

# ION EXCHANGE CHROMATOGRAPHY AND IMPLEMENTATION PROCESS.

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**Annotation:** *This article describes ion exchange chromatography, its implementation process and its advantages.*

**Key words:** *Ion chromatography, anion and cation chromatography, sulphonic and carboxylic group, quarternary ammonium group, polystyrene, formaldehyde, buffer solutions*

**Annotatsiya:** *Ushbu maqolada ion almashinuvi xromatografiyasi, uni amalga oshirish jarayoni va afzalliklari tasvirlangan.*

**Kalit so'zlari:** *Ion xromatografiyasi, anion va kation xromatografiyasi, sulfonik va karboksilik guruh, to'rtlamchi ammoniy guruhi, polistirol, formaldegid, bufer eritmalari.*

**Introduction.** Thanks to the discovery of chromatography method, organic chemistry, especially the chemistry of natural compounds, developed rapidly chromatography qualitative and quantitative analysis of multi-component systems, if they are isolated in pure form (including on an industrial scale), is of great importance. Rare metals are analyzed using chromatography also played an important role in the discovery of artificially prepared transuranic elements. With the help of chromatography 99 elements - Einsteinian (Es), 100 elements - Fermian (Fm) and 101 elements - Mendeleevian (Md) were separated. Chromatography is

of great importance in the determination of mixtures of air, water, soil, monomers, in the analysis of organic and petrochemical synthesis products, in the determination of the purity of medicines, and in criminalistics chromatography methods have also been introduced in the analysis of substances in the gas of spaceships, the gas of the Martian atmosphere, and the soil of the moon. Ion exchange analysis is a form of chromatography.

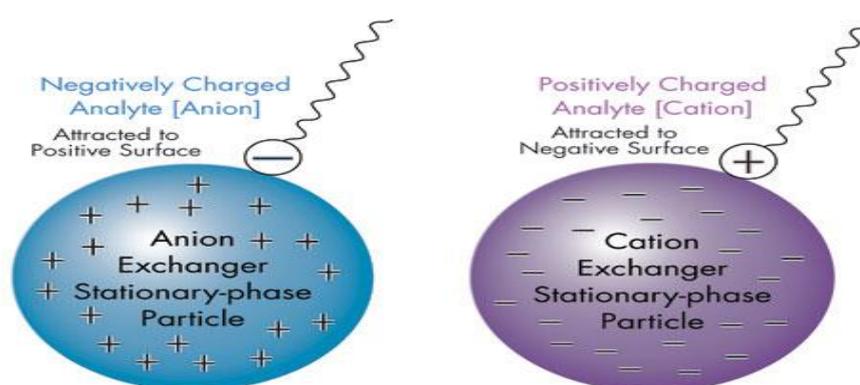
### **Analysis and Methodology**

**Ion chromatography** (or **ion exchange chromatography**) separates ions and molecules based on their affinity to the ion exchanger. It works on almost any kind of charged molecule—including large proteins, small nucleotids, and amino acids. However, ion chromatography must be done in conditions that are one unit away from the isoelectric point of a protein[1].

The two types of ion chromatography are

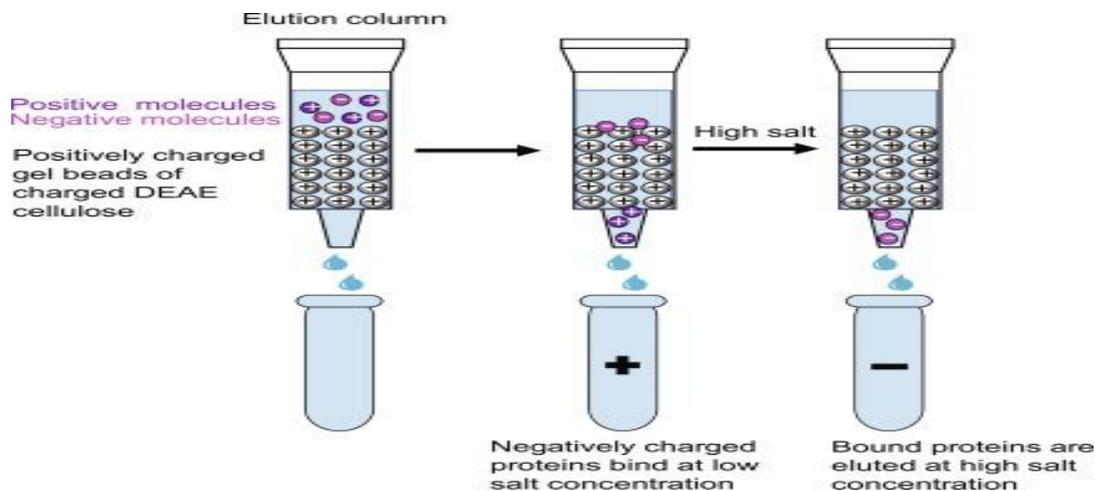
1- Anion-exchange ---anionic exchangers have positively charged groups that will attract negatively charge anions. These are also called "Basic ion exchange" materials[2].

2- Cation-exchange---possess negatively charged group, and these will attract positiv charged cations. These exchangers are also called "Acidic ion exchange" materials, their negative charges result from the ionization of acidic group.



Cation-exchange chromatography is used when the molecule of positively charged. The molecule is positively charged because the pH for chromatography is less than the pI (a/k/a p $H(I)$ ). In this type of chromatography, the stationary phase is negatively charged and positively charged molecules are loaded to be attracted

to it. Anion-exchange chromatography is when the stationary phase is positively charged and negatively charged molecules (meaning that pH for chromatography is greater than the pI) are loaded to be attracted to it. It is often used in protein purification, water analysis, and quality control. The water-soluble and charged molecules such as proteins, amino acids, and peptides bind to moieties which are oppositely charged by forming ionic bonds to the insoluble stationary phase. The equilibrated stationary phase consists of an ionizable functional group where the targeted molecules of a mixture to be separated and quantified can bind while passing through the column—a cationic stationary phase is used to separate anions and an anionic stationary phase is used to separate cations[3]. Cation exchange chromatography is used when the desired molecules to separate are cations and anion exchange chromatography is used to separate anions. The bound molecules then can be eluted and collected using an eluant which contains anions and cations by running higher concentration of ions through the column or changing pH of the column[4].



One of the primary advantages for the use of ion chromatography is only one interaction involved during the separation as opposed to other separation techniques; therefore, ion chromatography may have higher matrix tolerance. Another advantage of ion exchange is the predictability of elution patterns (based on the presence of the ionizable group). For example, when cation exchange chromatography is used, cations will elute out last. Meanwhile, the negative charged molecules will elute out first. However, there are also disadvantages

involved when performing ion-exchange chromatography, such as constant evolution with the technique which leads to the inconsistency from column to column. A major limitation to this purification technique is that it is limited to ionizable group[5].

In cation exchanger materials the acid group are sulphonic acid, carboxylic acid or phenolic, while anion exchanger resin the group are basic as amine, quaternary ammonium etc. On the basic strength of group, they are further divided into four categories:

- a) Strongly acidic cation exchange resin: sulphonic group.
- b) Weakly acidic cation exchange resin : carboxylic group.
- c) Strongly basic anion exchange resin : quaternary ammonium group.
- d) Weakly basic anion exchange resin : polystyrene, formaldehyde.

For strongly acidic and basic resin exchange capacity is independent of pH, and for weakly acidic basic resin exchange capacity is dependent on pH of the solution.

Buffers:-The composition of loading , wash and elution buffers is an important consideration for ion exchange chromatography. When a buffer contains the wrong counterion, it can prevent binding of the proteins of interest to the column resin. The charged species in buffers used for ion exchange chromatography should thus generally have the same sign as the charged species of the IEX resin. For example, although phosphate buffers are commonly used for protein purification, they are not appropriate anion exchange chromatography because the phosphate ion interacts strongly with positively charged anion exchange resins[6].

Common buffers for anion and cation exchange chromatography are

Type of ion exchanger	Buffer	Buffering Range
Cations	Acetic acid	4.8-5.2
	Citric acid	4.2-5.2
	Lactic acid	3.6-4.3

	Phosphate	6.7-7.6
	Tricine	7.8-8.9
	Bicine	7.6-9.0
	Diethanolamine	8.4-8.8
Cations	Di--ethylamine	9.5--11.5
	L-histidine	5.5--6.0
	Imidazole	6.6--7.1
	Pyridine	4.9--5.6
	Tricine	7.4--8.8
	Triethanolamine	7.3--8.3

**In conclusion :** We conclude that ion chromatography is an effective way of determining the components and ion chromatography process that separates ions and polar molecules based on their affinity to the ion exchanger. It works on almost any kind of charged molecule -- including large proteins, small nucleotides and amino acids.

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