

Indexed, Abstracted and Cited: [ISRA Journal Impact Factor](#), [International Impact Factor Services \(IIFS\)](#), [Directory of Research Journals Indexing \(DRJI\)](#), [International Institute of Organized Research and Scientific Indexing Services](#)  
[citefactor.org](http://citefactor.org) [journals indexing](#)  
 Directory Indexing of International Research Journals

## World Journal of Biology and Medical Sciences



Published by Society for Advancement of Science®

ISSN 2349-0063 (Online/Electronic)

Volume 2, Issue- 1, 93-101, January-March, 2015



WJBMS 2/02/04/2015

All rights reserved

A Double Blind Peer Reviewed Journal

[www.sasjournals.com](http://www.sasjournals.com)

[wjbmedsc@gmail.com](mailto:wjbmedsc@gmail.com)/[wjbms.lko@gmail.com](mailto:wjbms.lko@gmail.com)

### REVIEW ARTICLE

Received: 17/02/2015

Revised: 14/03/2015

Accepted: 14/03/2015

## Conventional Biotechnology in Purification of Proteins

Hamid Kheyroodin

Faculty of Desert Science, Semnan University, Semnan, Iran

### ABSTRACT

*A molecule composed of polymers of amino acids joined together by peptide bonds. It can be distinguished from fats and carbohydrates by containing nitrogen. Other components include carbon, hydrogen, oxygen, sulphur, and sometimes phosphorus. A protein is a linear polymer built from about 20 different amino acids. The type and the sequence of amino acids in a protein are specified by the DNA in the cell that produces them. This sequence of amino acids is essential since it determines the overall structure and function of a protein. A protein has several functions. It may serve as a structural material (e.g. keratin), as enzymes, as transporters (e.g. hemoglobin), as antibodies, or as regulators of gene expression. A protein may be classified based on its form and main functions: it can be a globular protein like most enzymes, fibrous protein which are for structural role; and membrane proteins that serve as receptors or channels for polar or charged molecule to pass through the cell membrane.*

**Key words:** Protein, Amino acid, Cell membrane DNA, and Peptide Bond.

### INTRODUCTION

Proteins are large, complex molecules that play many critical roles in the body. They do most of the work in cells and are required for the structure, function, and regulation of the body's tissues and organs (Fairhead, 2013). Proteins are made up of hundreds or thousands of smaller units called amino acids, which

are attached to one another in long chains. There are 20 different types of amino acids that can be combined to make a protein. The sequence of amino acids determines each protein's unique 3-dimensional structure and its specific function (Eith et al., 2002). Proteins can be described according to their large range of functions in the body, listed in

alphabetical order. Enzymes carry out almost all of the thousands of chemical reactions that take place in cells. They also assist with the formation of new molecules by reading the genetic information stored in DNA.

The collection of proteins within a cell determines its health and function. Proteins are responsible for nearly every task of cellular life, including cell shape and inner organization, product manufacture and waste cleanup, and routine maintenance. Proteins also receive signals from outside the cell and mobilize intracellular response. They are the workhorse macromolecules of the cell and are as diverse as the functions they serve cells rely on thousands of different enzymes to catalyze metabolic reactions (**Sakash and Kantrowitz, 2000**). Enzymes are proteins, and they make a biochemical reaction more likely to proceed by lowering the activation energy of the reaction, thereby making these reactions proceed thousands or even millions of times faster than they would without a catalyst. Enzymes are highly specific to their substrates. They bind these substrates at complementary areas on their surfaces, providing a snug fit that many scientists compare to a lock and key. Enzymes work by binding one or more substrates, bringing them together so that a reaction can take place, and releasing them once the reaction is complete. In particular, when substrate binding occurs, enzymes undergo a conformational shift that orients or strains the substrates so that they are more reactive protein methods study protein methods are the techniques used to study proteins. There are experimental methods for studying proteins (e.g., for detecting proteins, for isolating and purifying proteins, and for characterizing the structure and function of proteins, often requiring that the protein first be

purified). Computational methods typically use computer programs to analyze proteins. However, many experimental methods (e.g., mass spectrometry) require computational analysis of the raw data (**Robert, 1987**).

#### **Genetic Methods**

Experimental analysis of proteins typically requires expression and purification of proteins. Expression is achieved by manipulating DNA that encodes the protein(s) of interest. Hence, protein analysis usually requires DNA methods, especially cloning. Some examples of genetic methods include conceptual translation, Site-directed mutagenesis, using a fusion protein, and matching allele with disease states. Some proteins have never been directly sequenced, however by translating codons from known mRNA sequences into amino acids by a method known as conceptual translation. (See genetic code.) Site-directed mutagenesis selectively introduces mutations that change the structure of a protein. The function of parts of proteins can be better understood by studying the change in phenotype as a result of this change. Fusion proteins are made by inserting protein tags, such as the His-tag, to produce a modified protein that is easier to track. An example of this would be GFP-Snf2H which consists of a protein bound to a green fluorescent protein to form a hybrid protein. By analyzing DNA alleles can be identified as being associated with disease states, such as in calculation of LOD scores (**Small, 1989**).

#### **Protein Extraction from Tissues**

Protein extraction from tissues with tough extracellular matrices (e.g., biopsy samples, venous tissues, cartilage, skin) is often achieved in a laboratory setting by impact pulverization in liquid nitrogen. Samples are frozen in liquid nitrogen and subsequently subjected to impact or mechanical grinding. As water in the

samples becomes very brittle at this temperature, the samples are often reduced to a collection of fine fragments, which can then be dissolved for protein extraction. Stainless steel devices known as tissue pulverizers are sometimes used for this purpose. Advantages of these devices include high levels of protein extraction from small, valuable samples; disadvantages include low-level cross-over contamination (**Tatjana and Joachim, 2005**).

### Protein Purification

- Protein isolation
- Chromatography methods: ion exchange, size-exclusion chromatography (or gel filtration), affinity chromatography
- Protein extraction and solubilization
- Protein concentration determination methods
- Bradford protein assay
- Concentrating protein solutions
- Gel electrophoresis
- Gel electrophoresis under denaturing conditions
- Gel electrophoresis under non-denaturing conditions
- 2D gel electrophoresis
- Electro focusing

Ion exchange is an exchange of ions between two electrolytes or between an electrolyte solution and a complex. In most cases the term is used to denote the processes of purification, separation, and decontamination of aqueous and other ion-containing solutions with solid polymeric or mineralic 'ion exchangers'.

Typical ion exchangers are ion exchange resins (functionalized porous or gel polymer), zeolites, montmorillonite, clay, and soil humus. Ion exchangers are either cation exchangers that exchange

Ion exchange is widely used in the food & beverage, hydrometallurgical, metals finishing, chemical and petrochemical, pharmaceutical, sugar and sweeteners, ground and potable water, nuclear,

positively charged ions (cations) or anion exchangers that exchange negatively charged ions (anions). There are also amphoteric exchangers that are able to exchange both cations and anions simultaneously (**Jerde et al., 2000**). However, the simultaneous exchange of cations and anions can be more efficiently performed in mixed beds that contain a mixture of anion and cation exchange resins, or passing the treated solution through several different ion exchange materials.

Ion exchangers can be unselective or have binding preferences for certain ions or classes of ions, depending on their chemical structure. This can be dependent on the size of the ions, their charge, or their structure. Typical examples of ions that can bind to ion exchangers are:

- $H^+$  (proton) and  $OH^-$  (hydroxide)
- Single-charged monatomic ions like  $Na^+$ ,  $K^+$ , and  $Cl^-$
- Double-charged monatomic ions like  $Ca^{2+}$  and  $Mg^{2+}$
- Polyatomic inorganic ions like  $SO_4^{2-}$  and  $PO_4^{3-}$
- Organic bases, usually molecules containing the amine functional group - $RNH^+$
- Organic acids, often molecules containing - $COO^-$  (carboxylic acid) functional groups
- Biomolecules that can be ionized: amino acids, peptides, proteins, etc.

Along with absorption and adsorption, ion exchange is a form of sorption. Ion exchange is a reversible process and the ion exchanger can be regenerated or loaded with desirable ions by washing with an excess of these ions.

softening and industrial water, semiconductor, power, and a host of other industries (**Jackson and Paul, 1990**). Most typical example of application is preparation of high purity water for

power engineering, electronic and nuclear industries; i.e. polymeric or mineralic insoluble ion exchangers are widely used

for water softening, water purification, water decontamination etc.



Fig 1. Ion Exchanger.

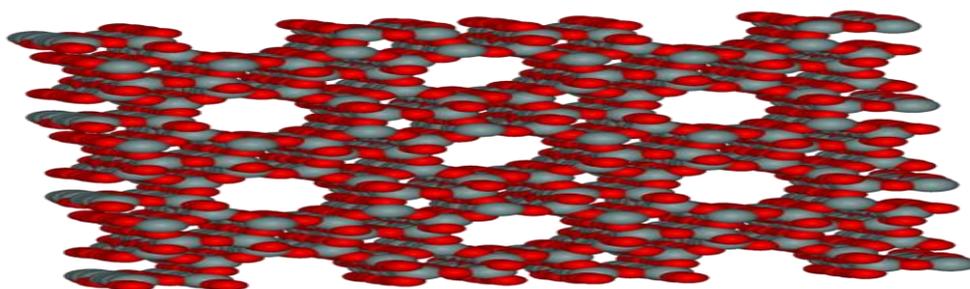


Figure 2. Nuclear magnetic resonance spectroscopy of proteins

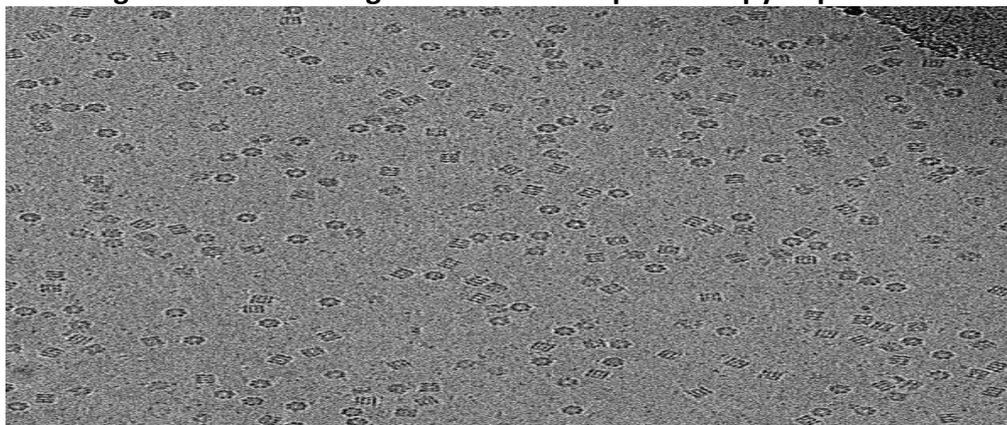


Figure 3. Study of protein by Cryo-electron Microscopy.

Ion exchange is a method widely used in household (laundry detergents and water filters) to produce soft water. This is accomplished by exchanging calcium  $\text{Ca}^{2+}$  and magnesium  $\text{Mg}^{2+}$  cations against  $\text{Na}^+$  or  $\text{H}^+$  cations (see water softening). Another application for ion exchange in domestic water treatment is the removal of nitrate and natural organic matter. Industrial and analytical ion exchange chromatography is another area to be mentioned.

Ion exchange chromatography is a chromatographical method that is widely used for chemical analysis and separation of ions. For example, in biochemistry it is widely used to separate charged molecules such as proteins. An important area of the application is extraction and purification of biologically produced substances such as proteins (amino acids) and DNA/RNA. Ion-exchange processes are used to separate and purify metals, including separating uranium from

plutonium and other actinides, including thorium, and lanthanum, neodymium, ytterbium, samarium, lutetium, from each other and the other lanthanides. There are two series of rare earth metals, the lanthanides and the actinides, both of whose families all have very similar chemical and physical properties. Using methods developed by Frank Spedding in the 1940s, ion-exchange used to be the only practical way to separate them in large quantities, until the advent of solvent extraction techniques that can be scaled up enormously. A very important case is the PUREX process (plutonium-uranium extraction process), which is used to separate the plutonium and the uranium from the spent fuel products from a nuclear reactor, and to be able to dispose of the waste products. Then, the plutonium and uranium are available for making nuclear-energy materials, such as new reactor fuel and nuclear weapons. The ion-exchange process is also used to separate other sets of very similar chemical elements, such as zirconium and hafnium, which is also very important for the nuclear industry. Zirconium is practically transparent to free neutrons, used in building reactors, but hafnium is a very strong absorber of neutrons, used in reactor control rods. Ion exchangers are used in nuclear reprocessing and the treatment of radioactive waste. Ion exchange resins in the form of thin membranes are used in chloralkali process, fuel cells and vanadium redox batteries. Ion exchange can also be used to remove hardness from water by exchanging calcium and magnesium ions for sodium ions in an ion exchange column. Liquid (aqueous) phase ion exchange desalination has been demonstrated. In this technique anions and cations in salt water are exchanged for carbonate anions and calcium cations respectively using electrophoresis.

Calcium and carbonate ions then react to form calcium carbonate, which then precipitates leaving behind fresh water. The desalination occurs at ambient temperature and pressure and requires no membranes or solid ion exchangers. Theoretical energy efficiency of this method is on par with electrodialysis and reverse osmosis.

#### **Other Applications**

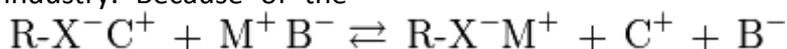
In soil science, cation exchange capacity is the ion exchange capacity of soil for positively charged ions. Soils can be considered as natural weak cation exchangers.

- In pollution remediation and geotechnical engineering, ion exchange capacity determines the swelling capacity of swelling or Expansive clay such as Montmorillonite, which can be used to "capture" pollutants and charged ions.
- In planar waveguide manufacturing, ion exchange is used to create the guiding layer of higher index of refraction.
- Dealkalization, removal of alkali ions from a glass surface.
- Chemically strengthened glass, produced by exchanging  $K^+$  for  $Na^+$  in soda glass surfaces using  $KNO_3$  melts.
- Ion chromatography  
Ion-exchange chromatography (or ion chromatography) is a process that allows the separation of ions and polar molecules based on their affinity to the ion exchanger. It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids. The solution to be injected is usually called a sample, and the individually separated components are called analytes. It is often used in protein purification, water analysis, and quality control.

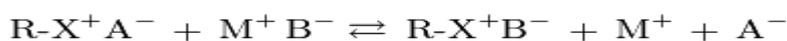
#### **History**

The boom of ion exchange chromatography primarily began between 1935-1950 and it was through the

"Manhattan project" that applications and IC where significantly extended. It was in the fifties and sixties that theoretical models were developed for IC for further understanding and it was not until the seventies that continuous detectors were utilized, paving the path for the development from low-pressure to high-performance chromatography. It was not until the year 1975 that "ion chromatography" was established as a name in reference to the techniques and was thereafter used as a name for marketing purposes. Today IC is of great importance in the investigation of aqueous systems such as the analysis of drinking water. Likewise, it is a popular method for analysis of anionic elements or complexes which serve for solving environmentally relevant problems. Likewise, it also has great uses in the semiconductor industry. Because of the



Anion exchange chromatography retains anions using positively charged functional group:



Note that the ion strength of either C+ or A- in the mobile phase can be adjusted to shift the equilibrium position and thus retention time. The ion chromatogram shows a typical chromatogram obtained with an anion exchange column.

#### Typical Technique

A sample is introduced, either manually or with an autosampler, into a sample loop of known volume. A buffered aqueous solution known as the mobile phase carries the sample from the loop onto a column that contains some form of stationary phase material. This is typically a resin or gel matrix consisting of agarose or cellulose beads with covalently bonded charged functional groups. The target analytes (anions or cations) are retained on the stationary phase but can be eluted by increasing the concentration of a similarly charged species that will displace the analyte ions from the stationary

abundant separating columns, elution systems and detectors available, chromatography has developed into the method of choice for ion analysis.

#### Principle

Ion-exchange chromatography retains analyte molecules on the column based on coulombic (ionic) interactions. The stationary phase surface displays ionic functional groups (R-X) that interact with analyte ions of opposite charge. This type of chromatography is further subdivided into cation exchange chromatography and anion-exchange chromatography. The ionic compound consisting of the cationic species M<sup>+</sup> and the anionic species B<sup>-</sup> can be retained by the stationary phase. Cation exchange chromatography retains positively charged cations because the stationary phase displays a negatively charged functional group:

phase. For example, in cation exchange chromatography, the positively charged analyte could be displaced by the addition of positively charged sodium ions. The analytes of interest must then be detected by some means, typically by conductivity or UV/Visible light absorbance. In order to control an IC system, a chromatography data system (CDS) is usually needed. In addition to IC systems, some of these CDSs can also control gas chromatography (GC) and HPLC

#### Proteins Separation

Proteins have numerous functional groups that can have both positive and negative charges. Ion exchange chromatography separates proteins according to their net charge, which is dependent on the composition of the mobile phase. By adjusting the pH or the ionic concentration of the mobile phase,

various protein molecules can be separated. For example, if a protein has a net positive charge at pH 7, then it will bind to a column of negatively charged beads, whereas a negatively charged protein would not. By changing the pH so that the net charge on the protein is negative, it too will be eluted. Elution by increasing the ionic strength of the mobile phase is a more subtle effect - it works as ions from the mobile phase will interact with the immobilized ions in preference over those on the stationary phase. This "shields" the stationary phase from the protein, (and vice versa) and allows the protein to elute. Separation can be achieved based on the natural isoelectric point of the protein. Alternatively a peptide tag can be genetically added to the protein to give the protein an isoelectric point away from most natural proteins (e.g. 6 arginines for binding to cation-exchange resin such as DEAE-Sepharose or 6 glutamates for binding to anion-exchange resin). Elution from ion-exchange columns can be sensitive to changes of a single charge- chromatate focusing. Ion-exchange chromatography is also useful in the isolation of specific multimeric protein assemblies, allowing purification of specific complexes according to both the number and the position of charged peptide tags.

#### **Study of Protein Structures**

X-ray crystallography is a tool used for identifying the atomic and molecular structure of a crystal, in which the crystalline atoms cause a beam of incident X-rays to diffract into many specific directions. By measuring the angles and intensities of these diffracted beams, a crystallographer can produce a three-dimensional picture of the density of electrons within the crystal. From this electron density, the mean positions of the atoms in the crystal can be determined, as well as their chemical

bonds, their disorder and various other information. Since many materials can form crystals—such as salts, metals, minerals, semiconductors, as well as various inorganic, organic and biological molecules—X-ray crystallography has been fundamental in the development of many scientific fields. In its first decades of use, this method determined the size of atoms, the lengths and types of chemical bonds, and the atomic-scale differences among various materials, especially minerals and alloys. The method also revealed the structure and function of many biological molecules, including vitamins, drugs, proteins and nucleic acids such as DNA. X-ray crystallography is still the chief method for characterizing the atomic structure of new materials and in discerning materials that appear similar by other experiments. X-ray crystal structures can also account for unusual electronic or elastic properties of a material, shed light on chemical interactions and processes, or serve as the basis for designing pharmaceuticals against diseases.

In a single-crystal X-ray diffraction measurement, a crystal is mounted on a goniometer. The goniometer is used to position the crystal at selected orientations. The crystal is bombarded with a finely focused monochromatic beam of X-rays, producing a diffraction pattern of regularly spaced spots known as reflections. The two-dimensional images taken at different rotations are converted into a three-dimensional model of the density of electrons within the crystal using the mathematical method of Fourier transforms, combined with chemical data known for the sample. Poor resolution (fuzziness) or even errors may result if the crystals are too small, or not uniform enough in their internal makeup. X-ray crystallography is related to several other methods for determining atomic

structures. Similar diffraction patterns can be produced by scattering electrons or neutrons, which are likewise interpreted as a Fourier transform. If single crystals of sufficient size cannot be obtained, various other X-ray methods can be applied to obtain less detailed information; such methods include fiber diffraction, powder diffraction and small-angle X-ray scattering (SAXS). If the material under investigation is only available in the form of nanocrystalline powders or suffers from poor crystallinity, the methods of electron crystallography can be applied for determining the atomic structure. For all above mentioned X-ray diffraction methods, the scattering is elastic; the scattered X-rays have the same wavelength as the incoming X-ray. By contrast, inelastic X-ray scattering methods are useful in studying excitations of the sample, rather than the distribution of its atoms. Nuclear magnetic resonance spectroscopy of proteins (usually abbreviated protein NMR) is a field of structural biology in which NMR spectroscopy is used to obtain information about the structure and dynamics of proteins, and also nucleic acids, and their complexes. Structure determination by NMR spectroscopy usually consists of several phases, each using a separate set of highly specialized techniques. The sample is prepared, measurements are made, interpretive approaches are applied, and a structure is calculated and validated. NMR (Figure 2.) involves the quantum mechanical properties of the central core ("nucleus") of the atom. These properties depend on the local molecular environment, and their measurement provides a map of how the atoms are linked chemically, how close they are in space, and how rapidly they move with respect to each other. These properties are fundamentally the same as those used in the more familiar

Magnetic Resonance Imaging (MRI), but the molecular applications use a somewhat different approach, appropriate to the change of scale from millimeters (of interest to radiologists) to nano-meters (bonded atoms are typically a fraction of a nano-meter apart), a factor of a million. This change of scale requires much higher sensitivity of detection and stability for long term measurement. In contrast to MRI, structural biology studies do not directly generate an image, but rely on complex computer calculations to generate three-dimensional molecular models. Currently most samples are examined in a solution in water, but methods are being developed to also work with solid samples. Data collection relies on placing the sample inside a powerful magnet, sending radio frequency signals through the sample, and measuring the absorption of those signals. Depending on the environment of atoms within the protein, the nuclei of individual atoms will absorb different frequencies of radio signals. Furthermore the absorption signals of different nuclei may be perturbed by adjacent nuclei. This information can be used to determine the distance between nuclei. These distances in turn can be used to determine the overall structure of the protein. A typical study might involve how two proteins interact with each other, possibly with a view to developing small molecules that can be used to probe the normal biology of the interaction ("chemical biology") or to provide possible leads for pharmaceutical use ("drug development"). Frequently, the interacting pair of proteins may have been identified by studies of human genetics, indicating the interaction can be disrupted by unfavorable mutations, or they may play a key role in the normal biology of a "model" organism like the fruit fly, yeast, the worm *C. elegans*, or mice. To prepare

a sample, methods of molecular biology are typically used to make quantities by bacterial fermentation. This also permits changing the isotopic composition of the molecule, which is desirable because the isotopes behave differently and provide methods for identifying overlapping NMR signals.

#### Study by Cryo-electron Microscopy

Cryo-electron microscopy (cryo-EM), or electron cryomicroscopy, is a form of transmission electron microscopy (EM) where the sample is studied at cryogenic temperatures (generally liquid nitrogen temperatures)[citation needed]. Cryo-EM is developing popularity in structural biology. The popularity of cryoelectron microscopy stems from the fact that it allows the observation of specimens that have not been stained or fixed in any way, showing them in their native

#### ACKNOWLEDGEMENTS

Research presented in this manuscript is supported by Semnan University, Iran is gratefully acknowledged.

#### REFERENCES

**Eith, Claudia, Kolb Maximilian, and Seubert Andreas (2002).** "Introduction." Practical Ion Chromatography An Introduction. Ed. Viehweger Kai. Herisau: Metrohm. 160.

**Sakash, J.B. and Kantrowitz, E.R. (2000).** "The contribution of individual interchain interactions to the stabilization of the T and R states of Escherichia coli aspartate transcarbamoylase." *J Biol Chem* 275 (37): 28701–7. doi:10.1074/jbc.M005079200. PMID 10875936.

environment, in contrast to X-ray crystallography, which generally requires placing the samples in non-physiological environments, which can occasionally lead to functionally irrelevant conformational changes. In practice, the resolution of cryo-electron microscopy maps is not high enough to allow for unambiguous model construction on the basis of EM maps only, and models obtained by protein crystallography are used to interpret the cryo-EM maps. However, the resolution of cryo-EM maps is improving steadily, and some virus structures obtained by cryo-EM are already at a resolution that can be interpreted in terms of an atomic model. A version of electron cryomicroscopy is cryo-electron tomography (CET) where a 3D reconstruction of a sample is created from tilted 2D images (Figure 3).

**Fairhead, M. (2013).** Plug-and-Play Pairing via Defined Divalent Streptavidins, *J. Mol. Biol.* 426 (1): 199–214. doi: 10.1016/j.jmb.2013.09.016. PMID 24056174.

**Robert E. Smith (1987).** Ion Chromatography Applications. CRC Press. ISBN 978-0-8493-4967-6.

**Small Hamish (1989).** Ion chromatography. New York: Plenum Press. ISBN 0-306-43290-0.

**Tatjana Weiss and Weiss Joachim (2005).** Handbook of Ion Chromatography. Weinheim: Wiley-VCH. ISBN 3-527-28701-9.

**Jerde Douglas, T. Fritz and James S. (2000).** Ion Chromatography. Weinheim: Wiley-VCH. ISBN 3-527-29914-9.

**Jackson Peter Haddad and Paul R. (1990).** Ion chromatography: principles and applications. Amsterdam: Elsevier. ISBN 0-444-88232-4.

Corresponding author: Dr. Hamid Kheyroodin, Faculty of Desert Science, Semnan University, Semnan, Iran.

Email: [hkheyroodin@yahoo.com](mailto:hkheyroodin@yahoo.com)