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R E V I E W A R T I C L E

Affinity chromatography

Dr. K. Padmalatha, *D. Vijaya Durga, T. Deepika, B. Roopalatha, G. Prashanthi

Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada

A B S T R A C T

Affinity chromatography is one of the most diverse and powerful chromatographic methods for purification of a specific molecule or a group of molecules from complex mixtures. It is based on highly specific biological interactions between two molecules, such as interactions between enzyme and substrate, receptor and ligand, or antibody and antigen. These interactions, which are typically reversible, are used for purification by placing one of the interacting molecules, referred to as affinity ligand, onto a solid matrix to create a stationary phase while the target molecule is in the mobile phase. Successful affinity purification requires a certain degree of knowledge and understanding of the nature of interactions between the target molecule and the ligand to help determine the selection of an appropriate affinity ligand and purification procedure. With the growing popularity of affinity purification, many of the commonly used ligands coupled to affinity matrices are now commercially available and are ready to use. However, in some cases new affinity chromatographic material may need to be developed by coupling the ligand onto the matrix such that the ligand retains specific binding affinity for the molecule of interest. In this chapter, we discuss factors which are important to consider when selecting the ligand, proper attachment chemistry, and the matrix. In recent years, matrices with unique features which overcome some of the limitations of more traditional materials have been developed and these are also described. Affinity purification can provide significant time savings and several hundred -fold or higher purification, but the success depends on the method used. Thus, it is important to optimize the purification protocol to achieve efficient capture and maximum recovery of the target.

Keywords: Affinity chromatography, enzyme, receptor, substrate and ligand

A R T I C L E I N F O

Corresponding Author
D. Vijaya Durga
Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada
MS-ID: JPBMAL4409

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1. Introduction

Most samples in nature consist of a complex mixture of many sub□ stances. This fact has led to the development of chemical separation methods such as liquid chromatography to purify, analyze, or examine the components of such samples. The variety of chemical interactions and formats that can be employed in these separations, as based on the types of stationary phases and mobile phases that are used, has resulted in the creation of many types of liquid chromatography. For instance, reversed phase chromatography or nor□ mal phase chromatography can be utilized to separate chemicals based on their polarity, ion exchange chromatography makes use of ionic interactions, and size exclusion chromatography separates chemicals based on their size. Such methods can also be classified as low-performance or high-performance techniques based on the support materials that are used and the types of column efficiencies that are obtained.

The large variety of such methods has resulted in liquid chromatographic methods being employed for the separation or analysis of many types of chemicals in liquid-phase samples. As a result, it is not surprising that liquid chromatography is a common liquid-phase separation method that is found in both industrial set□ tings and research laboratories perhaps the most selective, versatile, and complex form of liquid chromatography is the method of affinity chromatography. This method is a type of liquid chromatography that uses a biologically related agent as the stationary phase. As this definition suggests, affinity chromatography is based on the reversible and specific binding that is often found in biological interactions. Examples of these interactions include the binding of an antibody with its target (or antigen), the binding of a hormone with its receptor, and the interaction of an enzyme with its substrate. This type of selective binding is used in affinity chromatography by placing within the column one of the interacting agents and immobilizing this agent to a support for use as a stationary phase. This immobilized binding agent is called the "affinity ligand," and it forms the basis by which the complementary tar□ get can be isolated or purified by the affinity column.

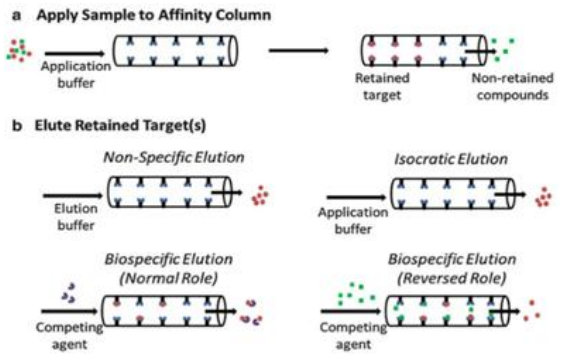

Fig.1: Examples of typical (a) application and (b) elution sequences for affinity chromatography. The isocratic elution method in (b) uses the same solution for both sample application and elution from the column. The nonspecific elution method in (b) uses a separate solution for elution that has a different pH, ionic strength, polarity or temperature from the solution used for sample application. The bio specific elution methods in (b) make use of an elution buffer that contains an agent that will compete with the affinity ligand for binding to the target (normal role) or that competes with the target for binding to the affinity ligand (reversed role).

Principle of Affinity Chromatography

Affinity chromatography takes advantage of specific binding interactions between the analyte of interest (normally dissolved in the mobile phase), and a binding partner or ligand (immobilized on the stationary phase). In a typical affinity chromatography experiment, the ligand is attached to a solid, insoluble matrix--usually a polymer such as agarose or polyacrylamide--chemically modified to introduce reactive functional groups with which the ligand can react, forming stable covalent bonds. The stationary phase is first loaded into a column to which the mobile phase is introduced. Molecules that bind to the ligand will remain associated with the stationary phase. A wash buffer is then applied to remove non-target biomolecules by disrupting their weaker interactions with the stationary phase, while the biomolecules of interest will remain bound. Target biomolecules may then be removed by applying a so-called elution buffer, which disrupts interactions between the bound target biomolecules and the ligand. The target molecule is thus recovered in the eluting solution. Affinity chromatography does not require the molecular weight, charge, hydrophobicity, or other physical properties of the analyte of interest to be known, although knowledge of its binding properties is useful in the design of a separation protocol. Types of binding interactions commonly exploited in affinity chromatography procedures are summarized in the table below.

Table 1. Typical biological interactions used in affinity chromatograph

S.No	Types of ligand	Target molecule
1	Substrate analogue	Enzymes
2	Antibody	Antigen
3	Lectin	Polysaccharide
4	Nucleic acid	Complementary base sequence
5	Hormone	Receptor
6	Avidin	Biotin/Biotin-conjugated molecule
7	Calmodulin	Calmodulin binding partner
8	Glutathione	GST fusion protein
9	Proteins A and G	Immunoglobulins
10	Metal ions	Poly-histidine fusion protein

Components of Affinity Chromatography Matrix

- The matrix is an inert support to which a ligand can be directly or indirectly coupled.
- In order to for the matrix to be effective it must have certain characters:
- Matrix should be chemically and physically inert.

- It must be insoluble in solvents and buffers employed in the process
- It must be chemically and mechanically stable.
- It must be easily coupled to a ligand or spacer arm onto which the ligand can be attached.
- It must exhibit good flow properties and have a relatively large surface area for attachment.
- The most useful matrix materials are agarose and polyacrylamide.

Spacer arm

- It is used to improve binding between ligand and target molecule by overcoming any effects of steric hindrance.

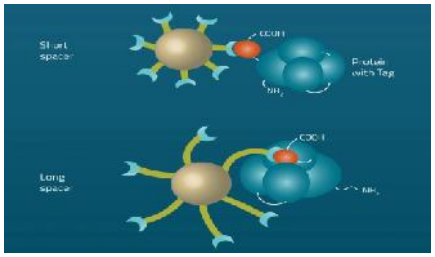

Fig 2. Spacer arm

Table 2: Overview of different spacers for affinity beads.

Chemical	Chain length
Cyanogen bromide	C1
Epoxide	C3
Epoxide with C6 acid	C10
Diamin	C10

Ligand

It refers to the molecule that binds reversibly to a specific target molecule.

The ligand can be selected only after the nature of the macromolecule to be isolated is known.

When a hormone receptor protein is to be purified by affinity chromatography, the hormone itself is an ideal candidate for the ligand.

For antibody isolation, an antigen or hapten may be used as ligand.

If an enzyme is to be purified, a substrate analog, inhibitor, cofactor, or effector may be used as a the immobilized ligand.

Table 3: Typical ligands used in affinity chromatography

Ligand	Target
Antibody	Antigen
Iron-, aluminum-ions	Phosphoproteins
Avidin	Biotin
Glutathione	GST
Chelator + Ni-, Co-ions	His-tagged proteins

The Mobile / Liquid Phase

Affinity chromatography resins or matrices are typically agarose or magneticagarose beads that are covalently coupled to a molecule that specifically binds to the affinity tag. There is a great variety in tag-resin partner chemistries and interaction types.

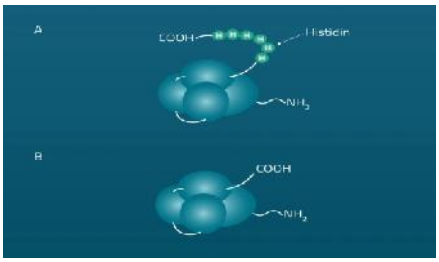

Fig 3. The mobile /liquid phase

Batch and column setups

Binding to the solid phase may be achieved by column chromatography whereby the solid medium is packed onto a column, the initial mixture run through the column to allow settling, a wash buffer run through the column and the elution buffer subsequently applied to the column and collected. These steps are usually done at ambient pressure. Alternatively, binding may be achieved using a batch treatment, for example, by adding the initial mixture to the solid phase in a vessel, mixing, separating the solid phase, removing the liquid phase, washing, re-centrifuging, adding the elution buffer, re-centrifuging and removing the elute. Sometimes a hybrid method is employed such that the binding is done by the batch method, but the solid phase with the target molecule bound is packed onto a column and washing and elution are done on the column. The ligands used in affinity chromatography are obtained from both organic and inorganic sources. Examples of biological sources are serum proteins, lectins and antibodies. Inorganic sources as moronic acts, metal chelates and thiazine dyes.]A third method, expanded bed absorption, which combines the advantages of the two methods mentioned above, has also been developed.

The solid phase particles are placed in a column where liquid phase is pumped in from the bottom and exits at the top. The gravity of the particles ensures that the solid phase does not exit the column with the liquid phase. Affinity columns can be eluted by changing salt concentrations, pH, pI, charge and ionic strength directly or through a gradient to resolve the particles of interest. More recently, setups employing more than one column in series have been developed.

The advantage compared to single column setups is that the resin material can be fully loaded since non-binding product is directly passed on to a consecutive column with fresh column material. These chromatographic processes are known as periodic counter-current chromatography (PCC). The resin costs per amount of produced product can thus be drastically reduced. Since one column can always be eluted and regenerated while the other column is loaded, already two columns are sufficient to make full use of the advantages [8]. Additional columns can give additional flexibility for elution and regeneration times, at the cost of additional equipment and resin costs.

Various affinity media

Many different affinity media exist for a variety of possible uses. Briefly, they are (generalized) activated/ functionalized that work as a functional spacer, support matrix, and eliminates handling of toxic reagents. Amino acid media is used with a variety of serum proteins, proteins, peptides, and enzymes, as well as rRNA and dsDNA. Avidin biotin media is used in the purification process of biotin/avidin and their derivatives. Carbohydrate bonding is most often used with glycoproteins or any other carbohydrate-containing substance; carbohydrate is used with lectins, glycoproteins, or any other carbohydrate metabolite protein. Dye ligand media is nonspecific but mimics biological substrates and proteins. Glutathione is useful for separation of GST tagged recombinant proteins. Heparin is a generalized affinity ligand, and it is most useful for separation of plasma coagulation proteins, along with nucleic acid enzymes and lipases Hydrophobic interaction media are most commonly used to target free carboxyl groups and proteins .Immuno affinity media (detailed below) utilizes antigens' and antibodies' high specificity to separate; immobilized metal affinity chromatography is detailed further below and uses interactions between metal ions and proteins (usually specially tagged) to separate; nucleotide/coenzyme that works to separate dehydrogenases, kinases, and transaminases. Nucleic acids function to trap mRNA, DNA, rRNA, and other nucleic acids/oligonucleotides. Protein A/G method is used to purify immune globulins. Specialty media are designed for a specific class or type of protein/co enzyme; this type of media will only work to separate a specific protein or coenzyme.

Immuno affinity

Another use for the procedure is the affinity purification of antibodies from blood serum. If the serum is known to contain antibodies against a specific antigen (for example if the serum comes from an organism immunized against the antigen concerned) then it can be used for the affinity purification of that antigen. This is also known as Immuno affinity Chromatography. For example,, if an organism is immunized against a GST-fusion protein it will produce antibodies against the fusion-protein, and possibly antibodies against the GST tag as well. The protein can then be covalently coupled to a solid support such as agarose and used as an affinity ligand in purifications of antibody from immune serum.

For thoroughness, the GST protein and the GST-fusion protein can each be coupled separately. The serum is initially allowed to bind to the GST affinity matrix. This will remove antibodies against the GST part of the fusion protein. The serum is then separated from the solid support and allowed to bind to the GST-fusion protein matrix. This allows any antibodies that recognize the antigen to be captured on the solid support. Elution of the antibodies of interest is most often achieved using a low pH buffer such as glycine pH 2.8. The eluate is collected into a neutral tris or phosphate buffer, to neutralize the low pH elution buffer and halt any degradation of the antibody's activity. This is a nice example as affinity purification is used to purify the initial GST-fusion protein, to remove the undesirable anti-GST antibodies from the serum and to purify the target antibody.

Monoclonal antibodies can also be selected to bind proteins with great specificity, where protein is released under fairly gentle conditions. This can become of use for further research in the future A simplified strategy is often employed to purify antibodies generated against peptide antigens. When the peptide antigens are produced synthetically, a terminal cysteine residue is added at either the N- or C-terminus of the peptide. This cysteine residue contains a sulfhydryl functional group which allows the peptide to be easily conjugated to a carrier protein (e.g. Keyhole limpet hemocyanin (KLH)).

The same cysteine-containing peptide is also immobilized onto an agarose resin through the cysteine residue and is then used to purify the antibody. Most monoclonal antibodies have been purified using affinity chromatography based on immunoglobulin-specific Protein A or Protein G, derived from bacteriaImmuno affinity chromatography with monoclonal antibodies immobilized on monolithic column has been successfully used to capture extracellular vesicles (e.g., exosomes and exomeres) from human blood plasma by targeting tetraspanins and integrins found on the surface of the EVsImmuno affinity chromatography is also the basis for immune chromatographic test (ICT) strips, which provide a rapid means of diagnosis in patient care. Using ICT, a technician can make a determination at a patient's bedside, without the need for a laboratory ICT detection is highly specific to the microbe causing an infection

Immobilized metal ion affinity chromatography

Immobilized metal ion affinity chromatography (IMAC) is based on the specific coordinate covalent bond of amino acids, particularly histidine, to metals. This technique works by allowing proteins with an affinity for metal ions to be retained in a column containing immobilized metal ions, such as cobalt, nickel, or copper for the purification of histidine-containing proteins or peptides, iron, zinc or gallium for the purification of phosphorylated proteins or peptides. Many naturally occurring proteins do not have an affinity for metal ions therefore recombinant DNA technology can be used to introduce such a protein tag into the relevant gene. Methods used to elute the protein of interest include changing the pH, or adding a competitive molecule, such as imidazole.

Recombinant proteins

Possibly the most common use of affinity chromatography is for the purification of recombinant proteins. Proteins with a known affinity are protein tagged in order to aid their purification. The protein may have been genetically modified so as to allow it to be selected for affinity binding; this is known as a fusion protein. Tags include hexa histidine (His), glutathione-S-transferase (GST) and maltose binding protein (MBP). Histidine tags have an affinity for nickel, cobalt, zinc, copper and iron ions which have been immobilized by forming coordinate covalent bonds with a chelator incorporated in the stationary phase. For elution, an excess amount of a compound able to act as

a metal ion ligand, such as imidazole, is used. GST has an affinity for glutathione which is commercially available immobilized as glutathione agarose. During elution, excess glutathione is used to displace the tagged protein.

Lectins

Lectin affinity chromatography is a form of affinity chromatography where lectins are used to separate components within the sample. Lectins, such as concanavalin A are proteins which can bind specific alpha-D-mannose and alpha-D-glucose carbohydrate molecules. Some common carbohydrate molecules that is used in lectin affinity chromatography are Con A-Sepharose and WGA-agarose. Another example of a lectin is wheat germ agglutinin which binds D-N-acetyl-glucosamine. The most common application is to separate glycoproteins from non-glycosylated proteins, or one glycoform from another glycoform. Although there are various ways to perform lectin affinity chromatography, the goal is extract a sugar ligand of the desired protein

Specialty

Another use for affinity chromatography is the purification of specific proteins using a gel matrix that is unique to a specific protein. For example, the purification of E. coli β-galactosidase is accomplished by affinity chromatography using p-aminobenyl-1-thio-β-D-galactopyranosyl agarose as the affinity matrix. P-aminobenyl-1-thio-β-D-galactopyranosyl agarose is used as the affinity matrix because it contains a galactopyranosyl group, which serves as a good substrate analog for E.Coli-B-Galactosidase. This property allows the enzyme to bind to the stationary phase of the affinity matrix and is eluted by adding increasing concentrations of salt to the column

Alkaline phosphatase

Alkaline phosphatase from E. coli can be purified using a DEAE-Cellulose matrix. A. phosphatase has a slight negative charge, allowing it to weakly bind to the positively charged amine groups in the matrix. The enzyme can then be eluted out by adding buffer with higher salt concentrations

Boronate affinity chromatography

Boronate affinity chromatography consists of using boronic acid or boronates to elute and quantify amounts of glycoproteins. Clinical adaptations have applied this type of chromatography for use in determining long term assessment of diabetic patients through analysis of their glycated hemoglobin

Steps in Affinity Chromatography

Affinity medium is equilibrated in binding buffer.

Sample is applied under conditions that favor specific binding of the target molecule(s) to a complementary binding substance (the ligand). Target substances bind specifically, but reversibly, to the ligand and unbound material washes through the column.

Elution is performed specifically, using a competitive ligand, or non-specifically, by changing the pH, ionic strength or polarity. Target protein is collected in a purified, concentrated form.

Affinity medium is re-equilibrated with binding buffer.

These events can be summarized into the following three major steps:

1. **Preparation of Column**
   - The column is loaded with solid support such as sepharose, agarose, cellulose etc.
   - Ligand is selected according to the desired isolate.
   - Spacer arm is attached between the ligand and solid support.
2. **Loading of Sample**
   - Solution containing a mixture of substances is poured into the elution column and allowed to run at a controlled rate.
3. **Elution of Ligand-Molecule Complex**
   - Target substance is recovered by changing conditions to favor elution of the bound molecules.

Weak affinity chromatography

Weak affinity chromatography (WAC) is an affinity chromatography technique for affinity screening in drug development. WAC is an affinity-based liquid chromatographic technique that separates chemical compounds based on their different weak affinities to an immobilized target. The higher affinity a compound has towards the target, the longer it remains in the separation unit, and this will be expressed as a longer retention time. The affinity measure and ranking of affinity can be achieved by processing the obtained retention times of analyzed compounds. The WAC technology is demonstrated against a number of different protein targets – proteases, kinases, chaperones and protein–protein interaction (PPI) targets. WAC has been shown to be more effective than established methods for fragment based screening.

Mechanism of Affinity Binding

A commonly used metaphor to illustrate affinity binding is the lock and key analogy. A unique structure present on the surface of a protein is the key that will only bind to the corresponding lock, a specific ligand on a chromatographic support.

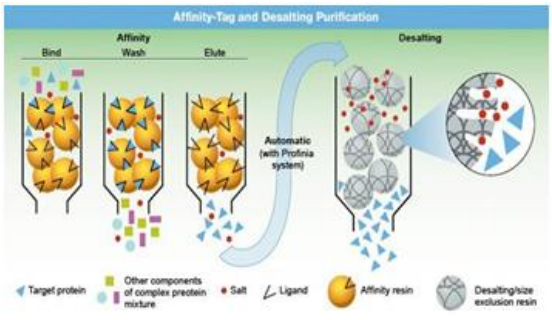

Fig 4

Affinity-tagged purification

In two-step affinity-tagged protein purification, a protein is first purified by affinity chromatography, then desalted. In some medium pressure chromatography systems, such as

the NGC medium pressure chromatography systems, these two steps can be automated. In the first step, a recombinant protein mixture is passed over a chromatography support containing a ligand that selectively binds proteins that contain an affinity-tag sequence (typically His or GST). Contaminants are washed away, and the bound protein is then eluted in pure form.

Affinity tags have different advantages. In immobilized metal affinity chromatography (IMAC), His binds with good selectivity to Ni2+ or other transition metals immobilized to the ligand; the tagged protein can be selectively eluted with imidazole. Proteins tagged with GST bind to glutathione as the ligand, and are eluted with solutions of glutathione. Proteins with an enzymatically active GST fusion tag can only be purified under native conditions. In contrast, polyhistidine-tagged proteins may be purified under native or denaturing conditions.

During the second step of desalting, affinity-purified samples can simultaneously undergo buffer exchange to remove salts in preparation for downstream applications. A number of desalting techniques, including size exclusion chromatography, dialysis, and ultrafiltration, also allow buffer exchange. Desalting often includes the removal not only of salt, but also of other foreign substances, such as detergents, nucleotides, and lipids.

Affinity chromatography can be broadly divided into two method types: The first method uses a naturally occurring structure or sequence of amino acids on the protein as the binding site. Examples include the affinity of Affinity Gel Blue support binding for albumin's bilirubin-binding site and the binding of protein A in the Affinity Gel and Affinity Prep protein A supports to the Fc region of IgG. An important consideration for antibody purification is to determine the affinity of your target antibody for protein A/G chromatography media, which varies widely.

The second method involves binding to a special amino acid sequence engineered into the protein of interest, commonly referred to as a "tag". A number of different tags are available. Two of the most commonly used protein tags are the polyhistidine tag, which binds to certain metal-containing complexes such as those in Profinity™ IMAC resins, and the glutathione s-transferase (GST) sequence, which binds to glutathione, found in Bio-Scale™ Mini Profinity™ GST media. Theoretically, any protein can be purified using the tagging method; however, many factors must be considered to design a process to purify tagged recombinant proteins. Bio-Rad offers ready to use affinity media and customizable or activated media.

Bind-Wash-Elute: The three steps of affinity purification
Most affinity purification protocols follow the same three steps:

1. Binding:
A complex solution containing the tagged protein is applied to the column and binds based on the affinity tag - matrix interaction

2. Wash:
Other proteins which bind unspecifically are washed away with suitable buffers

3. Elution:
Specifically bound protein is eluted from the column, typically by competitive binding of a similar molecule (e.g. histidine and imidazole), by cutting off the tag with a protease or by destabilization of the affinity tag - matrix interaction e.g. by a change of pH

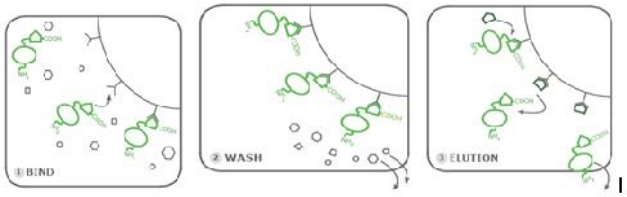

Fig. 5: Bind wash elute principle exemplified by an antibody-based affinity matrix (e.g. Rho1D4)

Affinity tag applications
While the term "affinity chromatography" implies that the protein of interest is being purified via the affinity tag, there are a number of applications that can be done in addition to purificaton. These include:

Detection: Specific antibodies are available for most affinity tags, so that tagged proteins can be detected in Western Blots, via immunostaining, in ELISA assays or other antibody-based applications.

Immobilization: Affinity tags can be used to immobilize tagged proteins, e.g. on surface plasmon resonance chips, on ELISA plates or other surfaces. The immobilized proteins can then be assessed e.g. for their ligand binding kinetics.

Pulldown: Affinity-tagged proteins can be pulled down from complex solutions e.g. via affinity magnetic beads. They can also be immobilized via affinity beads and used to pull down interaction partners from complex mixtures, such as cell lysates.

Different affinity chromatography methods in the lab

Magnetic Beads: Ligands bound to magnetic beads are a fast and smart way to purify proteins. To perform the purification you need a magnetic bead separator which purifies your protein of interest.

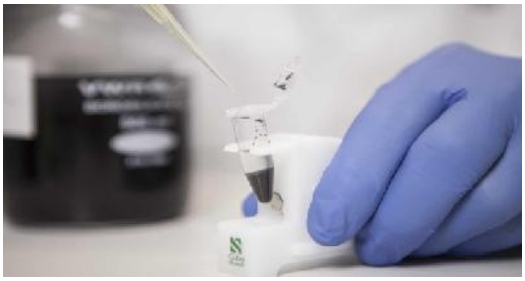

Fig. 6: A tube with magnetic beads.

Put your sample into the tube and mix well. Your protein of interest binds to the magnetic beads. With a magbead separator you can isolate your protein of interest from the rest of the sample.

Fast protein liquid chromatography (FPLC)

FPLC is the standard method for protein purification via affinity chromatography. The advantages of FPLC are that the buffer flow rate is controlled by a positive-displacement pump and the total flow rate of the buffer is kept constant. So FPLC is very suitable for method development and the results are reproducible. The used pressure is with typically less than 5 bar relatively low. The drawback of the method is that you need to buy a very expensive chromatography system.

Fig. 7: FPLC systems need many different solvents for protein purification.

Batch Spin

The batch spin method is done at ambient pressure. The solid phase is packed onto a column and the sample is added to bind the protein. It is a very simple method to get purified proteins in a short time.

Fig.8: This batch spin is done on ice to prevent protein denaturation.

Drip Columns

With drip columns you can work directly at your bench. They are small disposable plastic columns packed with chromatography affinity resin. In contrast to the FPLC method you cannot control pressure and flow rate.

Fig. 9: A drip columns filled with our INDIGO agarose.

The agarose filled in this columns have big diameters to prevent the column from clogging.

Applications of Affinity Chromatography

- Affinity chromatography is one of the most useful methods for the separation and purification of specific products.
- It is essentially a sample purification technique, used primarily for biological molecules such as proteins.

Its major application includes:

- Separation of mixture of compounds.
- Removal of impurities or in purification process.
- In enzyme assays
- Detection of substrates
- Investigation of binding sites of enzymes
- In in vitro antigen-antibody reactions
- Detection of Single Nucleotide polymorphisms and mutations in nucleic acids

Advantages of Affinity Chromatography

- High specificity
- Target molecules can be obtained in a highly pure state
- Single step purification
- The matrix can be reused rapidly.
- The matrix is a solid, can be easily washed and dried
- Give purified product with high yield.
- Affinity chromatography can also be used to remove specific contaminants, such as proteases.
- High sensitivity compared to TCD & FID.
- Affinity chromatography is used in the production of vaccines.
- Affinity chromatography is used in the purification of protein and enzyme.
- Affinity chromatography gives High specificity.
- Enzymes and other proteins are studied by affinity chromatography.
- To maintain the quality of the product, this chromatography is used in the pharmaceutical manufacturer in the production of vaccines.
- Affinity chromatography doesn't rely on ionic strength, pH, temperature, and composition of the buffer.
- The high degree of purity can be obtained by Affinity Chromatography.
- This is a very reproducible process.
- This is the simplification method.
- Used to increase the solubility.
- In genetic engineering, affinity chromatography is use

Disadvantages of Affinity Chromatography

- It takes a lot of skill to handle it.
- It interferes with the structure.
- Transfer and the leakage of metal ion lead to protein loss.
- Sometimes ligands leakage is observed.
- The volume of the sample is limited.
- The carrier gas used must be pure such as pure nitrogen.
- The ligands used in affinity chromatography are costly.

- Relatively low productivity.
- It has a non-specific adsorption.
- Degradation of the solid support.
- Metal-ion transfer and metal ion leakage lead to loss of protein.

2. Conclusion

Over the last one hundred years, affinity chromatography has grown from a method that could be used to isolate only a few enzymes or antibodies to a powerful technique that has a broad range of applications in chemical separations and analysis. This growth has been made possible by the availability of many binding agents, support materials, and immobilization methods that can now be used with this technique. These developments, in turn, have made it possible for affinity chromatography to become an important separation method for both the large- and small-scale purification of bio chemicals. Other applications that have appeared for this method have included its use in the direct or indirect detection of targets and its combination with other methods of chemical analysis. Affinity chromatography has also become a valuable tool for the study of biological interactions and has led to the creation of related affinity methods, such as the use of flow-based sensors to examine biological interactions and affinity capillary electrophoresis. As a result of the many current applications for this method, it is expected that affinity chromatography will continue to grow and develop in the future as a vital tool in areas that span from the production of biopharmaceuticals to clinical analysis, environmental testing, pharmaceutical testing, and biomedical research

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4. Reference

[1] International Union of Pure and Applied Chemistry. chromatography. http://wingate.merck.de/english/services/chromatographie/iupac/chrnom.htm.

[2] Hage DS. Affinity chromatography. Katz E Eksteen R Shoenmakers P Miller N eds. Handbook of HPLC 1998: Marcel Dekker New York. .

[3] Hermanson GT, Mallia AK, Smith Priniciple of affinity chromatography. New York: Academic Press, 1992: 454.

[4] Larsson PO. 1987;components of affinity chromatography.Crossref

[5] Hage DS..the mobile /liquid phase J Chromatogr 1998.Crossref

[6] De Frutos M, batch and colum setups Regnier FE. Tandem. Anal Chem 1993;.CrossrefPubMed

[7] Mayer TK, Freedman ZR.. various affinity mediaClin Chim Acta 1983;CrossrefPubMed

[8] Mallia AK, Hermanson GT, Krohn RI, steps in affinity chromatography Fujimoto EK, Smith PK Anal Lett 1981.Crossref

[9] Hjerten S, Li JP.steps invovel in chromatography. J Chromatogr 1990;.CrossrefPubMed

[10] Fluckiger R, lectins Woodtli T, Berger W. 1984.CrossrefPubMed

[11] Gould BJ, Hall PM, Cook JGH.boronate affinity chromategraphy. Clin Chim Acta 1982;. Crossref PubMed

[12] Klenk DC, Hermanson GT, Krohn RI, Fujimoto EK,weak affinity chromatography Mallia AK, Smith PK, Clin Chem 1982;.CrossrefPubMed

[13] Beck O, Boreus LO, LaFolie P, binding,wash,elution Jacobsson G J Chromatogr 1991;.CrossrefPubMed

[14] Kristensen K, Angelo HR,different affinity chromatography Blemmer T. Enantioselective J Chromatogr A 1994:CrossrefPubMed

[15] Chu Y-Q, Wainer IW. Magnetic beadsJ Chromatogr 1989;CrossrefPubMed

[16] Mangani F, Luck G, Fraudeau C, batch spin Verette E. J Chromatogr A 1997;CrossrefPubMed

[17] Jones DJ, Nguyen KT, McLeish MJ, applications Crankshaw DP, Morgan DJ. J Chromatogr 1996;Crossref

[18] Suzuki A, Takagaki S, Suzuki H, adavantages of affinity chromatography Noda K. J Chromatogr 1993;. CrossrefPubMed

[19] Fieger H, Blaschke. disadvantages of chromatography J Chromatogr 1992. Crossref Pub Med

[20] Silan L, Jadaud P, Whitfield LR, Wainer IW. disadvantages of affinity chromatography J Chromatogr 1990;CrossrefPubMed