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Review Article

ICP-OES: An Advance Tool in Biological Research

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Abstract

Spectroscopic analysis has been considered as a promising tool for the quantitative detection of elements in a biological sample. Inductively coupled plasma optical emission spectrometry (ICP-OES) is an advanced trace element analysis technique that uses the emission spectrum of an excited atom to detect and quantify the element present in the sample. The samples are introduced in the instrument in a fine liquid form and the conversion and extraction of pure inorganic components of a solid sample is known as sample preparation. The method of sample preparation is determined on the basis of chemical and structural differences between non-biotic and biotic samples. The environmental safety, bio-remediation, food quality check, diagnostic and biological research laboratories have been frequently using ICP-OES techniques because comparatively high throughput, simultaneous multi-element analysis, high precision, massive dynamic linear range, high throughput and low cost are major advantages of the technique. Many reports are available considering the use of ICP-OES in the field of environmental, petrochemical, metallurgical, geological and nano-technological studies but a collective review report is unavailable on the applications of ICP-OES specifically in biological samples.

Present review is a collection of reports available on types of biological samples, variation in sample preparation and advantages of ICP-OES over other methods. The report will develop a detailed understanding of the applications of ICP-OES in element analysis of biological samples.

Introduction

The metal contamination in soil water and air has become a serious concern to the health, farming and food safety [1]. Therefore, metal analysis in plants, animals and microorganisms is essential for eco-toxicological studies. Several diagnostic and therapeutic studies of health issues for instance decline in the immunological efficiency, cardiac disorders, fetal abnormalities, gastrointestinal cancer, redox reactions; cellular energetic and abnormal neurological activity patterns are also required to quantify the accumulation of trace metal in biological tissues [2]. The study of metals is an essential part of research in genetics, environmental toxicology, bioremediation, host-parasite relationship, nanotechnology, microbiology, structural biology, cytology, physiology, biomedicines and clinical biology. The interdisciplinary intrusion of metal study in biological science demands to build up more advanced, efficient and sophisticated analytical tools and techniques of metal analysis. A combination of biochemical and spectroscopic techniques has been used to study the mechanism of metal metabolism in biological material. In biological science laboratories ICP-MS, ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy), Flame Atomic Emission, Flame Atomic Absorption, Graphite Furnace Atomic Absorption and Atomic Absorption (cold vapor/ hydride generation) spectrometry techniques have been used for quantitative and qualitative analysis of metals. The selection of method for metal analysis depends on the range of element concentration in the sample. The ICP-MS is suitable if the range is part per trillion (ppt), furnace–AAS is used if the range is part per billion (ppb) for less than 5 elements as low dissolved solids in a sample. The flame-AAS is a good choice for analysis of fewer than 5 elements of part per million (ppm) and ICP-OES is selected for ppb as well as ppm high dissolved solid more than 5 multi-elements in a sample. Among them, ICP-OES is the most sophisticated multi-element analysis technique. The technique is widely used in official testing of drinking water, trace elements attached with proteins, food, field soil, soil-sediments, fossil fuel, bio-fuel and medicines in national and international testing laboratories [3]. A vast literature is available on the role of ICP-OES in soil, sediments, oil and other non-biological sample analysis but the application of the instrument in biological research warrant more critical analysis and compilation of research reports. The present review is an up-to-date collection of reports showing metal analysis in biological samples by ICP-OES.

Working principal of ICP-OES

ICP-OES is a method of optical emission spectrometry that

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uses the emission spectra of sample molecules to identify and quantify the elements present. The instrument has two parts one is inductively couple plasma and another one is an optical emission spectrometer. The major components of the ICP-OES instrument are sampler, pump, nebulizer, spray chamber, ICP torch, monochromator /polychromator, and detector arranged in a definite order.

The argon gas is supplied to the torch tube and simultaneously an electromagnetic field is generated by high frequency electric current in work coil positioned on the pointed end of the torch tube. The strong magnetic field induces the ionization of argon in torch tube. The ionized gas produced plasma with high electron density, temperature (10,000K) and energy. The newly produced bulk of the energy is used for excitation and emission of molecules in a test sample. The plasma provides a higher degree of sensitivity and stability by restrained formation of stable complex compounds that leads to simultaneous excitation of many elements.

The pretreated or digested liquid sample is introduced with a peristaltic pump into the nebulizer and then into the torch. The nebulizer turns the sample into tiny droplets like a fine mist. The larger droplets of the sample are going down in the drain but fine droplets are directed into the high plasma. The sample is introduced into the plasma in an atomized state through the narrow tube in the center of the torch tube. The sample goes to the plasma and hot plasma atomizes the molecules and excites them to a higher state. When the excited atoms return to low energy position, emission rays (spectrum rays) are released and the emission rays that correspond to the photon wavelength or emission lines are specific for each metal. The light is transferred to high resolution no purge seal optical system or spectrometer (OES). A radiofrequency generator (RF) produced an oscillation current in an induction coil located in the plasma tube. The tube develops an oscillating magnetic field in the sample ions during exposure to plasma gas. The prisms separate the light into the specific wavelength for the elements to be measured. This wavelength tracks the detector ray and the light intensity for each different wavelength is quantified by sophisticated analytical software that can convert them into concentration units [4]. The software also provides calibration, accuracy and precision limits after internal calculations. Thus ICP-OES is used to rapid and simultaneous analysis of elements present in the samples.

Biological sample preparation

The analysis of water, oil or non-living samples is differed from biological sample due to presence of organic and inorganic substances. The removal of organic matter and extraction of pure metal from a crude sample is known as the process of sample preparation. A fine homogenous solution of a sample is essential to eliminate interference and chocking of the nebulizer. Prior to sample preparation, the glassware is washed with non-ionic detergents (Tween or Triton), socked in 10% HNO_3 for 24 hours and subsequent oven-dried (750° C) to circumvent ionic contamination during analysis. The procedure of sample preparation purely depends on the nature of the source material. The biological samples have a high amount of organic content, salts or complex structures that can obstruct metal analysis so, acid digestion is used for the extraction of pure metals from the cell and cellular structure [5]. Three basic steps for sample preparation for ICP-OES analysis are digestion, evaporation and extraction. Table 1 illustrates the different methods of sample preparation reported for specific biological samples. Nitric acid (HNO_3) is used for acid digestion because hydrochloric (HCl) or sulfuric acid (H_2SO_4) can develop chlorides and sulfates of metal ions [5]. Acid digestion with sample-specific thermal treatment or sonication is used for the isolation of metal elements form organic substances. Digestion of biological samples with individual acid or combination of HCl and HNO_3 or HNO_3 and HF, with an oxidizing agent (hydrogen peroxide), is suggested by various researchers [6]. HF is specific for the total dissolution of silicon-rich plant material [7]. Researcher [8] has used strong alkaline substances (pH 13.4–14.7) tetramethylammonium hydroxide (TMAH) with EDTA for digestion of fatty acids, lignin and humic element in plant samples for ICP-OES analysis. Alkaline treatment reduces the time for digestion and facilitates the long-time storage of digested samples but at the same time, some reports show that alkaline digestion of organic substances may produce a high amount of organic residue which can cause an error in ICP-OES [6]. Enzymatic pretreatment for the hydrolysis of algal samples is also suggested by [9].

Metal analysis in prepared samples by ICP-OES

Preparation of standards: The verification of detection limit, instrument performance check, selection of wavelength and view are essential steps for metal analysis. The reference or pure metal standards are prepared in acidic matrix such as 5% HNO_3, 10% HCl, 0.8% HF solutions. A calibration curve is prepared by measuring intensity for the standard metal solution. The commercially available standard metal solution is used for calibration. Verification of detection limit is essential prior to formation of standard curve. The ideal correlation coefficient should be 1.0 which shows perfect fit curve.

Semiquantitative analysis: Prior to analysis of main element, semiquantitative analysis is also perform to find out accurate quantity of test metal at highest precision level. The semiquantitative analysis includes study of all elements at a time this information is useful to detect all elements and interference present in the sample. The ICP-OES is equipped with echellogram which allows detection of intensities and all peaks suggested in the method (38).

Preparation of instrument

Prior to the sample introduction, the instrument is standardized with software and model-specific protocol. The different temperature zone of plasma and ionization process gives slightly different signals. The intensity of signals can be emphasized by changing the view position. ICP-OES user has liberty to access radial, axial and dual view. The radial view or vertical view or side on view shows view from RF coil to tip of the plasma. It has wide dynamic range from low to high concentration of elements. The view is appropriate due to high tolerance to dissolved solids, and compatible to torch

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Table 1: Source Specific Methods of Sample Preparations for Metal Analysis by ICP-OES.

Biological Samples	Methods	Reference
Plants	**Digestion and Evaporation** Take a 50 ml digestion bottle with a screw. Put 0.5 g dried plant sample with concentrated HNO_3 (8ml) to destroy organic substances and keep it for 12 hours at ±25˚C in a fume hood. After the addition of 2 ml H_2O_2 digest the mixture at 95-110 ˚C on a hot plate. After evaporation at high temperature, the remaining dry mixture is left in the digestion bottle. **Extraction:** Add 5 ml of deionized water in dry material and keep it for heating till yellow fumes are formed. Again add 5 ml deionized water and maintain it to cool at room temperature. Filter the extract with Whatman no 2-filter paper. Makeup 50 ml volume of the extract with 0.1 M HNO3. The sample is ready for metal analysis.	[10]
Plants	**Digestion and Extraction** The 0.5 g dried sample mixed with 10 ml of HNO_3-HCl-H_2O_2 (8:1:1, v/v/v) in a digestion bottle and place it in the heating element at 120°C till the solid is completely converted in a fine solution. Make up the final volume (50 ml) of a fine extract with ultra-pure water. Samples can be stored in polyethylene containers.	[11]
Antarctic macro algae	**Digestion and Extraction** Take a 0.5 g oven-dried sample (80°C) with 2 ml H_2O_2, 8 ml HNO_3 and 2 ml HF and left the mixture for 24 hours. Add 2 ml HNO_3 and 2 ml $HClO_4$ and set it for microwave digestion cycles (250W-600 W). **Extraction** Digests are allowed to evaporate on hot plate. Add 2.5 ml of HNO_3 and 47.5 ml deionized water to the residue and store the clear solution for ICP-OES.	[7]
Fucus and Sargasso	**Digestion and Evaporation** Take 0.2 g of grounded algae with pepsin (prepared in 1% NaCl at pH1.0). Place the mixture on sonicator for 30 min at 37 °C. **Extraction** Centrifuge the mixture at 3000 rpm for 15 min. Take supernatant and made up the final volume with deionized water.	[9]
Fungi	**Digestion and Evaporation** Fungi samples dried at 110 °C for 90 hours and ground it with the mill. Take a 0.5 gm sample with HNO3 and keep them in the microwave for digestion. **Extraction:** The digest is filtered with filter paper and the final volume (15 ml) is made with deionized water.	[12]
Mushroom	**Digestion** A 0.31 gm mushroom sample is mixed with phosphoric acid and digested in an ultrasonic bath. **Extraction:** Filter the sample and make a final volume of 15.0 ml with deionized water.	[12]
Algae	**Digestion** A 50mg of the dried sample (at 85°C for 4h) is mixed into a vial having a mixture of 25% TMAH (1ml) and 0.2 mol EDTA. After 10 minutes continuous shaking vials are capped and placed in a thermostat oven set at 120 0C. Add 2 ml of water to the remaining solution and set the pH 8-9 with HCl. **Extraction** Add water to a final volume up to 5 ml. Centrifuge the solution at 1500 rpm for 3 min and the supernatant is used for metal analysis by ICP-OES.	[8]
Bacteria	The metal-rich bacterial samples are centrifuged at 7500 rpm for 10 minutes and the supernatant is directly used for metal analysis.	[13]
Microbial paleontological study	**Digestion** A 0.25 g of sediment sample is digested with concentrated HNO_3 at 150° C. **Extraction** Add concentrated HNO_3 and HCl (2:1) and heat it until brown fumes of NO_2 are produced. The mixture is filtered with Whatman No 42 and the final volume of filtrate is made up with deionized water. The sample is ready for metal analysis.	[14]
Blood, Serum, Urine, Tissue	**Digestion** Take 0.5 ml of the blood sample with a freshly prepared solution of hydrochloric acid and nitric acid (1:2 v/v) and left for 12 minutes. Digest the solution at 60 0C for 30 min. Add 2ml HNO and 0.5 ml H_2O_2 and again heat the solution at 78 ˚C till complete evaporation. The final volume (110 ml) is made up with Milli-Q water.	[15]
Pork liver, Bovine liver, Bovine muscle	Take 100 mg animal tissue sample and add 25% TMAH and 2.5 ml distilled water. Place it on an ultrasonic bath for half-hour than in microwave for 30 minutes. After cooling the digest final volume (20 ml) is made-up by deionized water.	[16]

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maintenance. The axial view or horizontal plasma detect signal from central path of plasma and hide the outer background therefore has ten times high detection limit than radial view. Some major disadvantages of the view are lower tolerance to dissolved solids and complex matrix. The torch life time is reduced by selection of axial view. The ICP-OES has a common facility called dual view which is perfect combination of qualities of radial and axial view. Thus, any view pattern can be selected for aquas and organic sample analysis but radial and dual view is a right choice if the sample has high amount of dissolved solid partials.

Radiofrequency power (W), gas flows (l min^{-1}), plasma, auxiliary, nebulizer, peristaltic pump speed (ml min^{-1}), stabilization time (s), number of replicates and detection wavelengths (nm) are standardized before sample introduction [9]. The argon pressure is set at 80-120 Psi and shear flow is 80 Psi. High temperature is produced during analysis therefore controlled temperature (20˚C) is mandatory for the functioning of ICP-OES [17]. The stability of instrument should be checked through analysis of 1ppm manganese (Mn) solution. The MN check with at least 10 replicates is essential to check accuracy and pression of the instrument. The relative standard deviation (RSD) is the absolute value of the coefficient of variation. The permissible RSD% is less than 2% for precision and sensitivity of an instrument. ICP-OES parameters are calibrated with internal standards scandium is used for Copper and Zinc whereas yttrium is used for Chromium, Vanadium, Strontium. Copper, Nickel, Aluminum, Boron, Barium. Generally, Scandium, Gallium, Germanium, Yttrium, Rhodium, Indium, Terbium, Bismuth are used as internal standards for standardization of metals analysis. The calibration curve for specific metal is prepared with calibration standards [18]. The analysis of calibration curve, linear regression, relative response between concentration in µg/mL and determine slope (m), intercept (b), and correlation coefficient (r) of the calibration curve is automatically performed by software attached with ICP-OES. The value for correlation coefficient (r) should be equal or higher than 0.995. The standardization and calibration processes should repeatedly perform till getting standard values.

Introduction of prepared samples

The prepared liquid samples are taken up by sampler and introduced into the system by peristaltic pump than optical analysis of metal spectrum, the study of spike recovery and checking of spectral interference provides detailed information of metal present in the biological sample. The samples should accompany with reagent blank, a negative control, a positive control of metal to be analyzed. The concentration of metal in prepared sample is calculated by formula C_S (ppb) = C_E x V_E x D/ W where C_E = Analyte concentration in final extract(µg/L) V_E = Final sample extract volume in milliliters (ml) D = Dilution factor (Diluted volume/aliquot volume) W = Weight of the sample in grams. The recoveries of fortified controls and samples can be analyzed by calculation of percentage recovery (%REC) using formula %Rec = (CF - CB) x W x 100/ VFS x CFS Where CF= Metal concentration in a fortified sample, CB = Metal concentration in a blank tissue from which it was prepared, in ppb (ng/g) W = Weight of fortified control, in grams, VFS = Volume of fortification standard added, in mL CFS = Concentration of fortification standard, in µg/L [19].

Application of ICP-OES reported in biological research

Metal analysis by ICP-OES is a rising trend in biological research. Table 2 illustrates a collection of reports available on metal detection in plants, animal and microbial samples. A vast literature survey indicates that Aluminium, Antimony, Arsenic, Barium, Beryllium, Bismuth, Bromide, Cadmium, Chloride, Chromium, Cobalt, Copper, Gold, Lead, Manganese, Mercury, Molybdenum, Nickel, Selenium, Silver, Thallium, Tin, Vanadium and Zinc are highly studied metals in biological samples by ICP-OES [20].

Advantages and limitations Of ICP-OES

The ICP-OES and ICP-MS are the two most advanced metal analysis techniques frequently used in biological sample analysis from last decay [39]. A comparative study of ICP-MS and ICP-OES is essential to establish the requirement, condition and significance of a method to obtain desired results [40]. Table 3 illustrates a comparative analysis of ICP-OES and ICP-MS. A critical analysis suggests that ICP-OES is a suitable choice for trace element analysis in biological samples.

The ICP-OES has following advantages which make it most accurate and promising than other analytical techniques:

1) Multi-element analysis: Simultaneous, sequential analysis of multiple elements by a combination of Charge Coupling Device (CCD) chip detector and echelle cross disperser is a unique character of ICP-OES. The speed of the instrument results in information of 72 elements within the least time range. Among them Zirconium (Zr), Tantalum (Ta), rare earth metals, Phosphorus (P) and Boron (B) are difficult to analyze with other spectrometry methods can be easily studied by ICP-OES.

2) Large analytical range with High sensitivity: Low detection limits ranging from parts per million (ppm) to parts per billion (ppb) is the main advantage of the ICP-OES technique.

3) High sample throughput: The system can deal with a large amount of data due to the automation of experiments in a small period of time.

5 Essay sample preparation: The solution of solid samples is prepared by dissolved or digested in solvents such as nitric acid, hydrochloric acid, Milli-Q water or specific organic solvents.

6) Higher Tolerance for TDS (Up To 30%): This instrument is highly suitable for ecotoxicological studies. The samples with high dissolved solids (TDS) or suspended solids such as water, industrial effluents, soil, groundwater and metal-enriched water from mines are analyzed in high regulatory limits with ICP-OES.

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Table 2: Collection of reports on metal analysis using ICP-OES reported in biological research.

Biological System	Biological Organism	Metals Detected	References
Plants	*Cordia salicifolia*, *Chiococca alba* (L.) Hitchc., *Echites peltata*	Cd, Co, Cr, Cu, Fe, Na, Zn, and Pb	[21]
	Amaranthus sp.	Cd	[22]
	Tetraena qataranse	Ba, Cd, Cr, Cu, Ni and Pb	[5]
	Mentha, *Ocimum*, *Eruca sativa*, *Coriandrum sativum* and *Petroselinum crispum*	Fe, Cu, Zn, Pb, Ni and Cr	[23]
	Trigonella foenum-graecum L.	Fe, Co, Ni, Cu, Zn, Cr, Al, Mn, Pb and Cd	[24]
	Tropical Marine Sedimentsa	Al, As, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn	[5]
	Plant species in herbal concoctions	Zn, Cr, Fe, Ni, Mn, Cu, Cd, Hg, Al, Pb.	[11]
	Green Macroalga Vaucheria sessilis	Zn, K, Ca, Mg, Na, Ba, Cu	[25]
	Laminaria and Porphyra seaweeds	Macro and Micro elements	[26]
Fungi	*Pleurotus ostreatus, Pleurotus eryngii, Pleurotus djamor, Pleurotus citrinopileatus, Pleurotus florida,* and *Pleurotus pulmonarius*	Al, As, B, Ca, Cd, Cr, Cu, Er, Fe, In, K, Lu, Mg, Mn, Na, Nd, P, Pb, Pt, Re, Rh, Sc, Se, Sr, Te, Th, Ti, Tm, U, Zn, and Zr	[12]
	Agrocybe cylindracea and *Hericium erinaceus*	Li	[27]
Animal	Fishes (Rohu, Mrigal, Thaila, Grass carp, Silver carp, Dolla, Pari, Mali, Tilapia, Maraki, Khagga, Singarhi)	Cd, Cr and Pb	[28]
	Blood and Urine	Ba	[29]
	Procambarus clarkii	**Cd, Cu, Fe, Ni, Pb, Zn**	[30]
	Pork liver, bovine liver, bovine muscle	Si	[16]
	Pepperbush, chlorella, hair, mussel, tea leaves, Sargasso, rice flour, and bovine liver	P, Zn, Ni, Al, Fe, Mn, Mg, Ca, Cu, Sr, Na, K	[6]
Bacteria	*Burkholderia fungorum FM*2	Zn (II), Pb (II), Cd(II)	[31]
	C. sakazakii MM045, Enterobacter *sp*. MM087	Ni, Cd, V, Pb	[32]
	Klebsiella variicola	Ni, Co and Cr	[33]
	*Escherichia coli ATC*25922 and *Staphylococcus epidermidis RP62A*	Ni, Cu, Pb,	[34]
	Bacillus licheniformis NSPA5, Bacillus cereus NSPA8, and Bacillus subtilis NSPA13	Cd (II), Cu (II), and Pb (II)	[35]
	Enterobacter cloacae	Hg (II), Pb (II), Cd (II, Cu (II) and Cr (VI)	[36]
	Microbacterium oxydans HG3, *Ochrobactrum* sp. *HG*16, *Lysinibacillus* sp. *HG*17, *Bacillus* sp. *CM*111, *Serratia marcescens* HG19, *Kocuria rosea* EP1, *Bacillus cereus* MM8.	As, Hg	[37]
	*Rhodomium marinum NW*16, *Rhodobacter sphaeroides KMS*24	Cd^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}	[38]

Table 3: A comparative analysis of ICP-MS and ICP-OES: Two frequently used metal analysis techniques in biological research.

S.No.	ICP-MS	ICP-OES
1	ICP-MS can detect 82 elements as it is also used for isotope ratio investigations.	ICP-OES is used to detect 73 elements of periodic table except radioactive elements, and halogen groups.
2	The metal detection limit is parts per trillion (ppt)	The lower limit of element concentration in parts per billion (ppb)
3	The tolerance for total dissolved solid is about 0.2% therefore it has low regulatory limits.	The tolerance for high total dissolved solids (TDS) or suspended solids is up to 30% with high regulatory limits.
4	A limited range of samples can be analyzed by ICP-MS. Useful for analysis of drinking water and less complex components of laboratory samples.	Multi-element analysis in a wide range of samples from soil, polluted water, soil waste, drinking water to complex biological samples can be done with ICP-OES
5	It has dynamic linear range 10^8 so the sample dilution is essential before the analysis with ICP-MS.	Sample dilution is not required because it has 10^6 dynamic linear ranges for the detection of the multi-element present with a wide concentration difference.
6	An ICP–MS system is costly due to the high consumption of argon.	ICP-OES is cheaper due to the lesser requirement of argon for study.
7	The installing, operating and maintaining cost of ICP-MS system is higher than ICP-OES.	ICP-OES is two to three times cheaper than ICP-MS system
8	Efficiently remove polyatomic spectral interferences using collision cell technology	Spectral interference is removed by using Fast Automated Curve-fitting Technique
9	Rapid semi-quantitative analysis is done by a separate method for high precision.	Rapid semi-quantitative analysis is done by using the echellogram which allows users to view the relative intensity of all peaks.
10	Sample enter in the instrument for ionization and passing of ions through plasma. Therefore, there are chances for sample deposition.	Only photons are measured from the sample after passing through the plasma. Therefore, the system is more stable than ICP-MS.
11	Each time new internal standards and new calibration curve required for accurate results.	The ICP-OES is a stable system therefore, various types of semi quantities analysis may be done using previously stored calibration curves.
12	A combination of HPLC/ICP-MS is used for speciation of elements.	Advanced version of ICP-OES is suitable for speciation analysis of sample element.

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7) Low sample volume and High Detection limit: ICP-OES is equipped with a detector that can detect the lowest amount of analyte in a sample with acceptable precision under the stated operating conditions of the methods. A small volume of samples can provide a large amount of data and information [41].

High detection limit, inter-element interference, cost of equipment, and lab setups with expert technical staff are some limitations but ICP-OES is the most promising technology for metal detection in biological researches.

Conclusion

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES Analysis) is a trace-level, elemental analysis technique that uses the emission spectra of a sample to identify and quantify the elements present. The major limitation is inter-element interference which is overcome by high resolutions chip and mathematical manipulation in data. Competitively low cost, simple operation, high stability, easy method development, capacity to evaluate refractory element, high throughput, high sensitivity and dealing with large samples per day and per run make it an excellent selection for biological laboratories.

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