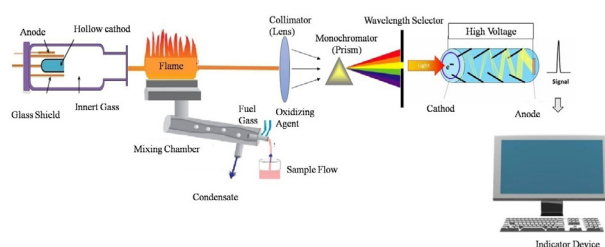

Figure 1: Basic illustration of atomic absorption spectrometer

wavelengths of lights depending on characterization of the metal itself. Nowadays, determination of more than a single element is possible by using the Multielement cathode lamps.

- **For example:** sodium vapor exhibits sharp absorption peaks at 589 & 589.6 nm in the yellow region of the visible spectrum leads to electronic excitation of the of molecular stats from 3s electron to two 3p states with slightly change in energy.

- **For example:** The various styrene derivatives are used such as chloromethylated polystyrene-PAN, CMPS-PAN separates various metal ions depending on the principle of chemically modified ion-exchanger affinity toward metal ions and used for the separation of Gold[Au (III)], Cadmium[Cd (II)], Cobalt[Co (II)], Chromium [Cr (III)], Copper[Cu (II)], Manganese [Mn (II)], Nickel [Ni (II)],Iron [Fe (III)],Palladium [Pd (II)], Lead [Pb (II)] and Zinc [Zn (II)] from aqueous specimen or sample solution. The metal ions estimation involved preconcentration of as sample solution and determination of heavy metal cations based by using atomic absorption spectrometry (AAS).[12-17]

Flame atomic absorption spectrometer (FAAS)

The quantitation limits of FAA is more as compared to AAS as FAA can detect part per million (ppm) concentration metal ions concentration in many metal or elemental sample solution. FAAS consist of various parts such as burner and flame of an air-acetylene and/or nitrous oxide flame connected with the atomizer which sprayed fine particles spray of feeded sample solution into micro particles.(Figure 2). The pneumatic type of nebulizer is widely used as nebulizer, nebulized sample solution on a flame to converts aqueous sample into its aerosolized micro particles. It requires very few sample solution for estimation using FAAS technique up to 10-15 s/sample. FAAS is more accurate, precise and have high repeatability, relatively low cost of sampling and errors can be easily corrected. Hence FAAS method is useful for the determination of heavy metals in complex macro as well as in micro biological matrices and soil sample solutions. But it limited to those samples which contain elements like to arsenic (As) and zirconium (Zr) as FAAS
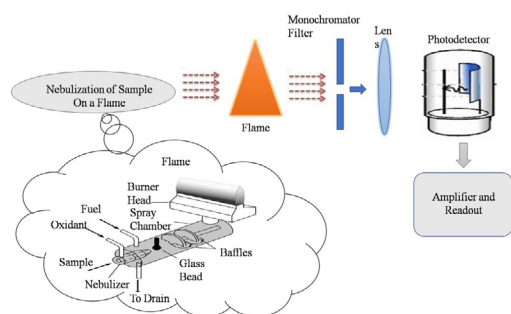
Figure 2: Nebulizer/expansion arrangement with flame burner head and Basic instrumentation of Flame atomic absorption spectrometer

atomization temperature is unable to induced complete atomization and thus molecular excitation of a sample solution.

The samples aerosolized through the capillary under the pressure of carrier gas (argon, at a pressure of 70-350kPa) escaping through two types of orifices exists between the outside of the capillary tube and the inside of the capillary tube and the outside of the glass concentric tube. The toroid (10-35μm in diameter) created by the two tubes for escaping argon gas and liquid sample in such a way that they will sufficiently produce a coarse aerosol (the Ventura effect). The expansion chamber, converts the coarse aerosol into finer aerosol and thus the rate of this nebulizer is between 0.5 and 4 ml per minute. (Figure 2) As compared to other methods FAAS is relatively inexpensive and easy to operate but has some drawbacks that limit the use of flame atomic absorption spectroscopy).[17-19]

- Large volumes of aqueous sample required in introduction system.
- The Contact time between metal ions and gaseous molecules is relatively high results in burning velocities of the gases, helps in estimation of higher amount of ion metals.

Requires aqueous sample preparation before analysis to analyze solid samples directly.

Graphite furnace atomic absorption spectroscopy (GFAAS)

GFAAS is an atomization technique used to determine analytes concentration up to parts per billion (ppb) levels. It involve steps such as stabilization of sample, for these purpose the sample is mixed with chemically modified which helps to stabilize the analyte. It can be used for macro molecules as well as for micro molecular matrices to make them more volatile prior to the atomization processes and dispensed into a graphite tube (atomizer). Both the end of this graphite tube is open and it has 5cm long and internal diameter 3-8mm as dimensions. At the middle part has a central hole as a feed inlet for introduction of sample with the help of a micropipette.

The inert gases such as Nitrogen and Argon gases (N_2 or Ar) used as carrier in graphite tube and their flow rate was maintained constant through-out the all steps to remove the residual extraneous material. The sample is travelled inside the graphite tube through a series of programmed heating stations or heaters which is connected to the electric current. The electrical supply is connected to both the end of graphite tube via water cooled contacts, so that the boiling temperature of water at 110°C for 30s is maintained so water will dry instantly. The dried particles formation is also called as ashing step, which includes further heating of the graphite tube at temperature between 350 and 1200°C for 45s. At the end of heating process, sample is then automized sample at graphite tube temperature in between 2000°C- 3000°C for 2-3s so that residual solvent will evaporated. The light source will pass radiation through sample containing graphite tube, the contact between sample and radiation light will increases the sensitivity and accuracy to estimate concentration of metal ions. The concentration of metal ions calculated on the basis of how much amount of light was absorption through radiation source is measured by the atomic vapor. The analysis of sample requires small quantity of sample up to 20μl. Figure 3 represents basic instrumentation of graphite furnaces. When their is a continuous changes in temperature, atomization of samples will achieve. That's reason the temperature is continuously changes during each different stages help to improve the reproducibility of analytical signals. But GFAAS have some disadvantages like slow analysis with a limited working range and high cost of sampling.[20, 21]

Hydride generation atomic absorption spectroscopy (HGAAS)

The HGAAS atomization technique is widely used to detect metalloids like antimony, arsenic, selenium, and tellurium. The sample is acidified and then allowed to react with a reducing agent such as sodium borohydride and will form volatile hydride. The formed hydride is dragged with neutral gasses such as argon gas and then transferred towards the heated quartz cell atomizer
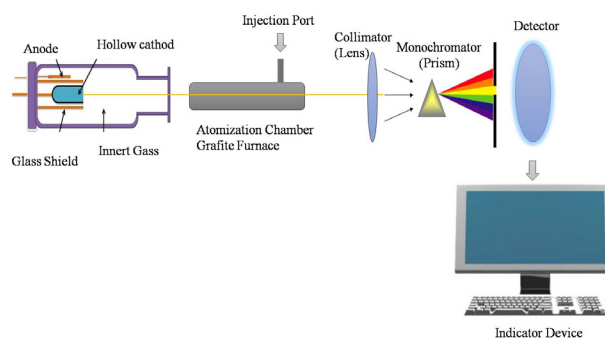
Figure 3: Basic Layout of a Graphite Furnace Instrument

and then to the optical axis of the conventional AAS as depicted in Figure 4. Under the quartz cell, the hydride metal transformed into gaseous metalloid analyte atoms since, the light source is continuously passed through sample quartz tube which acts as path length of a sample tube through light source passed. The electric signal is generated depending on the amount of light light absorbed by sample. HGAAS have limited used for certain metal ions, since certain elemental properties hindered estimation such as valence state of the analyte, configuration, gas pressures in the optical axis, acid concentration, stereo-structure nature and the cell temperature and to achieve high quality data, a highly skilled operator is required.[21,22]

Cold vapour atomic absorption spectrometer (CVAAS)

In CVAAS technique mercury vapor can be measured at room temperature and can be operated at normal temperature conditions. The CVAAS technique operated at cold temperature and can estimate mercury concentration in any sample. The mercury detection step involves the conversion of mercury to its elemental state by using strong reducing agent such as sodium borohydride or stannous chloride. The reduced form of mercury vapors were dragged in Neutral gasses such as argon (Ar) carrier gas and then transferred to the absorption cell kept in path of a light source of the spectrophotometer (Figure 5). Mercury specifically absorbs light energy at 253.7 nm, the amount of energy absorbed by mercury and the concentration of ions detected by using Beers Lambert law as the decreased in light proportional to the concentration of mercury in the sample. CVAAS has been

widely used to detect Hg in various pharmaceuticals, marine food, drug and variety of shark, fishes as well as in some ocean natural remedies.[23]

Voltammetry

Voltametric analysis is based on the change in the current in volts due to the chemical changes in ion concentration at the electrodes. Thus concentration of ions is directly proportional to the change in electrical discharge towards the readout system. Various electro-analytical techniques are used in small scale to measure change in voltage current of electrode potential (E) with respect to ions concentration is called as voltammetry. It has sensitivity from ppb to ppm or higher level of concentration of the sample. The differential pulse voltammetry or square-wave voltammetry has ability to measure current directly and can be used to detect higher concentrations (ppm or high ppb) levels in sample; whereas when these voltametric techniques are combined with a preconcentration step the lower concentrations of analyte can be measured and is known as stripping techniques.

Stripping Voltammetry

Stripping voltametric analysis estimation depends on accumulation, deposition or preconcentration or accumulation of metal ions at the different poles of the electrode. Anodic stripping voltammetry (Figure 6) measures the metal elements when sample is preconcentrated by means of stirring. During stirring metal ions get preconcentrated and metal elements separation achieve by means of convection transport. The preconcentration is achieved by applying small amount of potential energy 0.3–0.5V to achieve electro-deposition onto small-volume mercury electrode. The applied voltage currents are very small (negative) as compare to the peak potential for the metal ion determination. The estimation of metal ion is depending as few or 20 second to 20 minutes, how much amount of metal ions reduced and concentrated as Amalgam. During preconcentration, the linear voltage or pulse ramps potential energy applied anodically which results in reoxidation of amalgamated metals and stripped out of the electrode. As the metal elements starts to reoxidised as soon as the current starts to flows through the voltaic cell which is directly proportional to the concentration of the metal in the solution.

$$Mn+ + ne^- + Hg \rightarrow M (Hg)$$

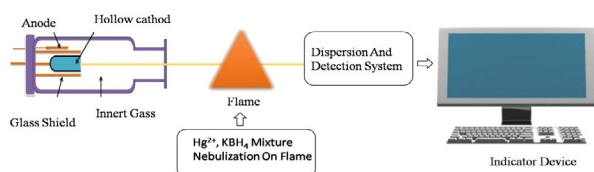[preconcentration] $M (Hg) \rightarrow Mn+ + ne^- + Hg\backslash$[stripping]
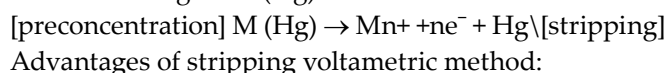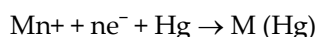
Advantages of stripping voltametric method:

- It is a sensitive to ppm concentration.
- Variety of elements estimation at a time.
- It has ability to differentiate metal elements depending on its oxidation state.
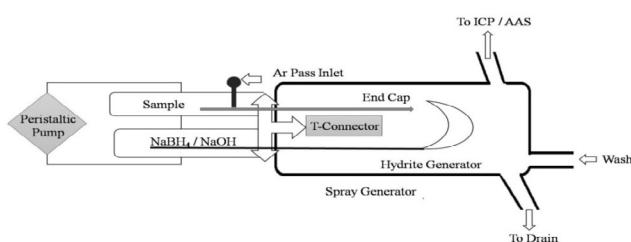
Figure 4: Basic Layout of Hydride generation atomic absorption spectroscopy

Figure 5: Basic Layout of Cold vapor atomic absorption spectrometer

- It is helpful for pharmaceutical, marine, agrochemical, soli and environmental sample analysis.[24-26]

X-ray fluorescence (XRF)

XRF technique work on the principle of a physical phenomenon where high intensity X-ray radiation UV-rays is collides with elemental electrons and thus, the electrons from inner orbit will jump to the unoccupied spaces in the outer orbit and becomes unstable and radiates energy called X-rays. (Figure 7)

Different elements have different electronic energy levels, the energy of the X-ray fluorescence peak is different for different elements and thus on these basis identified a specific element. Sodium, Halogens, Uranium and other metals easily estimated in specimen and matrices sample by X-ray fluorescence spectroscopy (XRFS) with minimal sample quantity. The instrumentation generally consists of radiation or light (X-ray) source, sample chamber and sample holder, detector system and a readout system as computer for data processing. X-ray fluorescence is also used to quantitation of metal ions level in soil samples collected from various industrialized areas such as Arsenic (As), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), vanadium (V), and zinc (Zn) can be estimated in a ppm level.[27,28]

Inductively Coupled Plasma Mass spectrometry (ICP-MS)

ICP-MS is a quantitative method used to estimate various elements in the system simultaneously. ICP-MS involves two steps. In the first step, the dissociation of molecules into its atoms or ions species using argon plasma source at high temperature (6000-1000K). Samples are introduced via peristaltic pump in either gaseous or aerosol with the help of sample nebulization to the plasma torch (Figure 8).
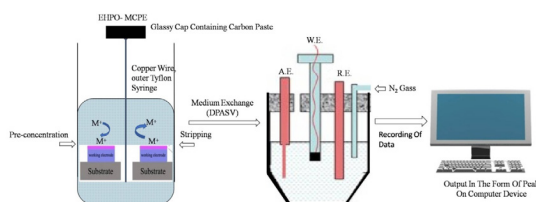
Figure 6: Basic Layout of Pre-concentration and stripping process of metal ion and detection by Anodic stripping voltammetry
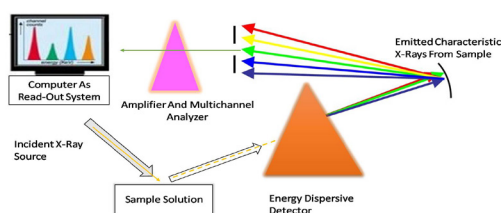

Figure 7: Basic Layout of X-ray fluorescence

Formed ions are attracted towards the magnetic deflector from the plasma and ions were distinguished on the basis of their atomic mass to charge ratio (m/e⁻) by using mass spectrometer – quadrupole mass analyzer or magnetic sector analyzer. The amount of light energy emitted by the metal ions is the measure of amount of metal ions concentration.

ICP-MS has parts-per trillion (ppt detection) sensitivity to detect various elements in variety of samples. ICP-MS also can be used to quantify the ultra-trace elements in sea water samples having higher saline matrices. The chemical complexes such as ammonium tetra methylene dithiocarbamate and methyl isobutyl ketone were used for sample preconcentration and to remove matrix prior to analysis, thus it will helps to minimize the interferences in analysis and improve the stability of sample. ICPMS is widely used to estimate trace elements Chinese and Malay medicines as well as in traditional herbal medicines such as Zinc(Zn),Manganese (Mn), Selenium (Se), Cupper (Cu) and Cadmium (Cd).[29,30]

Inductively coupled plasma/optical emission spectrometry (ICP OES)

Inductively coupled plasma/optical emission spectrometry (ICP/OES) work on the principle of detection of elements in a sample when they emits the photon energy when they collides with radiofrequency (RF) waves as radiation energy and get excited to emits energy. Prior analysis solid samples were required to digest with the Acids to improve sensitivity, but the gaseous and liquid samples not required such type of treatment and can injected directly into the plasma. The Injected sample is aerosolized with the help of nebulizers into micro particles and then transferred toward the center core of the plasma which has 10,000 K higher atomization temperature. The temperature is sufficient to convert plasma free atoms into the gaseous state. The formed atoms will collide with each other with small amount of kinetic energy in an ions and thus excitation of atoms from ground states. The excited ion will emits certain amount of photon radiation energy of a specific wavelength depending on specific elements. Depending on the amount of radiation energy emitted by an atoms will helps in estimate the concentration. The concentration of elemental composition is based on the relationship between the amount of radiated emery is directly proportional to the amount of elemental species present in sample (Figure 9). A variety of elements such as arsenic, Uranium, selenium, Cadmium and antimony detected by ICP-MS in combination with nebulization, hydride generation (HG) as well as electro thermal vaporization (ETV) and laser ablation methods.[13,30]
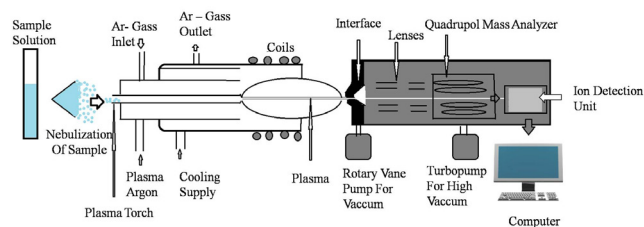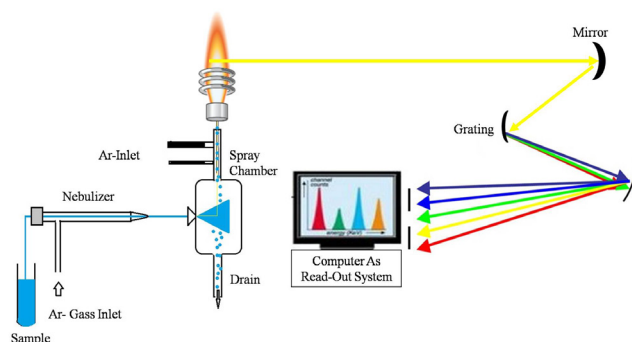
Figure 8: Basic instrumentation of Inductively Coupled Plasma Mass spectrometry

Neutron activation analysis (NAA)

NAA is the quantitative analytical technique implemented to estimate metal elements in a in different matrix samples. The quantitation of elements is based on intensities of gamma rays emitted from the radioactive isotopes which are proportional to the amount of the element in the sample. It includes collision of elements containing sample and neutral gases like Argon neutron flow after and desired radioactive isotopes of elements are generated. The unstable as well as excited metal elements radioactive isotopes decay to a lower energy state and emit delayed gamma rays with characteristic energies depending on variety of metal element. The radiated gamma rays intensities of the radioactive isotopes is calculated to estimate elemental concentration as it directly proportional to the concentrations of various elements in the sample (Figure 10). It is more superior, have higher sensitivity and selectivity for different elements (0.1 to 106 ppb) than other as these method have the sensitive to detect largenumber of elements simultaneously without affecting the sample quality and quantity in the sample, The estimation of elemental composition in Crab and shrimp , green tiger shrimp (*Penaeussemisulcatus*) and blue crab (*Portunuspelagicus*) from the Persian Gulf were estimated by NAA method.[30,31]

Thin Layer Chromatography

The Thin layer chromatography method has been developed on the principle on adsorption and separation of chemical species on adsorbent such as Silica gel-G and various mobile phases of different pH solutions for the separation of metal ions such as Tl (III), Cd (II), Cr (VI), As (III), Cr (III) and Hg (II) separation from binary, tertiary, quarterly and other multicomponent mixtures. The separations were achieved on the adsorbents surface with the chemical interaction and affinity of chemical elements with the mobile phase. The Silica gel-G and its modified congeners nowadays widely used as adsorbing material. The various organic, inorganic and aqueous phases combinations were used as mobile phase which helps to separate elements depending on pKa and pKb values. The metal elements separation is achieved by

Figure 9: Basic instrumentation of Inductively coupled plasma/ optical emission spectrometry

continuous changing and monitoring the mobile phase composition of pH and the Rf values of individual metal ions were studied by referring standard Rf values of metal ions. Among the all performed trials the optimized suitable conditions were selected for separation of metal ions from sample mixture. The buffer concentration or mobile phase pH 4 is ideally used for better separation of mixture components(Palatal LJ *et al.*, 2011).

Cu2+-V5+, U6+-V5+, U6+-Fe3+ , Fe3+-Cu2+ and tertiary mixture of U6+-Fe3+-Cu2+ were can be differentiated using TLC by implementing urea-formaldehyde polymer, organic, surfactants , acidic, cationic and anionic and neutral mobile phases.[33]

HPLC

The various metal ions having different electronic configuration, but having the same mass, metal ions which can't be resolved and indistinguishable by mass spectrometry such types of metal ions estimated by chromatographic method-using High performance liquid chromatography. But due to poor detectors sensitivity limits its use. To improve sensitivity, column effluent is mixed with derivatized with derivatizing agents such as luminal (5-amino-2, 3- dihydro-l, 4-phthalazinedione) and then allow to pass through hydrogen peroxide solution prior adition in solvent reservoir chamber. The oxidation of the luminal (5-amino-2,3- dihydro-l,4-phthalazinedione) metal ions to excitated oxidized chemiluminescence product such as 3-aminophthalic acid. The concentration of metal elements is estimated depends on chemiluminescence of the metal ions in solution. This derivatization process is widely used quantitatively to estimate the different types of trace metals in speciation solution as well as error in detection can be studied easily and neglected or masked.[34]

Ion Chromatography

Ion Chromatography used for separation of heavy metal ions on the basis of derivatization and complexation reaction. The column eluted is treated with buffers,

complexing agents (oxalic acid EDTA or tartaric acid as well as derivatizing agents so that ions will show Ligand-Metal complexation behaiour and help to improve sensitivity and selectivity for heavy metal ions by colour, Chemiluminescence, complexing, Colourimetric or photometric changes. These types of reaction were implied as well as post column derivatization reaction also used to improve detection of metal elements. In the precolumn techniques, sample concentration allowed for equilibration of the sample with column material and improving detection sensitivity of ions. Nowadays, the post-column reaction achieved by the combination with the chromophoric reagents the 4-(2-Pyridylazo) resorcinol (PAR) with Zn-EDTA which forms colored complexes with large no. of metal ions and improve detection and specific sensitivity of ions such as calcium and manganese ions.[35]

Laser-Induced Breakdown Spectroscopy with the Assistance of Electrical-Deposition:

It consists of coil made of 99.99% purity aluminum having length of 42 mm and 8 mm as a diameter was rotated around its axis by a stepping motor which acts as cathode with speed of 20 rpm. A platinum wire having spiral, twisted and helical shape having diameter of 1 mm, length of 50 mm and 30 mm inner diameter acts as anode and the central axis having aluminum rod placed and DC voltage current of 2–15 V was applied to start a voltaic cell current. A glass beaker having capacity of 1000mL acts as deposition cell which connected to the magnetic stirring apparatus. The sample solution was kept in glass beaker and allowed to stirred at about 600 rpm so that the metal elements will form uniform electrical-deposition. As current passes the aluminum rod starts to revolve around its axis and the electrical-deposition process occurs with the evolution of gas bubbles. After a short time interval, the process is stop and elemental deposited aluminum rod is then connected on the translation stage for elemental analysis by LIBS. The water sample of specimen sample having Cr^{3+}, Cu^{2+}, Mn^{2+}, Cd^{2+}, Zn^{2+} and Pb^{2+} metal elements were estimated quantitatively. But these methods have some disadvantages like time

Figure 10: Basic principle of gamma ray emission from the radioactive isotopes and its detection by Neutron activation analysis

consuming process, electrical deposition take time for electrical deposition and enrichment, hence required time for analysis.

Thus, to overcome this drawback nullifies by simultaneous multi-element analysis of sample, detection sensitivity improved of these methods are improved by using a dual-pulse LIBS setup, using optimized and more accurate, sensitive optical detection system and by improving the design of the electrical deposition cell.[36]

Nano-Silica Modified by Hematoporphyrin:

Nano-Silica Modified by Hematoporphyrin technique adopted for detection of metal ions such as Cu (II), Zn (II) and Mn (II) in speciation. The COOH and-OH groups groups were helps in complexation with Hematoporphyrin on the surface of silica at-Si-OSi and –OH N and form spherical shape of about 7.4nm called as a Nano-Silica Modified by Hematoporphyrin complex. The characterization of shape and pore diameter is ensure and measured by using FTIR, elemental analysis, Transmission electron microscope and EDAX technique. The used new sorbent Nano-HP silica material has good thermal stability, sensitivity, selectivity and have good preconcentration factor and can detect Zn, Cu and Mn metal ions in pharmaceutical and environmental samples.[37]

PDMS microfluidic device /Six-Electrode Microfluidic Device

Nowadays, more precise, accurate, sensitive and hyphenated technique such as inductively coupled plasma (ICP) hyphenated with atomic absorption spectroscopy (AAS) is widely used. But it's used limited due to high cost of analysis, as it is highly expensive, thus not convenient for daily use of this method. The chemical reagent polydimethylsiloxane (PDMS) microfluidic device used which consist of 6 gold working electrodes having 15nm layer of chromium followed by 200nm layer of gold on a clean glass substrate of a thickness of about 1 mm glass slide. This Glass slide was with the chromium or gold plating and tested for difference in voltage change by using an electrochemical method known as Differential Pulse Voltammetry. The change in current is measured in a series of discrete potential steps. The estimation of elemental deposition is a measure of change in current on Anodic Stripping Voltammetry (ASV). The change in current is directly proportional to the deposition of positively charged ions onto the negatively charged surface electrode. As change in potential current starts, metal ions starts to oxidize at a specific potential. The metal ions start to migrate or stripped from working electrode surface and will generate specific peak at a specific potential current for a particular metalion
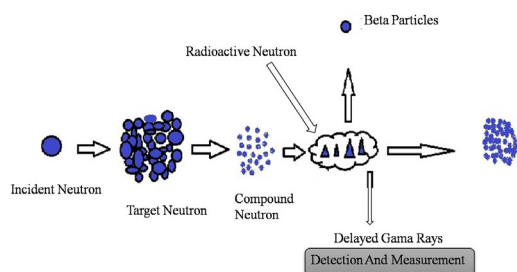
species. The bismuth lid is used to close the PDMS microfluidic device, also to improve the selectivity and sensitivity of devise and easily can detect Pb2+ and Cd2+ metal ions in a micro level of about parts per billion (µg/L) concentrations. In PDMS microfluidic device Photolithography also adopted to make a photoresist mold for fabrication of a polydimethylsiloxane (PDMS) layer and helps to separate individual channels for each electrode. To increase the bulk of PDMS microfluidic device PDMS layer also included.[38-40]

CONCLUSION

A wide variety of analytical methods are available for estimation of trace element and number of which have been explain in this review article. Analysis of pharmaceutical products for purity is the most important criteria for drug approval as well as therapeutic point of view. The above discussed analytical methods have been shown to be effective in detecting and measuring a number of elements in samples at extremely low concentration levels. None of the one method is ideal but can be used in combination to overcome limitation of one another.

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