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# **Ion Exchange Chromatography - An Overview**

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Additional information is available at the end of the chapter

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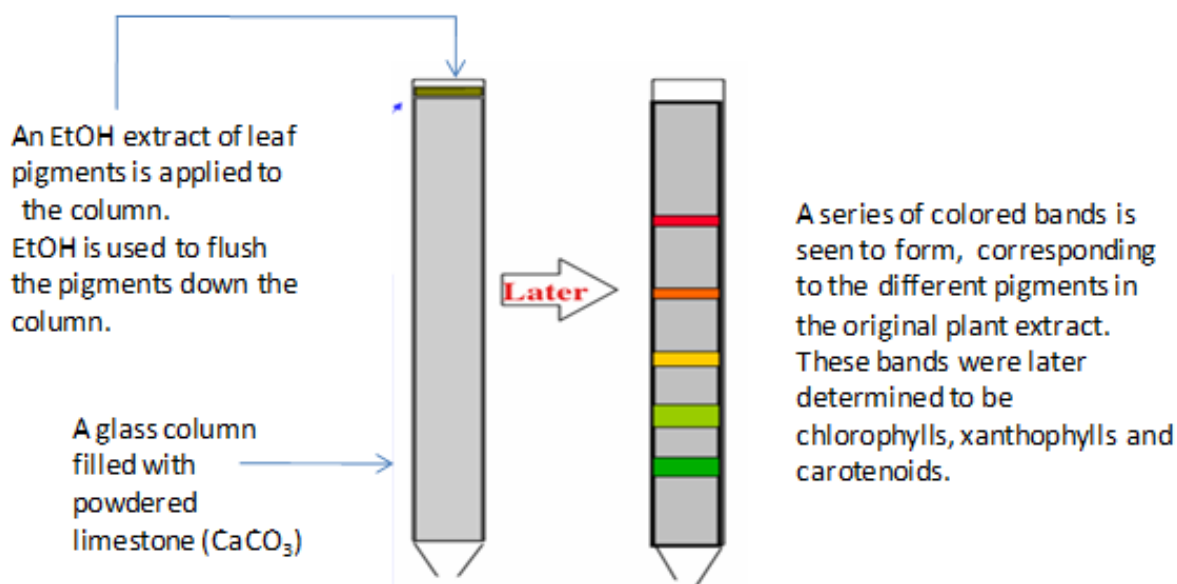
## **1. Introduction**

Chromatography is the separation of a mixture of compounds into its individual components based on their relative interactions with an inert matrix. However, chromatography is more than a simple technique, it is an important part of science encompassing chemistry, physical chemistry, chemical engineering, biochemistry and cutting through different fields. It is worth to be mentioned here that the IUPAC definition of chromatography is "separation of sample components after their distribution between two phases".

### **1.1. Discovery and history of chromatography [1, 2]**

M. Tswett (1872-1919), a Russian botanist, discovered chromatography in 1901 during his research on plant pigments. According to M. Tswett: "An essential condition for all fruitful research is to have at one's disposal a satisfactory technique". He discovered that he could separate colored leaf pigments by passing a solution through a column packed with adsorbent particles. Since the pigments separated into distinctly colored bands as represented in Figure 1, he named the new method "chromatography" (chroma – color, graphy –writing). Tswett emphasized later that colorless substances can also be separated using the same principle.

The separation results from the differential migration of the compounds contained in a mobile phase through a column uniformly packed with the stationary matrix. A mobile phase, usually a liquid or gas, is used to transport the analytes through the stationary phase while the matrix, or stationary phase, is generally an inert solid or gel and may be associated with various moieties, which interact with the analyte(s) of interest. Interactions between the analytes and stationary phase are non-covalent and can be either ionic or non-ionic in nature depending on the type of chromatography being used. Components exhibiting fewer interactions with the stationary phase pass through the column more quickly than those that interact to a greater degree.



**Figure 1.** Schematic diagram of the principles of chromatography as discovered by Tswett (1901).

Tswett's initial experiments involved direct visual detection and did not require a means of quantitation. Nowadays, chromatography is not only a separation technique. In most versions, it is hyphenated analytical techniques combining the separation with the identification and quantitative determination of the separated components. In this form, chromatography has become the most widely used technique in the chemical analysis of complex mixtures.

Many versions of chromatography are used. The various chromatographic techniques are subdivided according to the physical state of these two phases, the mobile and the stationary phases. These are: liquid chromatography including high performance, ion, micellar, electrokinetic, thin-layer, gel-permeation, and countercurrent versions; gas chromatography and supercritical fluid chromatography. Various forms of chromatography can be used to separate a wide variety of compounds, from single elements to large molecular complexes. By altering the qualities of the stationary phase and/or the mobile phase, it is possible to separate compounds based on various physiochemical characteristics. Among these characteristics are size, polarity, ionic strength, and affinity to other compounds. Chromatography also permits a great flexibility in the technique itself. The flow of the mobile phase might be controlled by gravity, pressure, capillary action and electro-osmosis; the separation may be carried out over a wide temperature range and sample size can vary from a few atoms to many kilograms. Also, the shape of the system in which the separation takes place can be varied, using columns of various length and diameter or flat plates. Through all this, evaluation chromatography has been transformed from an essentially batch technique into an automated instrumental method. Through its continuous growth, chromatography became the most widely used analytical separation technique in chemistry and biochemistry. Thus, it is not exaggeration to call it the technique of the 20th Century.

## 2. Ion chromatography

Classical liquid chromatography based on adsorption-desorption was essentially a non-linear process where the time of retardation (retention time) and the quantitative response depend on the position on the adsorption isotherm. Essentially, it was a preparative technique: the aim was to obtain the components present in the sample in pure form which could then be submitted to further chemical or physical manipulations [3].

Ion exchange chromatography (or ion chromatography, IC) is a subset of liquid chromatography which is a process that allows the separation of ions and polar molecules based on their charge. Similar to liquid chromatography, ion chromatography utilizes a liquid mobile phase, a separation column and a detector to measure the species eluted from the column. Ion-exchange chromatography can be applied to the determination of ionic solutes, such as inorganic anions, cations, transition metals, and low molecular weight organic acids and bases. It can also be used for almost all kinds of charged molecule including large proteins, small nucleotides and amino acids. The IC technique is frequently used for the identification and quantification of ions in various matrices.

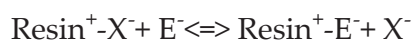
### 2.1. Ion chromatography process [4]

The basic process of chromatography using ion exchange can be represented in 5 steps (assuming a sample contains two analytes A & B): eluent loading, sample injection, separation of sample, elution of analyte A, and elution of analyte B, shown and explained below. Elution is the process where the compound of interest is moved through the column. This happens because the eluent, the solution used as the solvent in chromatography, is constantly pumped through the column. The representative schemes below are for an anion exchange process. (Eluent ion = ▲, Ion A = ■, Ion B = ●)

**Step 1:** The eluent loaded onto the column displaces any anions bonded to the resin and saturates the resin surface with the eluent anion.



This process of the eluent ion ( $E^-$ ) displacing an anion ( $X^-$ ) bonded to the resin can be expressed by the following chemical interaction:



**Step 2:** A sample containing anion A and anion B are injected onto the column. This sample could contain many different ions, but for simplicity this example uses just two different ions as analytes in the sample.

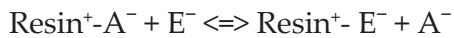


**Step 3:** After the sample has been injected, the continued addition of eluent causes a flow through the column. As the sample elutes (or moves through the column), anion A and anion B adhere to the column surface differently. The sample zones move through the column as eluent gradually displaces the analytes.

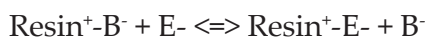
**Step 4:** As the eluent continues to be added, the anion A moves through the column in a band and ultimately is eluted first.



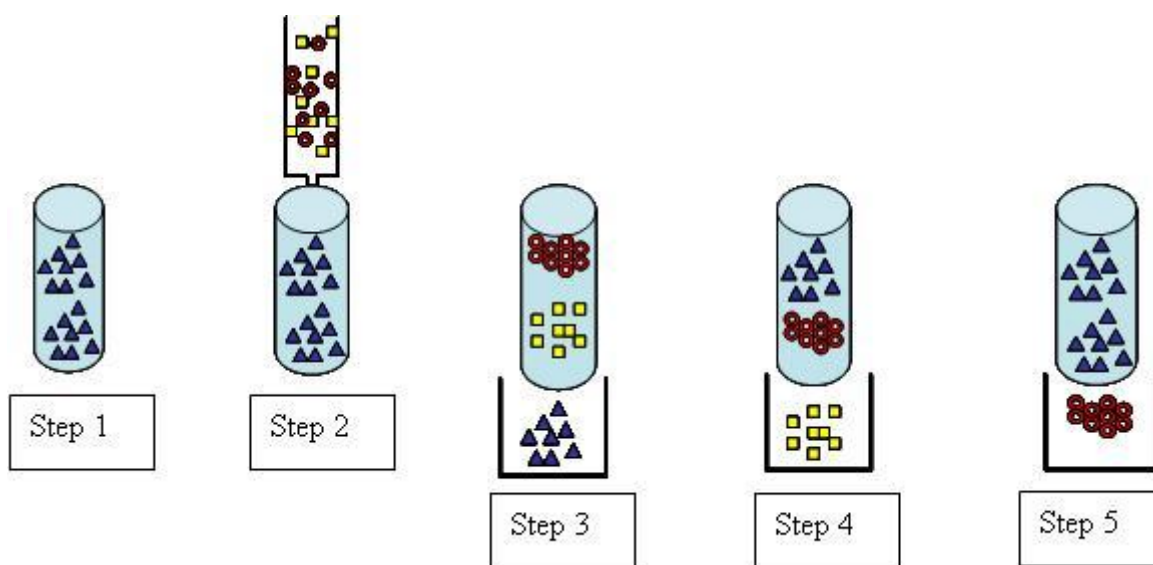
This process can be represented by the chemical interaction showing the displacement of the bound anion ( $A^-$ ) by the eluent anion ( $E^-$ ).



**Step 5:** The eluent displaces anion B, and anion B is eluted off the column.

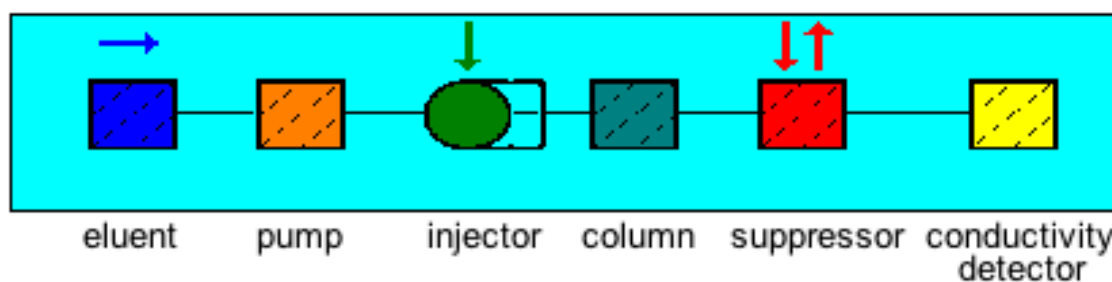


The overall 5 step process can be represented pictorially as shown in Figure 2:



**Figure 2.** Schematic representation of IC process.

A typical ion chromatography consists of several components as shown in Figure 3. The eluent is delivered to the system using a high-pressure pump. The sample is introduced then flows through the guard and into the analytical ion-exchange columns where the ion-exchange separation occurs. After separation, the suppressor reduces the conductivity of the eluent and increases the conductivity of the analytes so they are delivered to the detector. A computer and software are used to control the system, acquire and process the data. Since the introduction of ion chromatography in 1975, many developments were carried out to improve suppressor technology to provide better sensitivity and consistency for the analysis of a wide variety of compounds [5].



**Figure 3.** Schematic representation of Ion chromatography instrumentation.

### 3. Instrumentation [6-9]

Typical IC instrumentation includes: pump, injector, column, suppressor, detector and recorder or data system as represented in Figure 4.

compounds. As examples of organic acid separation and detection using ion chromatography, the analysis of hippuric acid, amines and its derivatives and phenolic compounds were mentioned.

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