INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD)
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A REVIEW ON SEPARATION AND QUANTIFICATION OF METAL IONS FROM LEAVES BY USING COLUMN CHROMATOGRAPHY-FLAME PHOTOMETRY

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Abtract: This article contains a review of the extraction and quantification of metal ions from leaf samples using flame photometry and column chromatography. Leafy vegetables provide essential minerals (e.g., sodium, potassium, calcium and magnesium) which are critical to the nutritional and physiological needs of humans. Column chromatography allows for efficient separation of the complex constituents of plants, based on how they are differentially adsorbed to or partitioned between a stationary phase and a mobile phase. Flame photometry allows for quick and selective analysis of alkali and alkaline earth metals via atomic emission spectrometry. This combination of techniques is relatively simple and inexpensive to perform, making it amenable to routine analyses of various types of plant materials with reasonable levels of sensitivity and specificity, although there are limitations such as: interference from other elements, limited ranges of detection, and the need for accurate calibration. Nonetheless, it provides a valuable tool for evaluating mineral composition, detecting heavy metals, and assuring the quality and safety of food by measuring this type of content in leafy vegetable samples.

KEYWORDS: Flame photometry, Column chromatography, Metal ions, Leafy vegetables, Mineral analysis, Sodium, Potassium, Calcium, Magnesium.

1.INTRODUCTION:

Food is regularly examined for a number of components to determine potential nutritional or toxicological effects, as well as to guarantee product quality or compliance with legal requirements. In a world where food shortages are a problem, leafy greens are generally accepted as an excellent source of nutrients and a complement to the diet. It is well known that leaves and leafy vegetables are a great source of vitamins and minerals [1-3].

Minerals play a crucial role in assessing the nutritional value of leaves. The primary ones are potassium, sodium, calcium, and magnesium. The significance of minerals like potassium, calcium, sodium, and others to human health is widely recognized. Essential quantities of these elements are necessary in the human diet to maintain a good, healthy life. Trace elements are crucial in human biology, as they are either poorly synthesized or not produced at all by the body. Small quantities of certain metals, such as manganese, copper, and zinc, are vital micronutrients and perform various biochemical roles in all forms of life. Although these components are crucial, they can become harmful when consumed in large amounts. Moreover, certain metals, such as lead, are not found naturally in the body, and their existence, typically due to workplace exposure or pollution, is detrimental to health, with children being more vulnerable to these metals than adults. Sodium (Na) is the primary cation outside cells, and it contributes to the distribution of body fluids. The level of sodium ions in the plasma (extracellular) ranges from 130 to 145 mmol/l. Elevated and diminished concentrations are known as hypernatremia and hyponatremia, respectively. Potassium is significant for mental function in addition to its role in physical processes. It aids in enhancing efficient cognitive performance by significantly contributing to oxygen delivery to the brain. Phytocomponents sourced from plants provide an excellent source of medicinal compounds, and due to their inherent complexity, it becomes essential to isolate and purify these individual substances for further examination and evaluation of their structural and functional characteristics. [3-6]

Flame atomic emission spectrometry (FAE) uses flame photometry to measure species as atoms. It works on the principle that alkali metals will become excited and give off light when sufficient energy has been absorbed by their alkali metal salts in a non-luminous flame. Hence, by measuring the intensity of the colours, you can determine how much energy has been used to evaporate the alkali metal salts into the gas form, as well as producing characteristic wavelengths of light.[30-31]

2.COLUMN CHROMATOGRAPHY: 7-8

In chromatography, one type of chromatography is called column chromatography and is used to separate one chemical from out of a mixture. Because each chemical will absorb to an adsorbent differently, chromatography can be used as a way to separate chemicals from each other. Each chemical will pass through the column at a different rate, and thus will be collected in different fracciones, thereby forming fractions using chromatography. Because a variety of adsorbing activities exist (the three main types are normal phase, reversed phase, and others), and because they can be used with many different types of solvents, chromatography can be run under a wide variety of conditions.

2.1 PRINCIPLE OF CHROMATOGRAPHY: 11-13

Different components in a mixture will have different affinities for and different retention factors, or partition coefficients, for the stationary phase (what is holding the material in place) and the mobile phase (what is moving and separating the mixture). This is the basic concept of column chromatography. In column chromatography, the mixture you want to separate is mixed with some type of solvent (liquid) and allowed to run through the column packed with the adsorbent (stationary phase). The type of mixture being separated and the type of adsorbent (stationary phase) used in the column will determine how quickly a component will adsorb onto the column (i.e., how quickly it will be separated). In column chromatography, the top part of the column is occupied by the compound that adsorbs with the greatest affinity; therefore it has the least adsorbable material in the bottom part of the column. The basis of column chromatography is selective adsorption. When the components of the adsorptive sample move through or migrate along the mobile phase, the create a definite band within the column and elute. The area within the column that contains one specific substance is referred to as a zone. The chromatographic separation process produces chromatograms and the different zones forming will be referred to chromatograms.

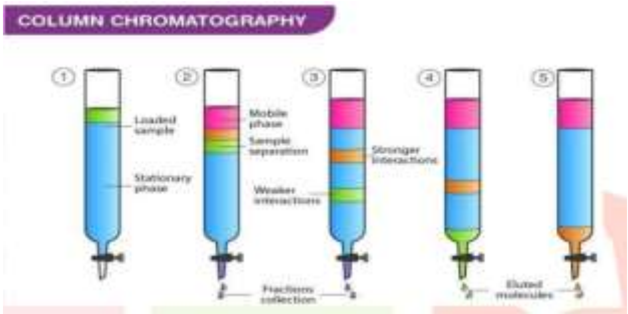

Figure 1: Basic working principle of column chromatography. [7]

2.2 HOW IT WORKS: 14-27

- **Stationary phase:** The adsorbent is the solid substance that serves as a stationary phase, and in column chromatography, the most commonly used stationary material is silica gel and the second most is alumina. Cellulose powder was used in previous experiments.
- **Mobile phase:** The mobile phase, or eluent, is a solvent or combination of solvents that are used to carry compounds through the column.
- **Separation:** The mobile phase enters and is allowed to travel through the column where the phytochemicals separate from each other and fall off.
- **Collection:** The components are divided into separate groups; they are collected in a small container.
- **Analysis:** The collected components underwent analysis through thin-layer chromatography.

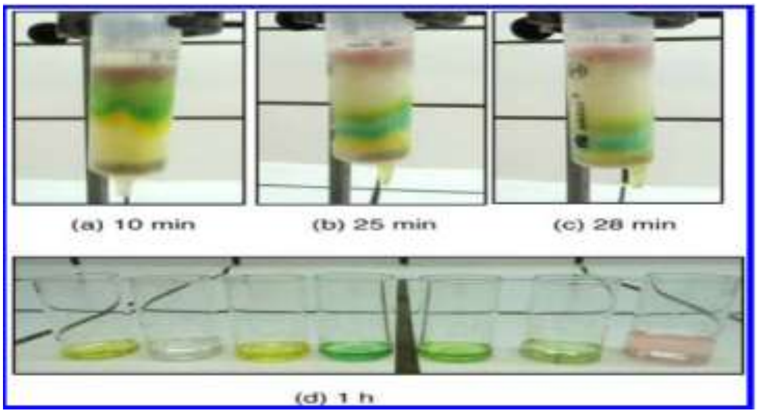

Figure 2: (a) Separation of β-carotene and xanthophylls on a potato starch column by elution with petroleum ether; (b and c) Separation of xanthophylls and chlorophyll a and b on the same column by elution with a mixture of petroleum ether/acetone 90:10; (d) all the fractions collected from leaves.[6]

Table:1. Types of chromatography:[15-25]

Type of Chromatography	Principle of Separation	Common Stationary Phase	Typical Application
Adsorption	Differential adsorption to a solid surface	Silica gel, alumina	Separation of non-polar compounds, natural products
Partition	Differential partitioning between two liquid phases	Liquid coated on solid support	Separation of complex mixtures like amino acids
Gel Filtration (Size Exclusion)	Molecular size (larger molecules elute first)	Porous beads (agarose, dextran)	Purification of proteins and other macromolecules
Ion Exchange	Charge differences (electrostatic interaction)	Ion-exchange resins (charged beads)	Purification of proteins, separation of metal ions
Affinity	Specific binding interaction with a ligand	Ligand immobilized on a matrix	Single-step isolation of specific biomolecules

2.3 LIST OF LEAVES PERFORMING IN COLUMN CHROMATOGRAPHY: [40-46]

- **Spinach Leaves:** It separates photosynthetic pigments (chlorophylls and carotenoids) based on polarity of the solvent. Using silica gel or alumina as the stationary phase and organic solvent as the mobile phase.
- **Ficus Benjamina (Weeping Fig) and Epipremnum Aureum (Devil's Ivy):** Both have listed chromatographic experiments but yield differing separation results depending on the solvent systems.
- **The Tradescantia pallida (purple heart):** yields purple pigment: Separation of pigments using chromatography might reveal otherwise green-brown pigments beneath the purple pigment.
- **Coloured autumn leaves:** They have the greatest amounts of carotene and xanthophyll pigments found in leaves, and, once chlorophyll has broken down, both carotene and xanthophyll become very visible to the naked eye.
- **Green leaf Ginkgo biloba:** It has a yellow band (from the xanthophyll pigment) chromatographically separated from the green chlorophyll pigment.
- **Lantana Camara:** It is a plant commonly used in studies to extract and isolate specific bioactive secondary compounds, not just as a source of the major photosynthetic pigments.
- **Mangifera Indica (Mango) and Piper Betle:** These leaves are studied for the purpose of separating and identifying secondary compounds within them through column chromatography.

2.4 BENEFITS OF COLUMN CHROMATOGRAPHY: [24-28]

- Column chromatography allows for the separation of various complex mixtures.
- Column chromatography can separate any quantity of mixture.
- There is a wide variety of mobile phases.
- In preparative chromatography, analytes can be isolated and reused.
- It may be feasible to implement automation.
- This is a solid method of operation.

2.5 DRAWBACKS OF COLUMN CHROMATOGRAPHY: [24-28]

- Separating the compounds requires additional time.
- Column chromatography has limited separation capabilities compared to advancements.
- Greater amounts of solvents are necessary, which incurs higher costs.

- Automation increases complexity and expenses.

3. FLAME PHOTOMETRY: [29-31]

When you add a metal into a flame, it will produce radiation. This is how flame photometry or flame emission spectroscopy works. The amount of energy given off is used to find out how much of the metal went into the flame, and the light produced will be different for each metal.

Metal atoms added to the flame during flame photometry become excited. After they have released their radiation, the excited atoms become less stable and will return back to their ground state over time. Flame photometry measures the radiation that is emitted from an excited atom once it returns to its ground state. So, the total energy emitted when excited atoms return to their ground state is the radiation lost in the flame. Also, the intensity of the emissions produced is affected by the temperature of the flame, therefore atomic absorption spectrometry is different from flame photometry.

3.2 PRINCIPLE: [31-33]

Spectrophotometric analysis is used to determine the presence of metallic elements by examining their spectral emission when a metal salt is introduced into a flame using flame photometry (a type of atomic spectrophotometry). The spectrophotometer detects these wavelengths (or the intensity of the color produced) in order to determine both what metal is present in an unknown sample and how much of that metal is contained in the sample. When the metal salt is burned, it generates a specific wavelength of light. Each metal has a unique color based on how they emit different wavelengths of light from the flame. Therefore, the color that is produced tells us which metal is in the solution and the intensity of that color provides an approximate measurement of the concentration of that particular element in the unknown sample. When a sample of liquid containing a salt solution is placed into a flame, the solvent evaporates prior to the solid salt particles vaporizing into gas. The flame heats the gas and can excite the neutral, free atoms or radicals resulting from the dissociation of the gas molecules. These excited (but unstable) atoms will produce light (photons) when they change to a lower energy state. Therefore, the basic concept behind flame photometry is to measure the amount of light emitted by the gas as it cools back to room temperature.

3.3 INSTRUMENTATION OF FLAME PHOTOMETRY: [32-39]

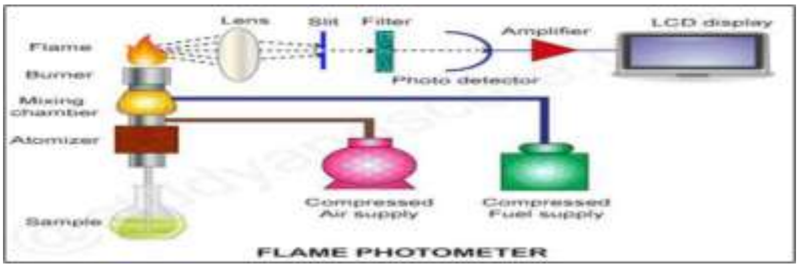

Figure 3: Instrumentation of flame photometry[32]

- **Sample delivery method:** The nebulizer uses a compressed gas to aerosolize the sample into thousands of small droplets through a process called nebulization. The atomization area has a flow path for the sample to enter.
- **Burner:** The flame must be hot enough to completely evaporate the liquid droplets of the sample solution and leave solid residues on the burner**.**

Types of Burners:

- **Meeker burner:** This is operated with natural gas and oxygen, generating low temperatures and excitation energy. Although it is now obsolete, it was particularly effective for alkali metals.
- **Total consumption burner:** Sample solutions are aspirated through a capillary under high pressure of fuel and oxidant. The liquid is drawn into the flame, where oxygen aspirates the solution, resulting in solid residue. This process leads to atomization and excitation, typically using hydrogen and oxygen as fuel and oxidant.
- **Premix burner:** In this burner, the sample and fuel oxidant are thoroughly mixed before reaching the flame, resulting in a uniform flame. However, a significant disadvantage is the heavy loss of mixture, which can be up to 90% during the process.
- **Lundengarph's burner:** In this burner, a sample is mixed in a chamber and sent to the fuel nozzle for atomization, with only about 5% of the total content reaching the flame.
- **Shielded burner:** When making measurements using a protected flame (a "shielded burner") that uses inert gas in order to provide a barrier against the surrounding atmosphere, increased sensitivity is necessary to achieve a more accurate measurement result from use of the burner.
- **Nitrous oxide-acetylene burner:** A combination of nitrous oxide and acetylene makes for a very effective means of producing free atoms of metals having reflective oxide coatings (e.g., titanium or aluminum) for use as references when determining the presence of other metals that may be present. However, due to the ease of ionization of alkali metals and their relatively high temperatures, they are not very useful in this application. Additionally, strong background emissions make it difficult to quantify the amount of metals emitted from the source under consideration due largely to flame-induced atomization processes and the difficulties that exist when measuring metal emissions generated in this manner

- **Mirror**: All directions in space receive the flame's release of radiation. A mirror is put in by the burner to reflect the radiation back to the monochromator's entrance slit in order to increase the total amount of radiation used in the analysis. Using this concave mirror allows for optimal use, in terms of maximizing the reflection of light away from the flame at all angles; thus, allowing for maximum utilization of the flame's dispersed radiation while also allowing for some radiation reflected back to the monochromator's entrance slit.

- **Slit:** To provide the best quality of radiative energy to pass through the optical system, the best tools are used for the entrance and exit slits just inside and outside of the dispersion elements. Because the slit blocks out all but a minimal amount of radiation from the flame and its reflected counterpart, only the flame's firing and the firing's returning reflections can be transmitted into the optical system; all other sources of radiative energy will be eliminated from reaching the system.
- **Monochromator:** The need for a filter monochromator within this apparatus arises because flame photometry detects the wavelengths of light emitted by substances, and the amount of light emitted is proportional to the intensity of the light.
- **Filter:** The material used to produce a filter has been chosen because it allows transmission at only a certain range of wavelengths. When a filter has been placed in position between the flame and the photodetector, all radiation emitted by the flame will enter into the photodetector with the exception of that which is filtered out by the filter. All unwanted wavelengths will be absorbed by the filter and therefore will not be counted.
- **Detector:** The detector monitors how much radiation hits the surface of the detector after it has been permitted to hit the detector from your optical system (including reflected light). The detector should have the ability to measure or detect all of the wavelengths of radiation that reach the detector and therefore should be a photomultiplier detector which produces an electrical signal when radiation hits it (for example, high-quality flame photometer.

3.4 A SYNOPSIS OF THE METHODS USED IN FLAME PHOTOMETER ANALYSIS IS PROVIDED BELOW: [33-37]

- **Desolvation:** The process of removing the moisture from a solution sample is referred to as "desolvation." The evaporation of the solvent occurs because the flame dries the metal particles within it.
- **Vaporization:** The metal particles from the sample undergo a process of dehydration by drying out in the flame. The net result of this process is that the solvent evaporates.
- **Atomization:** The term "atomization" refers to the breaking down of every single atom in the chemical compound into its component metal atoms as a result of application of heat by a flame. Because of the flame, the metal ions that exist in the sample are converted into their corresponding metal atoms.
- **Excitation:** It refers to the ability of an atom's electrons to absorb some amount of energy (due to the electrostatic nexus between the nucleus and the electrons) and, when they have been excited, transition to some higher energy state.
- **Emission:** It occurs when an atom has returned to its ground state or lower energy state to achieve stability, at which point the intermediate raised energy state is no longer considered stable. The energy radiated through atomic motions is emitted as radiation of some particular wavelength, which can be quantified by the photodetector.

Table 2: Specifics of the alkali and alkaline earth metals' detectable atomic flame emissions, including the wavelength of the emission and the colors that are created. [47]

SN	Element	Emission wavelength (nm)	Flame colour
1	Potassium(K)	766	Violet
2	Lithium (Li)	670	Red (Carmine)
3	Calcium (Ca)	622	Orange
4	Sodium (Na)	589	Yellow
5	Barium (Ba)	515	Lime Green

3.5 Benefits: [36-39]

- Basic quantitative analytical examination utilizing flame analysis.
- Affordable.
- The identification of alkali and alkaline earth metals has been made easier thanks to a reliable, efficient and accurate procedure.
- This procedure can identify reactive components using ppm to ppb standards and is an easy, quick and precise way to determine reactiveness.

3.6 Drawbacks: [36-39]

- The level of the metal ion in the solution cannot be determined precisely.
- A standard solution with established molarities is needed to identify the ion concentration that matches the emission spectra.
- Achieving precise results for ions at elevated concentrations is challenging.
- The molecular structure of the compound in the sample is undeterminable.
- Elements like carbon, hydrogen, and halides remain undetectable because they do not radiate.
- Therefore, flame photometry continues to be applicable for regular, economical measurement of primary mineral ions (notably Na, K, and other readily excited alkali/alkaline-earth metals) in leaf/plant tissue, despite specific limitations (interferences, detection thresholds, and the necessity for precise calibration).

3.7 APPLICATION: [32-33]

- **Qualitative application:**

Identify alkali and alkaline earth metals in samples according to the method of aqueous solution preparation. For example, sodium produces a yellow flame when burned. And it is difficult to identify nonradioactive elements such as carbon, hydrogen, and halides.

- **Quantitative Analysis:**

➢ An evaluation of soil suitability for growing crops considers the concentration of numerous alkali and alkaline earth metals. A measurement of sodium and potassium ion concentrations in body fluids are also used as an indicator of sodium and potassium ion activity in muscle, including the heart. Such analysis is conducted on many different types of water to determine their fitness for use as industrial and agricultural water, as well as for drinking and laundry water.

- **Other uses:**

➢ With the use of a flame photometer, lead has been found in gasoline. The same device is employed to examine both alcoholic beverages, fruit juices, as well as soft drinks. When you compare the level of intensity emitted for each solution using your regular standard against the emitted intensities you will create a calibration curve. Each ion of the desired chemicals will be plotted based on their concentration vs. the intensity emitted from a spectroscopic emission test.

3.8 LIMITATIONS: [33]

- Flame photometry is suitable for measuring metal concentrations; however, because of the relatively low energy produced by flames and the lack of energy needed to excite metallic atoms resulting in comparatively little emitted light from those metals (particularly for metals that require much energy to get excited), most samples would not yield satisfactory quantification results at flame photometer sensitivity levels.

- While flame photometry is capable of providing an overall measure of the amount of a metal in a given sample, the method does not provide data on the molecular form (i.e., how the metal exists) of that metal in the original sample.

- Flame photometry has never been used to directly identify or quantify rare gas, halide, or noble metal atoms.

- All of these elements need a greater energy input than is provided by a flame before they can become excited (i.e., "fluorescent").

- Flame photometry is limited because it can only report the total concentration of metals within a given sample but does not provide information on the molecular structure of the metal(s).

- Inert gases cannot be directly detected/determined by exercise; thus, they cannot be used to evaluate or compare analytical results.

- Only liquid samples will suffice for analysis; hence liquid heads and samples take a long time to prepare for analysis.

- There are limitations to flame photometry in terms of the number of metal atoms that can be measured using flame photometry; therefore, the number of metallic elements that may be measured using this method is also limited.

Conclusion:

Column chromatography and flame photometry can successfully produce results of the separation and quantification of sodium, potassium, calcium, and magnesium as metal ions in leaf tissue samples. Column chromatography is a reliable technique for separating components (a mixture) of plant tissue based on how they will displace or compete with each other in the two phases of chromatography – stationary and mobile phases. Flame photometry is a fast and specific measurement technique for the determination of a specific amount of a specific metal ion from the alkaline and alkaline earth metals (sodium, potassium, calcium, magnesium), which have become important for human nutrition. Together the combined use of column chromatography with flame photometry is a straightforward, low cost, easy-to-carry out procedure to be utilized for leafy vegetables that provide an appropriate sensitivity and specificity for measuring the mineral content of these types of vegetables. There are several limitations associated with using this combined technique, such as the interference of other competing elements; the limited number of elements that can be measured; and the need for proper calibration of the analytical method; however, it can be inferred that the uses of this combined method are apparent in measuring dairy products for minerals and detecting the presence of other metals in dairy products. These two methods collectively represent an effective tool for assessing nutritional status, ensuring food safety, and providing environmental monitoring for the purpose of ensuring the safety of the public.

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