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Review Article

HPLC for Peptides and Proteins: Principles, Methods and Applications

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ABSTRACT

Peptides and proteins are large biomolecules composed of long chains of L -amino acids and plays key functions in the living organisms. A variety of techniques are used to characterize these biomolecules and, among the techniques used, high performance liquid chromatography (HPLC) has been extensively employed both for the preparative as well as the analytical characterization of peptides and proteins. Various HPLC techniques developed for the separation of proteins rely on the differences in the adsorption characteristics, surface charge, ligand specificity, and molecular size of protein molecules. Advancements made in these HPLC techniques have contributed immensely to the development of peptides and proteins based pharmaceuticals. Present article summarizes the principle, methods, and applications of the most common HPLC techniques used in the field of peptides and proteins.

Key words: Affinity, HPLC, Ion-exchange, Reversed-phase, Size exclusion.

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INTRODUCTION

'Chromatography' refers to the process of separation of the individual components of a mixture based on their relative affinities towards stationary and mobile phases.[1] The credit for the discovery of chromatography goes to Russian botanist Mikhail Tswett who described the separation of plant leaf pigments in solution by passing through a column of solid adsorbents. He coined the term chromatography (derived from Greek words 'chroma' meaning colour and 'graphein' meaning to write), to denote the separation of color compounds. Column chromatography is the most common physical configuration in which the stationary phase is packed into a column through which the mobile phase is pumped. High performance liquid chromatography (HPLC) is a form of column chromatography used frequently in analytical chemistry to purify, identify, analyze, and quantify the compounds. Also, because of its inherent properties HPLC has also been used as the most popular technique for the analysis of biomolecules.

Different chromatographic methods are developed based on different principles. However, irrespective of the HPLC technique involved, a typical chromatography procedure for separation of molecules employs steps as depicted in Figure 1. The basic procedure in the HPLC is to flow the liquid mobile phase containing sample at high pressure driven by a pump through the column packed with suitable matrix. Separation is achieved through differential interaction of molecules with the column matrix and thus are separated by the time required to pass the column. The column structure provides a possibility to employ high pressure to drive the mobile phase flowing much faster and complete a separation within short time. The signals of separated molecules are detected by a suitable detector and data are analyzed on the computer system with processing software.

Proteins are polymeric biomolecules of L-amino acids and perform critical functions within organisms. The introduction of HPLC to the purification and analysis of peptides and proteins has played a critical role in both our understanding of biological processes and in the development of peptides and proteins based pharmaceuticals *i.e.* biopharmaceuticals. Minor differences between various proteins such as hydrophobicity, charge, bioaffinity, and size have been utilized to develop several basic types of chromatography techniques.[2] These techniques differ mainly in the types of stationary phase used to achieve the separation of protein molecules. This article describes principles, methods, and applications of the most common type of HPLC techniques for peptides and proteins *i.e.* reversed phase, ion-exchange, affinity, and size-exclusion.

Reversed-Phase Chromatography

The technique of reversed-phase chromatography (RPC) was originally developed for the separation of relatively small organic molecules. In the late 1970s the use of RPC was extended to the purification of proteins[3] and it has since then achieved considerable interest due to its high resolving power, robustness, and reproducibility. However, the mechanism of the interactions for peptides and proteins with column matrices deviates distinctly from that of the typical organic molecule. Separation of small molecules is usually achieved through partitioning while large molecules such as peptides and proteins are retained by adsorption to the stationary phase.

Principle

RPC involves the separation of protein molecules on the basis of hydrophobicity. The separation depends on the hydrophobic binding of protein from the mobile phase to the immobilized hydrophobic ligands attached to the stationary phase.[4] A schematic diagram showing the separation of peptides and proteins using RPC is shown in Figure 2. The protein mixture is initially applied to the stationary phase in the presence of aqueous buffers and proteins are eluted by the addition of organic solvent to the mobile phase. Elution can proceed either under isocratic conditions where the concentration of organic solvent remains constant, or by gradient elution whereby the amount of organic solvent is gradually increased over a period of time resulting in elution of solutes in the order of increasing hydrophobicity. All peptides and proteins carry

a mix of hydrophilic and hydrophobic amino acids, but those with high net hydrophobicity will be able to participate in hydrophobic interactions with the stationary phase. As mixtures of proteins are applied to the column, polar proteins will elute first while non-polar proteins will bind to the column. Elution of the bound hydrophobic protein can be accomplished by increasing the concentration of organic solvent.

Method

The RPC experimental system for the analysis of peptides and proteins usually consists of an n-alkyl silica-based sorbent from which the solutes are eluted with gradients of increasing concentrations of organic solvent such as acetonitrile containing an ionic modifier such as trifluoroacetic acid. Complex mixtures of peptides and proteins can be routinely separated and low amounts of material can be collected for further characterization. The process of RPC can be summarized in four steps. In the equilibration step the hydrophobic column is primed by applying the specific sample buffer. In second step, the sample protein is injected into the system under conditions that favor binding, typically using low concentration of organic modifier. Proteins in the mixture that have a high percentage of exposed hydrophobic amino acid residues will be adsorbed to the hydrophobic stationary phase and other proteins in the mixture will be washed out. Separations can be easily manipulated by changing the gradient slope, the operating temperature, the ionic modifier, or the organic solvent composition. Then bound hydrophobic protein is eluted by changing the buffer conditions during elution step. The most common way to do this is to use a gradient that slowly increases the hydrophobicity with increase in the concentration of organic solvent. Those molecules with the highest degree of hydrophobicity will be most strongly retained and eluted last. Finally, a wash step removes most of the tightly bound molecules at the end of elution and the column is then re-equilibrated to start conditions before the next run.

Applications

RPC is a very powerful technique for the analysis of peptides and proteins because it can provide excellent resolution under a wide range of chromatographic conditions for very closely related molecules. With RPC, it is very easy to manipulate the chromatographic selectivity through changes in mobile phase composition and characteristics. This HPLC technique generally results in high recoveries with excellent reproducibility. RPC is routinely used in the laboratory scale purification of peptides and proteins. However, RPC can cause the irreversible denaturation of protein samples thereby reducing the potential recovery of material in a biologically active form. Therefore for successful preparative purification, either an inactive protein must be adequate for the purpose or the protein must be able to withstand the harsh conditions of the RPC. For proteins, this technique is therefore mostly used for purity check and quality control analyses, when recovery of functionally active protein is not essential. RPC is widely used in the separation and analysis of therapeutic proteins in the pharmaceutical industry. Octadecylsilane (C_{18}) columns are most commonly used in the analysis, although octyl silane (C_8) and butyl silane (C_4) columns are also used in specific cases. Protein digestion using trypsin or other enzymes results in protein fragments averaging about 10 amino acids each. These protein fragments can be separated by RPC and provide important information on the character and nature of the protein. This is known as peptide mapping and commonly used for identification of biotherapeutic proteins. Analysis of tryptic digest fragments using RPC coupled with mass spectrometry provides information about the glycosylation pattern of proteins. RPC is also extensively used to analyze protein modifications, such as deamidation or oxidation which are considered as protein impurities. Such modifications often result in the loss of biological activity of the protein. Deamidation of protein results in conversion of an asparagine to an aspartic acid (or isoaspartic acid) resulting in addition of an acidic group to the protein. Under acidic conditions aspartic acid is slightly more hydrophobic than asparagine and therefore, during RPC analysis fragment containing the aspartic acid will elute slightly later than a fragment containing asparagine. Methionine residues in proteins may be oxidized through metal catalysis, oxygen and light. Oxidation causes a protein to become more hydrophilic and oxidized proteins elute before the unoxidized form in RPC. Examples of such modifications include deamidation of insulin and oxidation of filgrastim which may be analyzed using pharmacopoeia methods based on RPC.[5,6]

Ion-Exchange Chromatography

Ion-exchange chromatography (IEXC) was introduced in the 1960s for the separation of charged biomolecules such as peptides, proteins, and nucleic acids. IEXC is a highly selective chromatographic technique which is capable of resolving proteins which differ by only a single charged amino acid. IEXC can be subdivided into two types: in cation exchange chromatography positively charged ions bind to a negatively charged resin while in anion exchange chromatography negative ions bind to the immobilized functional group with positive charge.

Principle

IEXC separates biomolecules based on reversible charge-charge interactions between the charged groups on proteins and an ion-exchange support which carries the opposite charge. Once the solutes are bound, the column is washed with low ionic buffer. Neutral proteins or those carrying the same charge as the ion-exchange support are not attracted towards the medium and are easily removed during a washing step. The bound protein molecules of interest are eluted either by increasing the ionic strength of the buffer or pH of the buffer can be modified so as to alter the ionic interactions between the biomolecules of interest and ion-exchange media.[8] Figure 3 illustrates the IEXC separation process that follows. In case of peptides and proteins, ionized lysine, cysteine, and histidine provide positive charges and negative charges are principally provided by aspartate and glutamate residues. At a certain pH point, called isoelectric point (pI), the total positive charges of a protein equal to the total negative charges and the net charge is zero.[9] In IEXC separation a protein will not have any net charge at its isoelectric point (pI) and will not interact with the charged medium. However, at a pH above its pI a protein will bind to a positively charged medium or anion exchanger and, at a pH below its pI a protein will bind to a negatively charged medium or cation exchanger. In addition to the ion exchange interaction other types of binding due to van der Waals forces and non-polar interactions may occur but these effects do not contribute significantly.

Method

An IEX medium comprises a matrix of spherical particles substituted with ionic groups that are negatively or positively charged. Anion exchange resins have a positive charge and are used to retain and separate negatively charged compounds while cation exchange resins have a negative charge and are used to separate positively charged molecules. The matrix is usually porous to give a high internal surface area. The medium is packed into a column to form a packed bed and equilibrated with buffer which fills the pores of the matrix and the space in between the particles. The pH and ionic strength of the equilibration buffer are selected to ensure that, when sample is loaded, proteins of interest bind to the medium and impurities remain unattached. The proteins which bind are effectively concentrated onto the column while proteins that do not have the correct surface charge pass through the column, eluting during or just after sample application. When the sample has been loaded,

conditions are altered in order to elute the bound proteins. Most frequently, proteins are eluted by increasing the ionic strength of the buffer or less frequently by changing the pH. As ionic strength increases, the salt ions (typically Na^+or Cl^-) compete with the bound components for charges on the surface of the medium and one or more of the bound species begin to elute and move down the column. The proteins with the lowest net charge at the selected pH will be the first ones eluted from the column as ionic strength increases. Similarly, the proteins with the highest charge at a certain pH will be most strongly retained and will be eluted last. The higher the net charge of the protein, the higher the ionic strength that is needed for elution. By controlling changes in ionic strength using different forms of gradient, proteins are eluted differentially in a purified and concentrated form.

The mobile phase in IEXC is aqueous solution with proper ionic strength and pH. The pH value determines the charge property of protein. It should be noted that in IEXC the interaction between a protein and an ion exchanger depends more on the charge distribution of the protein surface than the net charge. In practice, the pH is typically at least 1 unit higher or lower than pI of target protein to ensure the protein has an expected surface charge. The most commonly used strategy is to accelerate the exchange of protein by increasing ion strength in initial buffer and sodium chloride is the most widely used agent. It is convenient to increase the cation Na^+ and anion Cl^- at same time and without significantly change pH value of solution. Proteins could be eluted by linear or stepwise gradient ion strength or combination of them. Another elution method is to change the surface charge of proteins by changing pH value of the elution buffer. Typically, in cation IEXC, increased pH value decreases the surface positive charge and the interaction between proteins and exchangers is weakened. Reversely the pH value is decreased in anion IEXC to elute protein. Proteins are eluted at the pH value close to their pI. It should be noted that, change of pH could also alter the charge property of weak exchangers in certain ranges, so the weak exchanger possibly gives different resolution in these ranges.

Applications

The major advantages of IEXC are high resolution, speed, and good recoveries of desired protein. However, this chromatography technique require specific corrosion resistant chromatographic equipment due to the use of ionic buffers. IEXC technique has high resolving power and is capable of separating two proteins differing by one charged amino acid. The technique is extensively used in protein purification and characterization. IEX is one of the most frequently used chromatographic techniques for the protein separation at industrial scale and is used as a polishing step following affinity purification. IEXC is commonly used to separate protease, alkaline phosphatase, and biotinylated proteins. During the production and purification of biopharmaceuticals, proteins may exhibit changes in charge heterogeneity. These changes may result in altered activity and stability of the biopharmaceutical product. Also, these charge variants can induce adverse immunological reactions in the human population. Therefore, identification and monitoring of charge variants in the development and manufacturing of protein based therapeutics is critical to the production of safe and effective drugs. As an analytical tool IEXC is extensively used for protein profiling by characterization of the charged variants, particularly those of monoclonal antibodies.[10,11]

Affinity Chromatography

Affinity chromatography was first used in 1968 for the purification of enzymes and it has since been extended to purification of other biomolecules also including receptor proteins, immunoglobulins, glycoproteins and nucleic acids. This technique separates proteins on the basis of reversible interaction between a protein and a specific ligand coupled to a chromatography matrix. The technique is ideal for a purification protocol and can be used whenever a suitable ligand is available for the protein of interest. In a single step, affinity purification can offer immense time-saving over less selective multistep procedures. The high specific biorecognition gives the technique an extremely high selectivity, by which a protein or a group of proteins could be separated from a crude sample in one step and reaches to a satisfying purify.

Principle

The biological function of proteins often involves specific interactions with other molecules, called ligands. Most of the proteins have an inherent recognition site that can be used to select the appropriate affinity ligand. Key steps in affinity chromatography are depicted in Figure 4. Separation of a desired protein using affinity chromatography relies on the reversible interactions between the protein to be purified and the affinity ligand which is covalently coupled to an inert chromatographic matrix.[12] The sample containing mixture of proteins is applied under conditions that favor specific and reversible binding of the target protein to the ligand. An interacting protein has binding sites with complementary surfaces to its ligand. The binding can involve a combination of electrostatic or hydrophobic interactions as well as short-range molecular interactions such as van der Waals forces and hydrogen bonds. Since only the intended protein is adsorbed from the extract passing through the column, other substances will be washed away. The interaction of ligand-protein can be reversed, either specifically using a competitive ligand, or non-specifically by changing the pH, ionic strength or polarity to elute the target protein in bioactive form.

Method

In general, the procedure for immobilization of a ligand consists of three steps. First the matrix is activated to make it reactive toward the functional group of the ligand. Thereafter the ligand is covalently coupled through chemical reaction. Finally, residual unreacted groups are blocked by a large excess of a suitable low molecular weight substance. This provides a higher degree of certainty that all binding will be between the ligand and the sample. For affinity chromatography applications, the ideal gel material should have suitable chemical groups to which the ligand can be covalently coupled and have a relatively large surface area available for attachment. Also the matrix must be both chemically and mechanically stable and inert to harsh solvents and buffers that are employed during the elution of the protein. Hydrophilic and neutral matrices are preferred to prevent the proteins from interacting nonspecifically with the gel matrix itself. Agarose is used as the most popular base for affinity matrices. A number of synthetic organic and inorganic porous bead matrices such as cross-linked dextrans, polystyrene, polyacrylamide, cellulose, porous glass, and silica are also available.[13]

In affinity chromatography, the ideal binding buffer conditions are optimized to ensure that the target molecules interact effectively with the ligand and are retained by the affinity medium while nonspecific interactions are minimized. In most cases the binding buffer is also used to wash unbound substances from the column without eluting the target molecules. In the elution phase buffer conditions are changed to break the interaction between the target molecules and the ligand and thereby eluting target molecules from the column. The principle of desorption is to change the binding equilibrium for the adsorbed substance from the stationary to the mobile phase. This can be achieved in many different ways, either specifically or non-specifically. It is important that the elution buffer work quickly and do not change the function or activity of the desired protein. The most frequently used method for eluting strongly bound substances non-specifically is by decreasing the pH of

the buffer. Often a pH value around 2 is needed for desorption, but the chemical stability of the matrix, ligand and adsorbed protein determine low pH values to be used. Sometimes an increase in pH may also be effective. An increase in the ionic strength of the buffer can also be useful for elution of proteins bound by electrostatic interactions and 1 M NaCl is frequently used for this purpose. When the binding is dominated by strong hydrophobic interactions, rather drastic methods of elution such as reducing the polarity or including a denaturing agent in the buffer are employed. This type of elution is typical for immunosorbent based on immobilized polyclonal antibodies. In specific elution, bound proteins are desorbed from the ligand by the competitive binding of the eluting agent that binds either to the ligand or to the target molecule. The addition of a free ligand works by challenging the matrix-bound ligand for the target molecule. If the target molecule forms a stronger binding with the free ligand, it will desorb from the matrix-bound ligand and elute with the free ligand. The addition of a competitor works almost exactly like the free ligand, but instead of binding to the target molecule, the competitor binds to the matrix-bound ligand. By having a higher affinity for the matrix-bound ligand, or being in a higher concentration, the competitor replaces the target molecule which is then eluted.

Applications

Affinity chromatography is a rapid and efficient purification technique capable of giving high concentrations of proteins, even from complex mixtures, in one simple process step. Applications of the technique are limited by the availability of immobilized ligands. Since affinity chromatography relies on functional properties of proteins, the technique offers a unique feature that proteins in biologically active forms often can be separated from denatured or functionally different forms. One form of affinity chromatography commonly uses Protein A or Protein G as ligands for purification of immunoglobulins from various species. These ligands are cell wall proteins from *Staphylococcus* or *Streptococcus* with high affinity for the constant region of IgG. Binding is usually achieved at physiological pH values and lower pH values of around 3 is usually needed for elution. Similarly, immobilized lectins are used for separation of glycoconjugates such as glycoproteins, polysaccharides, and glycolipids. Concanavalin A is the mostcommonly used lectin which interacts strongly with mannose and glucose residues. Substances bound to the immobilized lectin can be displaced with a competitive substance such as the monosaccharide for which the lectin has an affinity. The binding can also be reversed with a borate buffer, which forms a complex with glycoproteins or nonspecifically by a change in pH. In a similar way, heparin interacts strongly with the DNA binding proteins such as histones, nucleosomes, transcriptional activators, replicases and extensively used in their affinity purification. Receptor proteins can also be purified by affinity chromatography with the use of the effector molecules as the immobilized ligand. Examples of receptor proteins that are routinely purified in this way are steroid, esterogen, transferrin and androgen receptors. For isolation of an enzyme, the ligand may be the substrate, a competitive reversible inhibitor or an allosteric activator. In purification of recombinant proteins, the gene encoding the target protein is genetically fused with a gene encoding a protein, known as purification tag. When the chimeric protein is expressed the tag allows for specific capture of the fusion protein. Examples of commonly used purification tags are Glutathione S-transferase, maltose-binding protein, His-tag, FLAG tag, S-tag, Strep tag, and calmodulin binding peptide.

Size Exclusion Chromatography

Size exclusion chromatography (SEC) is used primarily for the analysis of large molecules such as proteins or polymers.[14] In contrast to other chromatography techniques which are based on interaction of proteins with the column matrices, in SEC separation is achieved according to molecular size and shape of the molecules while passing through the porous matrix.[15] Molecules do not bind to the surface of media but are retarded by the porous structure of media. However, as compared to adsorption chromatography the pore size of SEC matrix is much smaller and not uniform.

PRINCIPLE

Separation in SEC is achieved based on the molecular sieve properties of the porous matrix. SEC matrices consist of a range of beads with slightly different pore sizes. As shown in Figure 5, smaller molecules run longer and more winding paths in media rather than running straight paths outside the media as larger molecules do resulting in more retardation of small molecules as compared to large molecules.[15] Although the separation in SEC is generally assumed to be according to molecular weight, it is more accurate to claim that it is achieved by the differential exclusion or inclusion within porous particles. There are two basic types of SEC. One is gel permeation chromatography, which uses a hydrophobic column packing material and organic solvent as mobile phase to measure the molecular weight distribution of synthetic polymers. The other is gel filtration chromatography, which uses a hydrophilic packing material and an aqueous mobile phase to separate and measure the molecular weight distribution of water soluble molecules such as proteins.[16]

Method

The support chemistry used for SEC matrices is chosen to reduce adsorptive properties. The matrices used in SEC are often composed of cross-linked polymers such as agarose, dextran, or polyacrylamide. Different pore sizes can be obtained by differing the degree of cross-linking. The surface of these supports contains predominantly hydroxyl groups and provides a good environment for hydrophilic proteins. Macroporous silica has also been employed in SEC but must then be coated with a hydrophilic layer to prevent denaturation of proteins. The size of the pore in the gel plays a key role in exclusion of all molecules above a certain size. Matrices are usually classified based on their capacity to separate different sizes of a hypothetical globular protein. The upper range is the range at which larger molecules are completely excluded from the channels and thus allowing for no separation. The lower range is the range at which small molecules are able to enter all channels and molecules that are even smaller will not have any extra channels to access and there is thus no selection below this size. The linear range between these two extremes is what usually is reported for each matrix. It is important to select a gel with suitable separation range. The media with narrow linear range often employed in group separations, by which solutes are simply separated into two groups. On the contrary, the media with wide linear range usually used to separate similar proteins such as IgG and albumin.

In contrast to other types of chromatography, mobile phase in SEC is considered as carrier phase and the selectivity of a SEC matrix is not adjustable by changing the composition of the mobile phase. In SEC, mobile phase plays an important role as the pH and ionic composition of mobile phase preserves the structure and biological activity of the protein being studied. Elution of proteins is carried out isocratically as single mobile phase is used and gradient pumps are not needed. The height of packing bed (column length) affects both resolution and the separation time. Larger bed height often gives a better resolution, but takes more time to run a separation.

APPLICATIONS

The SEC finds its applications owing to the facts that it is simple to perform and provides unique features not found in other chromatography techniques. SEC is widely used both in preparative and analytical protein purifications. The principal advantage of SEC is its gentle non-

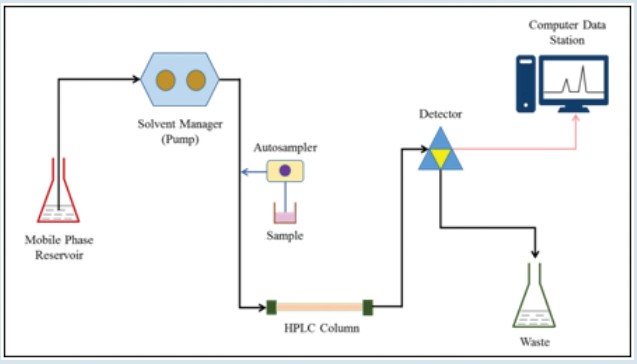

Figure 1: A typical chromatographic procedure.

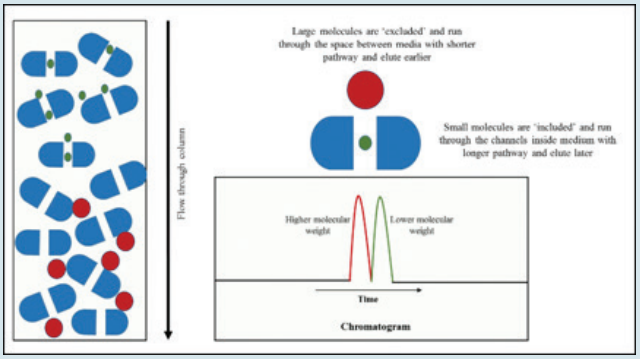

Figure 5: Separation of molecules in SEC based on the size.

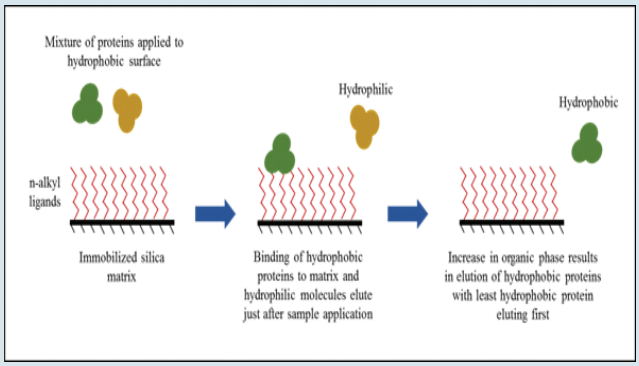

Figure 2: Schematic representation of the separation of hydrophobic and hydrophilic proteins in RPC.

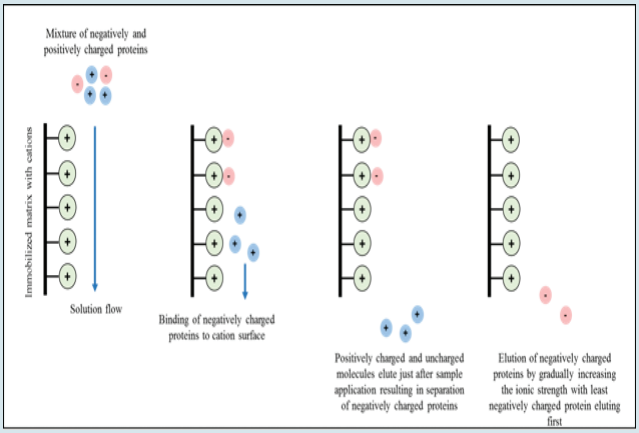

Figure 3: Steps in ion (anion) exchange chromatography.

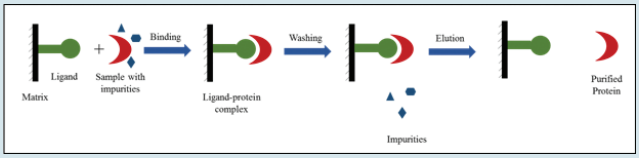

Figure 4: Steps in typical affinity chromatography purification.

interaction with the sample, resulting in preservation of biological activity of the proteins. Moreover, since the separation is not dependent on any adsorptive property of the molecule, SEC provides a method for separating protein aggregates that are not easily distinguished by other chromatographic methods. SEC is preferably used as a final polishing step when sample volumes have been reduced. Since SEC is carried out under mild conditions and mobile phase composition remains unchanged during the process, SEC becomes a good method of choice for separating proteins that are unstable to alterations of pH value, ionic strength or polarity. SEC is used in protein purification directly after other chromatography techniques since the buffer composition will not generally affect the final separation.

SEC is used in group separation mode to remove small molecules from a group of larger molecules and as a fast, simple solution for buffer exchange. Buffer exchange or desalting refers to the situation where low molecular weight components such as salt molecules of the sample are exchanged for another buffering substance. SEC can preferably be used for buffer exchange as the low molecular weight salt molecules are easily separated from the much larger proteins. Small molecules such as excess salts are easily separated from larger molecules. SEC is also used in fractionation mode to separate multiple components in a sample on the basis of differences in their size. The goal can be to isolate one or more of the components, or to analyze the molecular-weight distribution in the sample. High-resolution fractionation by SEC is well-suited for the final polishing step in a purification scheme where monomers are easily separated from aggregates. In analytical laboratories, SEC is also employed to quantitate the aggregates in biopharmaceuticals as aggregates are often considered as impurity e.g. determination of higher molecular aggregates in erythropoietin.[16]

CONCLUSION

It is evident from the above description that HPLC is a highly successful technique for the separation and characterization of peptides and proteins owing to its inherent characteristics of resolution, reproducibility, robustness, and high recoveries. Also, the elution of desired protein can be easily manipulated under different forms of HPLC techniques by altering the interactions of desired protein with stationary phase matrices. Recently we have witnessed tremendous growth in the field of recombinant DNA technology which has resulted in rapid growth in the development of therapeutic proteins under classes of cytokines, growth factors, enzymes, hormones, blood products, and monoclonal antibodies. Therefore it is expected that HPLC in its various forms will continue to contribute significantly in the purification and analysis of peptides and proteins for a long period of time.

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CONFLICT OF INTEREST

Authors declare no conflicts of interest.

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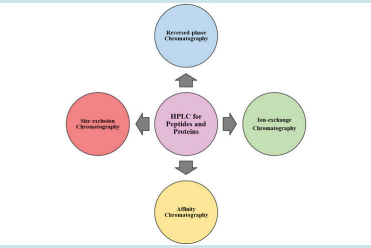
PICTORIAL ABSTRACT



SUMMARY

- High performance liquid chromatography (HPLC) in its various forms such as reversed-phase, affinity, ion-exchange and size-exclusion is used both for preparative and analytical characterization of peptides and proteins.
- Advancements made in HPLC techniques have contributed immensely to the development of peptides and proteins based pharmaceuticals, called biopharmaceuticals.
- The principle, methods, and applications of the most common HPLC techniques used in the field of peptides and proteins are discussed here.

ABOUT AUTHOR

Dr. Gaurav Pratap Singh Jadaun obtained his Ph. D. degree in Biotechnology from National JALMA Institute for Leprosy, Agra and currently working as Junior Scientist at National Institute of Biologicals, Noida. He has research experience in molecular genomics of mycobacterial drug resistance and molecular epidemiology. Currently his work focusses on quality evaluation of biotherapeutics proteins and biologicals.