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Ion Chromatography: Principles and Instrumentation

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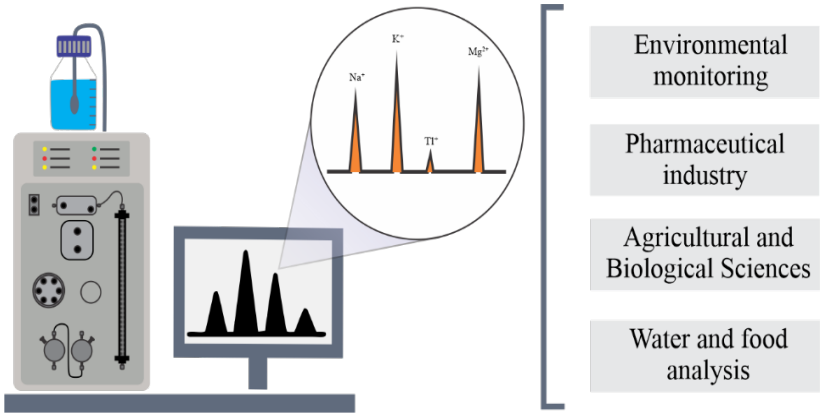
Ion chromatography (IC) is one of the most used analytical methods for the determination of inorganic cations and anions, as well as organic ions. It is considered a method of separation with simple steps, low cost and with good efficiency in chromatographic separation. In addition, it has good validation metrics such as sensitivity, selectivity, precision, robustness, low detection limit and quantification. IC easily replaces conventional wet chemistry methods, which tend to have more analysis steps. The easy extraction of samples, in most cases, does not require organic solvents, facilitating the disposal of waste. Since its creation, IC has accumulated advantages and good acceptance in the food, pharmaceutical, environmental monitoring industries, in addition to increasing implementation in academic research laboratories.

Graphical abstract



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1. Introduction

Ion chromatography (IC) is one of the most used techniques for the determination of organic and inorganic ions. Since the development of this chromatographic technique by Small [1], it has obtained analytical applications in environmental monitoring [2, 3], food analysis [4, 5], criminal forensics [6, 7], analysis in water quality control procedures [8, 9] and pharmaceutical industry [10].

IC has good selectivity, robustness and sensitivity that allow the separation of ions in the sample and simultaneous quantification of inorganic anions and cations, as well as complexes that present charge and organic substances that can exist in ionic form such as the pesticide glyphosate and its metabolite aminomethylphosphonic acid (AMPA) [11].

The technique is based on electrostatic interactions between charged fragments on the surface of molecules and oppositely charged functional groups bound to a stationary phase. It is a surface process that occurs between the contact of an ionic solid and a solution [12,13]. This process can replace conventional chemical methods of analysis, such as: gravimetry, volumetry or completion. These methods, which, in large numbers of samples, are time-consuming and difficult to automate, decreasing sensitivity and, occasionally,

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susceptible to interference and sample contamination by manipulation by the analyst [14,15]. Due to the advantages and rapid improvement of the technique, it can be expected that its applicability will extend into new areas in the near future, especially hyphenated to other techniques to improve performance.

2. Technique history

The name chromatography is derived from the Greek "*Chroma + graphein*", which means writing of color. At the beginning of the 20th century, Michael S. Tswett developed a technique to extract a variety of plant compounds in glass columns filled with calcium carbonate and passed through the solvent petroleum ether, thus verifying the separation of the pigments [16].

The first observations recorded in the literature that refer to ion exchange were made by Way and Thompson in 1850. The researchers found that the soil had the ability to remove ammonia ions (NH_4^+) when leached by ion solutions (Ca^{2+}), replacing them by equivalent amounts [17].

It was not until the end of World War I that ion exchange was used for analytical purposes, when Folin and Bell obtained the separation and quantification of ammonia in urine using an artificial ion exchanger, synthetic aluminum silicate. In 1935, Adams and Holmes made synthetic ion exchange resins. The basis of their synthesis was the condensation polymerization of polysubstituted benzene compounds or formaldehyde. In 1940, exchange resins based on the copolymerization of styrene and divinylbenzene were developed for use as a water treatment medium. However, the first report of this method occurred only in 1944 by Russell, Svartout, Hume and Kettle, first published in the open literature in 1947. In 1975, Small, Stevens and Bauman performed the separation and detected the samples using a conductivity meter and incorporating a suppressor column between the separator and a conductivity detector to increase sensitivity by reducing background [18].

In the 1980s, Gjerde and colleagues used the ion chromatography system without the suppression device with very low conductivity eluents. Both suppressed and unsuppressed ion chromatography modes can be applied to analyze diverse samples. However, the application of suppressed ion chromatography is more commonly used. Since then, many innovations have improved the performance of the technique [19].

3. Ion Chromatography Instrumentation

The diversity of CI application has expanded the different types of inflamed tenches that can be used as instruments. From small, portable systems to industrial analytical systems have been adapted. However, the ion chromatography system is simple. It basically comprises an eluent for sample drag, pump systems that operate alternately maintaining constant pressure, an injector, a separation column, a chemical suppressor and a detector for generating the chromatograms (Fig. 1).

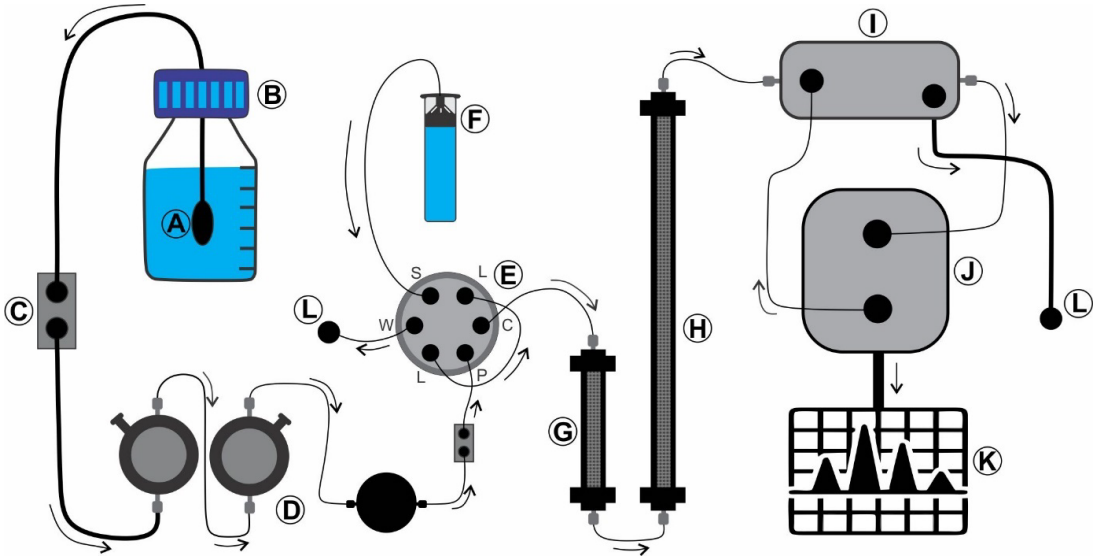

Fig. 1. Schematic of an ion chromatography system. A-Filter; B-Bottle with eluent; C-Degasser; D-Pump system; E-Injection System; F-Vial Sample; G-Guard column; H-Separation Column; I-Chemical suppressor; J-Detector; K-Chromatogram; L- Waste.

3.1 Mobile phase

The eluent is the mobile phase that has a fixed or variable concentration during the chromatographic run using an eluent generator cartridge, which contains the appropriate concentrated electrolyte solution for the eluent being generated. The sample is initially dissolved in this eluent, which is then led to the stationary phase. This allows for the separation of different components or elements that are contained in a sample solution [20].

Depending on the analysis of anions or cations, different eluent solutions are used. The most common eluent for anion analysis is a dilute buffer solution containing sodium bicarbonate and sodium carbonate. Alternatively, sodium or potassium hydroxide may be used as the eluent. In cation analysis, the eluent is normally a solution of dilute acid such as sulfuric, acetic, citric, sulfonic and carboxylic acids [21].

3.2 Degasser

The quality of the eluent significantly affects chromatographer performance and wear is a way to ensure high quality of the eluent. The degasifier is a high pressure gas removal device that removes electrolysis gases created

during the generation of eluents, preventing the formation of bubbles caused by the gases outlet in the eluent ratio valves, pumps "heads" and detector cell, which may interfere with the chromatographic process [22].

3.3 Pump systems

In general, alternative double piston pumps are used to reduce noise in the chromatogram base line, as it provides the constant and pulses free flowing phase needed for sensitive detectors such as conductivity, UV/VIS and amperometry. Therefore, an electronic circuit in combination with pulse shock absorbers is used to reduce the maximum residual pulse [23].

Some pumps operate only in isocratic mode or with isocratic capacities and gradients. While isocratic is the standard choice for routine cationic analysis, gradient elution allows the separation and analysis of a considerably larger range of ions [24].

3.4 Injector

The sample is injected into the system through a valve injector. The injection valve typically contains six ports in a two-position change format. One position is used to load the sample in the injection circuit (Fig. 2A) and, after that, there is a change of the injection valve, this being the second position, where the sample is transported by the mobile phase to the guard column and, in then to the separation column (Fig. 2B).

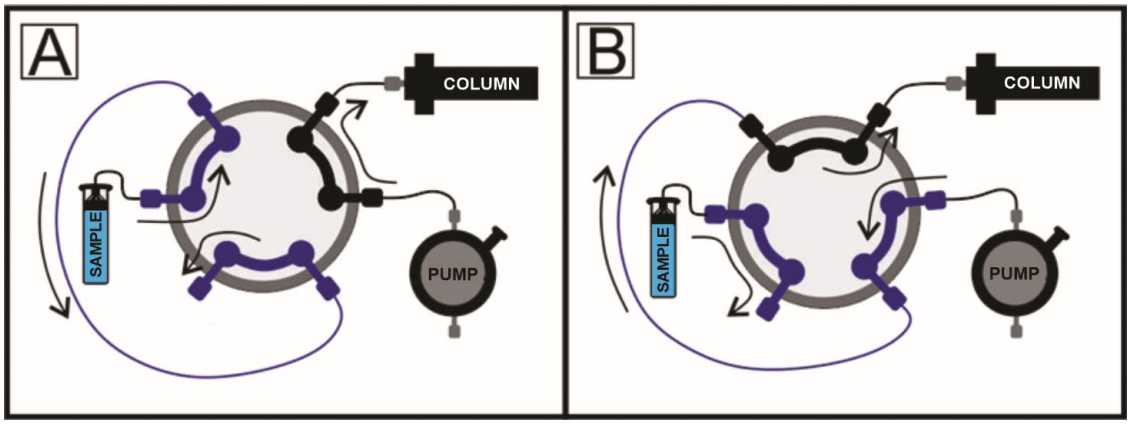

Fig. 2. Sample injection system. Loop loading with sample (A) and drag the sample through the eluent into the separation column (B).

3.5 Column or Stationary Phase

Separation columns or chromatographic columns, also known as stationary phase, are supports packed in cylindrical tubes of different diameters and lengths, which have inlet and outlet at their ends. The internal contents of these columns are generally porous and solid substrate particles with positively charged or negatively charged ionic functional groups on their surface [25]. An overview of stationary phases used for ion chromatography was described by Weiss and Jensen [26] illustrated in figure 3.

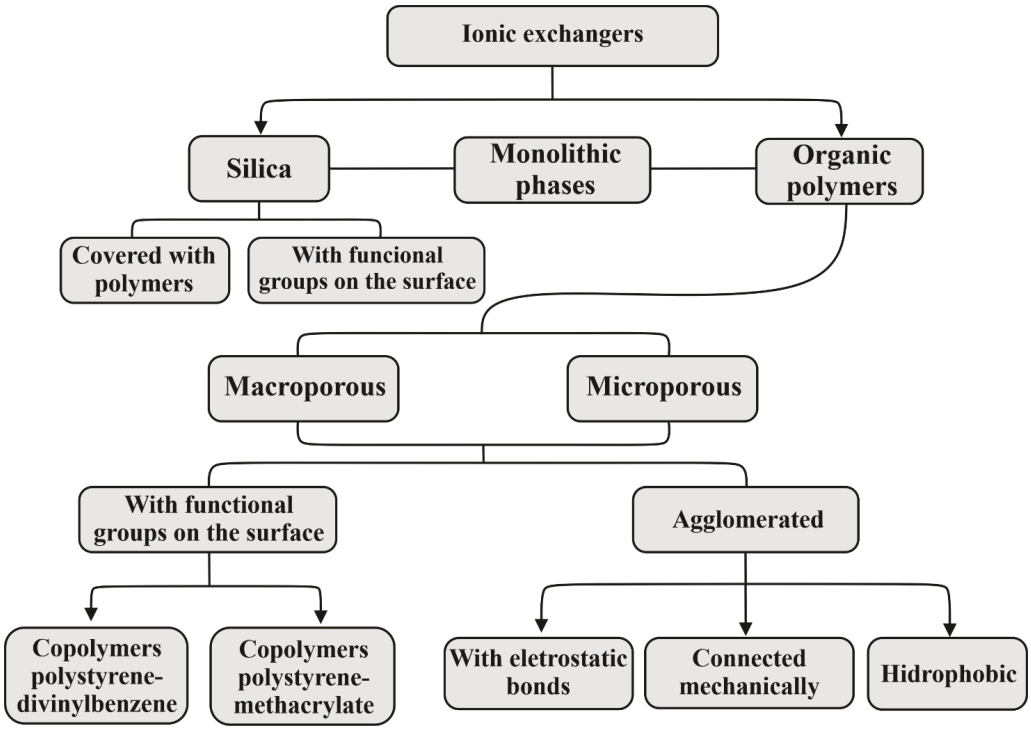

Fig. 3. Stationary phases used in Ion Chromatography.

The ion exchange resin must be stable and insoluble in common solvents, and can be organic or inorganic [18, 20, 27]. Organic resins are the most used ion exchangers. Those with a silica base have greater efficiency in chromatographic separations. The most common are polystyrene, which easily forms an addition polymer with divinylbenzene [17, 28].

There are also resins with inorganic characteristics, whose ion exchange properties are favorable. This resin offers greater thermal stability. The most used inorganic ion exchange are hydrated oxides, acid salts of polyvalent metals, salts of heteropoly acids, insoluble ferrocyanides and aluminosilicates [17].

3.6 Ionic exchangers

Functional groups have three different types of ion exchangers. In cation exchange chromatography, negatively charged ligands bond to positively charged molecules (Fig. 4A), whereas the opposite occurs for anion exchange chromatography (Fig. 4B), whereas Zwitterionic ligands have both charges in a single column (Fig. 4C).

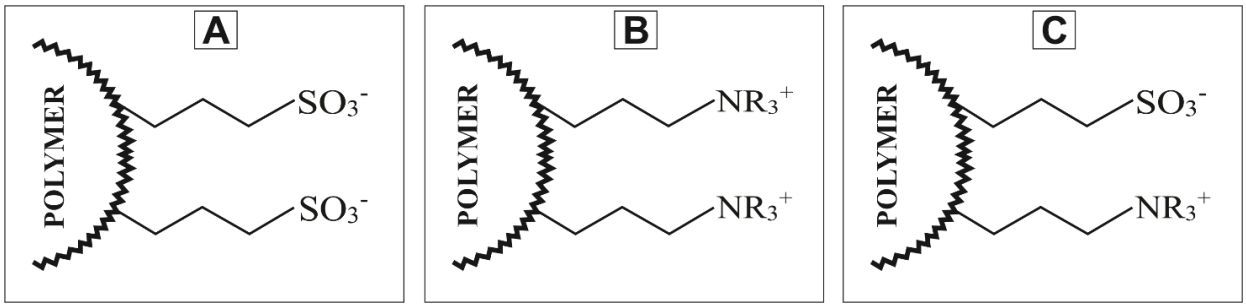

Fig. 4. Types of ion exchangers. Cationic exchanger with a negatively charged functional group (A), anion exchanger with a positively charged functional group (B) and a Zwitterionic exchanger with both functional groups (C).

3.6.1 Anion Exchanger

Anion exchangers are resins that are capable of capturing anions. There are strong base resins that are derived from the reaction of tertiary amines with styrene-DVB and weak base resins that are functionalized with primary and secondary amine groups. They can be applied in the adsorption of strong acids, but the kinetics is prolonged. Amine resins have good yield and are regenerated by NaOH, NH_4OH or $NaCO_3$ [29, 30].

3.6.2 Cationic Exchanger

Cationic ion exchangers are composed of a strong or weak organic polymeric matrix. Strong acid resins are produced by sulfonation, whose functional group is sulfonic acid. These resins work in a good pH range, but require a greater amount of regenerant. This is the most chosen for almost all water analyses. Weak character exchangers are carboxylic resins that have exchange capacity and are used to treat larger organic ions [29, 31].

3.6.3 Zwitterionic Exchanger

Zwitterionic ion exchangers are those stationary phases used in the multi-selective retention mechanism. These exchangers have both positive and negative charges, so they carry a net zero charge. Zwitterionic stationary phases accumulate equal amounts of oppositely charged groups, fixed close to the surface or within the volume of the stationary phase. Ideally, the zwitterionic phase can be considered as a phase containing an equivalent amount of strongly acidic and strongly basic groups [11, 32].

3.7 Suppressors

After sample separation and before detection there is a suppressor cell. The main function of the electrochemical suppressor system as part of the detection unit is to reduce the background signal of the mobile phases, increasing the detectability of sample ions. Thus, the high background conductivity of the electrolytes in the eluent is reduced through a selective exchange membrane, lowering the baseline and enabling detection at low concentrations [23,33], as illustrated in Fig. 9.

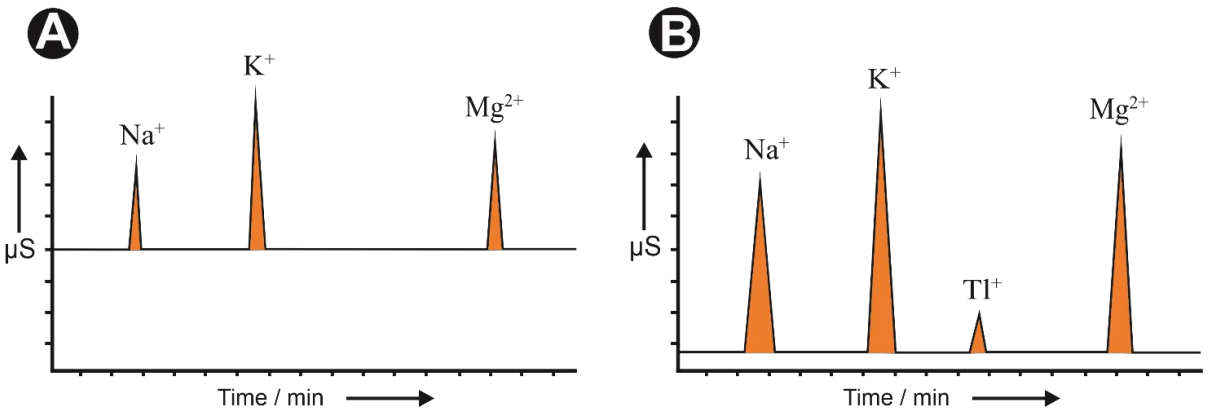

Fig. 5. Baseline reduction using an electrochemical suppressor. A – Chromatographic run without the use of an electrochemical suppressor. B- Chromatographic run with the use of an electrochemical suppressor.

3.8 Detectors

Passing through the suppressor, the analyte is conducted to the detector of the apparatus. There are several types of detectors, they can be conductivity, amperometric, potentiometric, spectrophotometric or fluorescence [33]. Conductivity cells are the most used for IC, where anions and cations are determined through electrical conductance (Fig. 6A), producing a signal based on the physical or chemical properties of the analyte, generating the chromatogram when processed by the computer system (Fig. 6B) [34].

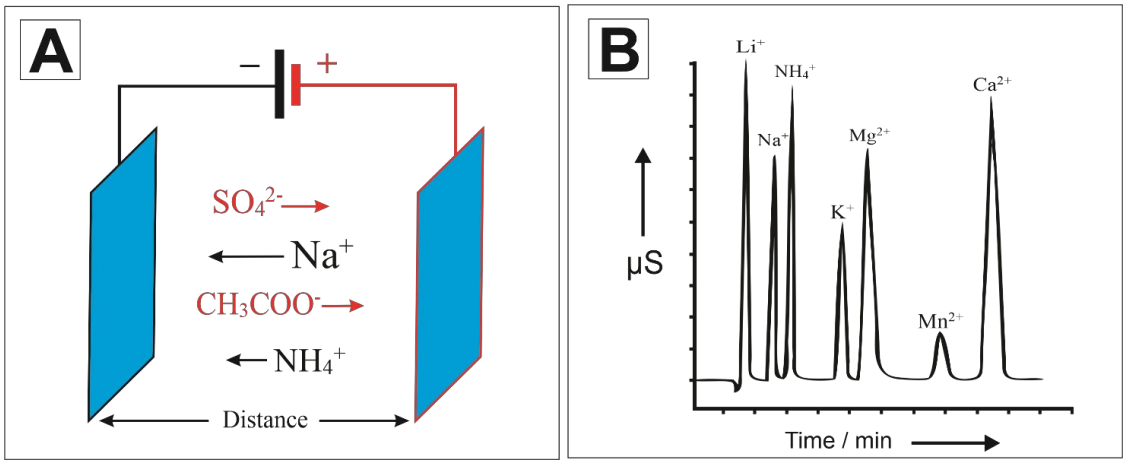

Fig. 6. Detection of ions passing through a conductivity meter generating analytical signal peaks. Conductometric detector with polarized plates to attract ions in solution (A) and chromatogram formed by capturing the detected signal (B).

UV-VIS detection is one of the most popular modes for high performance liquid chromatography, but its application in ion chromatography is limited [35]. The fluorescence detector is rarely used in IC, as only some ions have a fluorescent appearance [36]. Amperometric detection can be used for samples with pK values greater than 7 [37, 38]. Refractive index detection is rarely used in IC [38]. One of the most efficient detection methods used is mass spectrometry (MS) [39, 40]. However, it is not popularly found coupled to the IC due to the high cost compared to the other detectors mentioned.

4. Sample preparation

The sample preparation procedure is necessary before IC analysis in order to protect the separation columns, obtaining reliable and reproducible results. A sample that is not properly prepared can cause increased column back pressure, reduced retention times, low peak efficiency, poor resolution, poor reproducibility, uneven baseline, and fouling in detector cells, altering instrument performance [41–43].

The choice of sample preparation method should provide good analyte recovery and remove as much of the interferents as possible at the same time [44]. These methods range from relatively simple processes, such as sample dilution, filtration, or pH adjustment, to more complicated procedures involving multiple steps, such as analyte extraction from solid materials, analyte pre-concentration, and sample cleaning steps to eliminate headquarters.

In IC, samples containing strong acids or bases cannot be analyzed directly, so samples digested with acids must be partially neutralized before injection. However, the addition of a base for neutralization prevents the determination of the added cation and leads to an increase in the salt load, which can also interfere with chromatographic separations. One solution is the use of ion exchangers (in microcolumn or membrane-based configurations), which in their acidic or basic forms can neutralize alkaline or basic solutions, respectively, without reagent addition and without dilution [45].

5. Conclusions

Ion exchange chromatography has been improving the technique and accounting for several advantages. It has become increasingly popular for analysis of water, pharmaceuticals, food, environmental monitoring and with increasing use in recent years. The technique has good precision and accuracy, a wide variety of applications, many detection modes, high selectivity, speed, separation efficiency and low consumable cost. However, like any technique, it has some limitations depending on the application of the method, which can be mitigated with the hyphenation of other techniques.

Author Contributions

Alexandre Varão Moura: conceptualization, investigation, project administration, writing – original draft, visualization. José Domingos Santos da Silva: writing – review & editing, supervision. Priscila Gubert: writing – review & editing, project administration, supervision.

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