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A REVIEW ON IMMUNOAFFINITY CHROMATOGRAPHY

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Abstract: Affinity chromatography is a type of liquid chromatography that makes use of biological-like interactions for the separation and specific analysis of sample components. This review describes the basic principles of immunoaffinity chromatography and examines its use in the testing of clinical samples, with an emphasis on HPLC based methods. Some traditional applications of this approach include the use of baronage, lectin, protein A or protein G, and immune affinity supports for the direct quantification of solutes. Newer techniques that use antibody-based columns for on- or off-line sample extraction are examined in detail, as are methods that use affinity chromatography in combination with other analytical methods, such as reversed-phase liquid chromatography, gas chromatography, and capillary electrophoresis. Indirect analyte detection methods are also described in which immune affinity chromatography is used to perform flow-based immunoassays. Other applications that are reviewed include affinity-based chiral separations and the use of affinity chromatography for the study of drug or hormone interactions with binding proteins. Some areas of possible future developments are then considered, such as tandem affinity methods and the use of synthetic dyes, immobilized metal ions, molecular imprints, or aptamers as affinity ligands for clinical analytes. Immunoaffinity chromatography is the process in which the binding affinity of an antigen to a parent antibody is utilized as a basis of separation. Owing to their avidity and specificity, monoclonal antibodies have become indispensable for both protein characterization and purification. The article describes the basic procedure of immunoaffinity chromatography. The support matrix upon which the antibody is immobilized and the activation chemistry used to couple the antibody to the matrix affect the immunosorbent performance. Support matrices available for Immunoaffinity chromatography and their activation chemistries including the recent advances have been reviewed. The paper also discusses the developments in the applications of this technique in analysis as well as extraction.

Keywords: Affinity chromatography, baronage, lectin, protein A, synthetic dyes, monoclonal antibodies



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INTRODUCTION

Antibodies or immunoglobulin's represent glycol proteins having carbohydrate-recognition motifs and they play an important role in biochemistry, biotechnology and bio therapeutics. Antibody based bio therapeutics and in-vivo diagnostics are gaining wider approval from many health authorities all over the world and they represent the largest number of molecules in clinical trials today. The worldwide consumption of human IgG nearly tripled between 1992 and 2003 from 19.4 to 52.6 tons. Among the most popular of affinity derived technologies is IAC on antibody columns to purify antigens. One of the reasons for the rapid growth of IAC is the rapid advancement in the field of molecular biology and biotechnology. One of the first uses of IAC was reported in 1951 by Campbell et al. who used immobilized bovine serum albumin on *p*-amino benzyl cellulose to purify anti albumin antibodies. Since then, there has been a great expansion in the applications of IAC for analytical, clinical and diagnostic purposes. Immuno affinity chromatography is one of the most powerful techniques for single-step isolation and purification of individual compounds or classes of compounds from liquid matrices. Is based on the highly selective interaction between Abs and their Ags. Because of the high affinity and high selectivity of the Ag-Ab interaction, the method provides a high degree of molecular selectivity. IAC is operated as a column chromatographic process. Key

consideration in any chromatographic process is the resolution in less time for analytical purpose and resolution along with concentration of the analyte form preparatory purpose. Since only protein molecules containing the epitopes recognized by the immobilized antibody will be retained on the column, IAC has a very high resolving power.

The principle of immuno affinity or immuno adsorption chromatography is based on the highly specific interaction of an antigen with its antibody.

IAC involves three steps

- a) preparation of the Ab matrix, followed by packing the sorbent as a column where the adsorption/desorption chromatography will be carried out;
- b) Binding the Ag to the Ab matrix; and
- c) Elution of the Ag.

In the first step, the antibody specific to the protein of interest is immobilized onto a rigid solid support to yield an active immunosorbent.

A complex mixture of proteins is then passed over the immuno sorbent that favour adsorption whereby the antibody captures the protein of interest and unwanted proteins are removed from the column by washing.

In the last step, Ab-Ag interaction is dissociated and the Ag is released into the

eluate that favors desorption. IAC can be performed off-line or on-line, and it can be coupled to a wide variety of separation techniques and analytical methods such as

liquid chromatography (LC), gas chromatography (GC), and Immuno Chemical analysis (eg. ELISA).

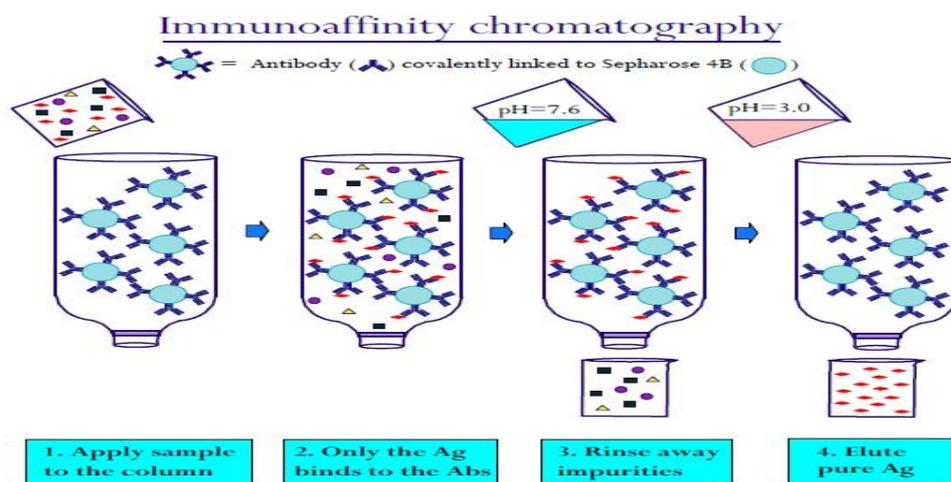


Fig.1. Immunoaffinity chromatography

INSTRUMENTATION

The chromatographic process involves a stationary phase (also called matrix or support) and a mobile phase. The stationary phase retains the analyte depending on its affinity for the analyte thus concentrating the analyte which is later eluted to give pure product. The mobile phase serves as a carrier for the analyte. In IAC the mobile phase is an aqueous buffer with different compositions so as to facilitate binding of analyte and its elution.

Stationary Phase

The stationary phase in IAC consists of antibody or antibody fragments immobilized on matrix or support. For the immobilization of the antibodies or its fragments the supports are activated so that they couple with the antibodies. Choice of the support, the chemistry of their activation as well as the type of the antibodies or antibody fragments used is important for the selectivity and efficiency of IAC.

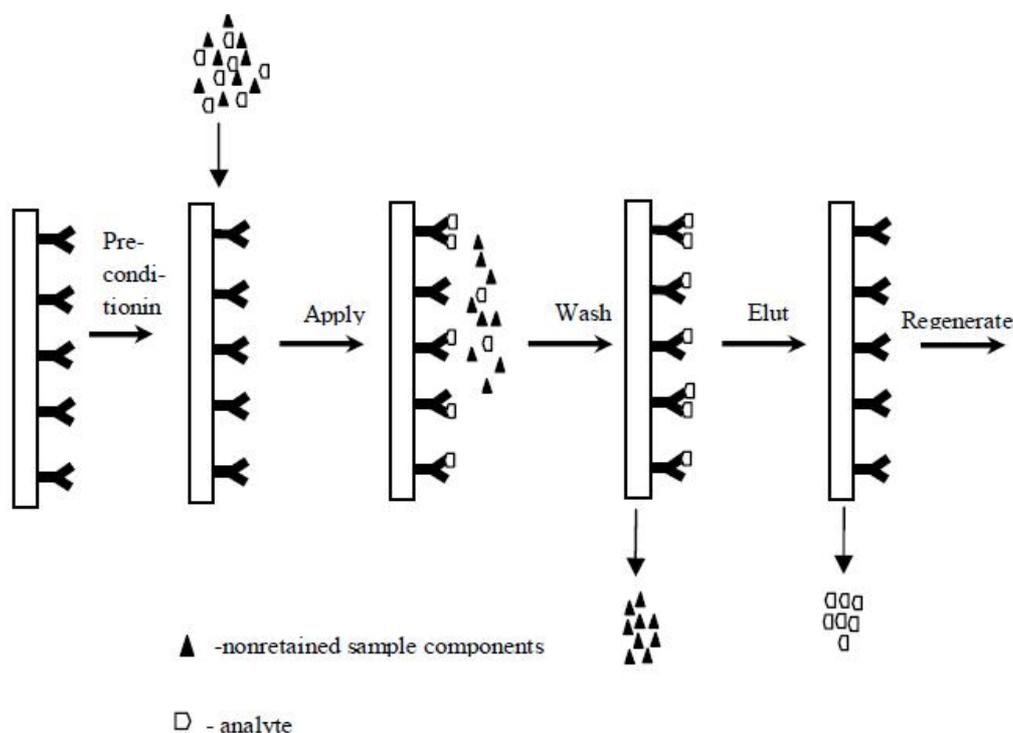


Fig.2: General Scheme of IAC

Supports and Support Activation

In IAC the antibodies immobilized on supports are used for separation process. The support material used is an important consideration in the development of successful IAC method.

Ideal characteristics Supports

- Uniform size-** Narrow size distribution is essential to prevent the blockage of column.
- The immune affinity matrix onto which the antibody ligand will be attached should be inexpensive, readily available, easy to use, and highly stable: the support material and the attached ligand should not react

with the solvents used in the purification process.

- Supports with pore sizes of 300-500Å, which is approximately three to five times the diameter of an antibody, allow for maximum antibody coverage, as well as for suitable binding of immobilized antibodies to many small or medium sized targets (100-150 kDa).
- Stable-** The analyte sample for the purification or analysis may have very high or very low pH. The matrix should withstand these extremes of pH. Also the matrix should be stable to allow the use of denaturing reagents in the elution stage.
- Hydrophilic-** The support should be hydrophilic because hydrophobicity induces

nonspecific binding hence unwanted molecules are retained in the column.

f) **Show low nonspecific absorption-** The aim of IAC is selective elution of the analyte of interest. Hence the matrix should have the least nonspecific absorbance as possible so that only the analyte is retained and concentrated in the IAC column.

g) **Readily derivatized-** In IAC the antibodies are immobilized on the matrices. Hence matrices should possess functional groups that can be easily activated so as to react with and immobilize antibodies.

h) **Rigid-** IAC can be operated using HPLC (High Performance Liquid Chromatography). As this technique is executed under high pressures, the matrix should be rigid and resist compression at high flow-rates in columns.

Classification of supports or matrices

Depending on their efficiency, the supports or matrices are classified into 3 types. They are

1. Low performance supports
2. High efficiency supports
3. Affinity membranes

Low performance supports

They are generally beads of carbohydrate-related materials or synthetic organic supports.

The low back pressure of these supports facilitates the operation under gravity flow

with a slight vacuum or peristaltic flow applied. These matrices include carbohydrate-based media (agarose, dextrose, or cellulose), synthetic organic supports such as acrylamide polymers, polymethacrylate derivatives, polyether sulfone matrices, or inorganic materials such as silica and zirconia. The use of these gels for IAC is relatively simple and inexpensive. The main disadvantage of these materials is their slow mass transfer properties and their limited stability at high flow rates and pressures.

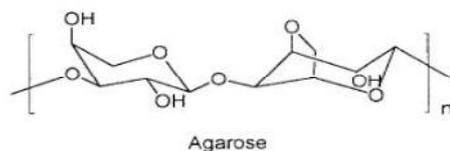
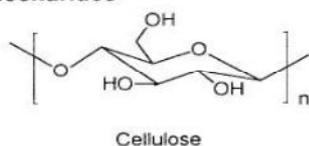
High efficiency supports

These supports facilitate use of IAC as HPLC method. The mechanical stability and efficiency of these materials facilitates use with standard HPLC equipment which improves speed and precision for analytical applications of IAC. These supports include derivatized silica, glass and organic matrices such as azalactone beads or polystyrene based perfusion media. The use of these supports along with an antibody or related ligand is referred to as high performance immunoaffinity chromatography (HPIAC).

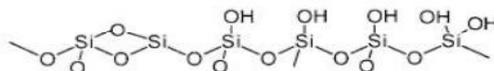
Affinity Membranes

Affinity membranes, owing to their high mechanical strength, do not suffer the problems of compaction as encountered with beaded matrices. These devices are used in the form of stacks of membrane filters or hollow-fiber ultra filtration cartridges^{15, 16} in IAC. Antibodies are immobilized on the support.

A. Polysaccharides



B. Silica



C. Synthetic polymers

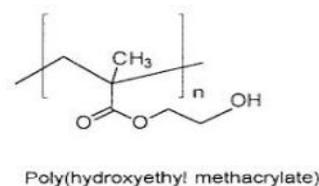
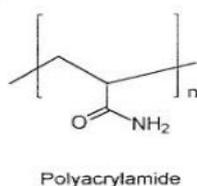


Fig.3. Chemical structures of some commonly used supports employed to immobilize antibodies

Antibody

Antibodies (or immunoglobulin's) are proteins produced by an animal in response to the presence of an exogenous substance called antigen. Each antibody possesses specificity for its antigen. IAC utilizes antibodies immobilized to stationary support. The high specificity and avidity of antibodies is used for better separation. These antibodies act as ligands where specific analytes from complex mixture bind to it with high avidity to resolve the mixture. Antibodies or immunoglobulin's

consist of pairs of disulfide-linked heavy (H) - and light (L)-chain dimers. The heavy and light chains contain variable (V) and constant (C) domains. The variable domains of both H and L chains form the binding sites for interaction with the antigen. (Huse) This technique mainly employed to either polyclonal antibodies or Monoclonal antibodies. But Monoclonal antibodies are preferred because the polyclonal antibodies are never specific for a single antigen but reflect the variety of immunological challenges.

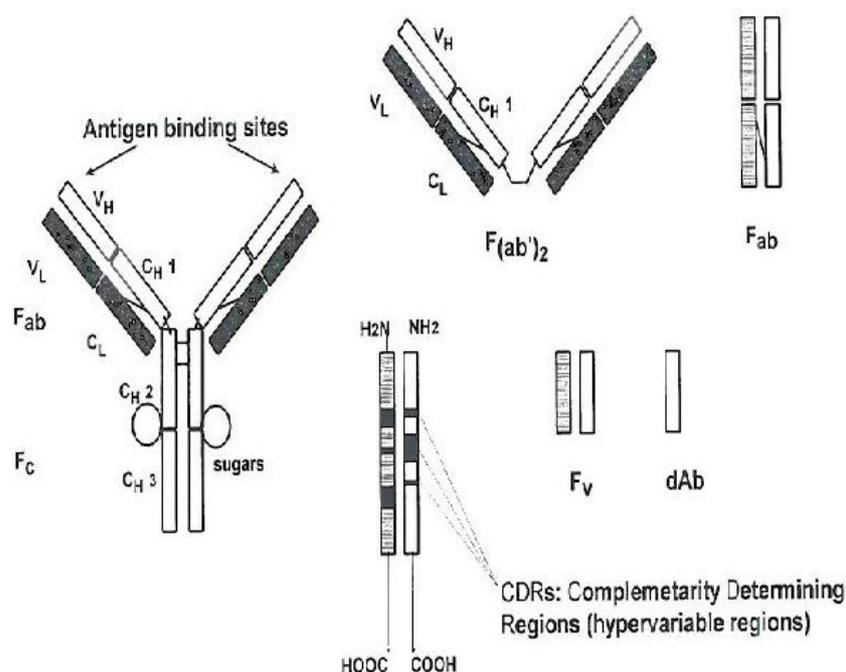


Fig.4: Structure of immunoglobulin G and its important fragments used in IAC

Immunoaffinity chromatography relies on the exquisite binding between an antibody and an antigen, the result of four different types of non-covalent (and therefore reversible) interactions: ionic interactions, hydrogen bonds, van der Waals interactions, and hydrophobic interactions. Our ability to manipulate antibodies and antibody-antigen interactions offers great potential for the use of IAC in research as well as for therapeutic and diagnostic applications. Moreover, the advent of recombinant antibody production has paved the way for even more advances in manipulating antibodies to our advantage.

Choice of Antibody

The primary isolation of specific antibodies is necessary for the subsequent purification of specific antigens. Antibodies used as ligands can be purified by precipitation with dextran or ammonium sulfate, or by isolation on a Protein A, Protein G, or Protein L column.

Ideal antibody for use in immunoaffinity chromatography should possess two properties

(a) High intrinsic affinity, since an antibody attached to a solid phase has no room for cooperative binding. This is especially important when using a diluted antigen source, where quantitative antigen capture is hard to achieve. Quantitative binding of

antigen to the immunoabsorbent along with a low background (non-specific interactions) are insured when using an antibody with an affinity $\geq 10^8$ and two hours of antigen-antibody contact. When the antibody affinity is $\leq 10^6$, some antigen will be left in solution, and exposure to the antibody column will have to be repeated.

(b) Ease of elution: This depends on the type and number of antigen-antibody bonds: the fewer types of interactions involved, the easier all of them can be destabilized. Polyclonal antibodies were once used for IAC but due to high non-specific absorption and with the production of MAbs their use declined. In IAC purification, the choice of MAb used is of utmost importance for determining the success or failure of the system. For a MAb to be used as an IAC ligand, MAb must retain its biological activity during the coupling reaction and then under the conditions necessary to achieve elution of the antigen.

Antibody Immobilization

The key factor in antibody immobilization onto the affinity matrix is to tightly bind the antibody to the support medium without interfering with the activity and accessibility of the antigen binding site. There are many different methods for antibody immobilization to both low- and high performance supports. Immobilization of antibodies is most usually achieved by coupling through the free amino groups of lysine residues, hydroxyl, carboxyl or

sulfhydryl groups on the **There are 3 types of approaches,**

1. Covalent Immobilization:

- a) Non-site-selective
- b) Site selective

2. Non covalent immobilization

3. Other approaches

1. Covalent immobilization: In this technique the antibodies are immobilized by formation of covalent bonds with the activated supports.

The two types of covalent immobilization are

a. Non-site-selective:- It is done by reacting the free amine groups on antibodies with activated supports (supports are activated using agents such as N, N'-carbonyl diimidazole, cyanogens bromide, N hydroxysuccinimide and tresyl chloride/tosyl chloride or with supports that have been treated to produce reactive epoxide or aldehyde groups on their surface). Though the use of antibody amine groups serves the easiest route for immobilization it can give rise to less than optimum activity because of the random orientation or denaturation of the immobilized antibody.

b. Site-selective:- Antibodies, or antibody fragments can also be covalently immobilized through site-selective methods. For example –

i. Free sulfhydryl (-SH) groups generated during the production of antibody Fab fragments can be used to couple these fragments to supports using appropriate activation chemistry.

For example: divinylsulfone, epoxy, maleimide, iodoacetyl/bromoacetyl TNB-thiol, or tresyl chloride/tosyl chloride can be used to activate the sulfhydryl group.

ii. Carbohydrate residues of antibodies can be used to couple them after mild oxidation of these residues using periodate or enzymatic systems to produce the aldehyde residues. These aldehyde groups can be reacted with a hydrazide or amine-containing support for antibody immobilization. This results in IAC columns that have higher relative binding activities than comparable columns made by amine-coupling methods.

rface of the protein.

2. Noncovalent immobilization:-

Noncovalent immobilization involves the adsorption of antibody to the support.

Site selective approaches in non-covalent immobilization are

a. Oxidizing the carbohydrate residues of the antibodies to produce the aldehyde group which after reaction with the biotin-hydrazide can bind the antibodies non covalently to an immobilized streptavidin support.

b. Adsorbing the antibody to secondary ligands such as protein A or protein G which bind strongly as well as selectively to the stem region of antibodies leaving the antigen binding site free to interact, under physiological conditions. This binding can be disrupted by decreasing the pH of the surrounding solution.

3. Other approaches:- An alternative coupling method involves first generating free sulfhydryl groups in either intact or fragmented MAb. The MAb or F(ab')₂ fragments may be reduced with β-mercaptoethylamine to produce free -SH groups at the hinge region of the MAb. The fragments may then be immobilized to a matrix containing iodoacetyl groups to give a stable thioether link or to maleimide groups. The effect of site directed immobilization on improving the performance of IAC support does not provide any evidence to arrive at any conclusion.

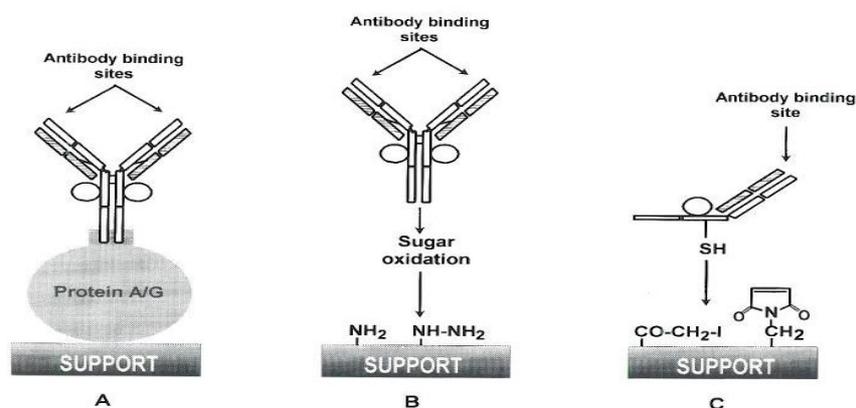


Fig.5: Antibody oriented immobilization methods A. protein A, B. oxidation of sugar residues, C. thiol groups generated from antibody fragments

CHROMATOGRAPHIC SEPARATION

The separation of the analyte using IAC involves three steps, viz. binding, washing and elution

Binding

Binding is an essential step that has a profound influence on the purity of the final product of an IAC process. antibody is bound to a solid phase, adsorption conditions should maximize antigen-antibody interaction. The efficiency of binding is related to the strength and the kinetics of this interaction, which in turn depend on the amount of immobilized antibody, the concentration of applied target, and the flow rate used for binding. Binding can be performed in column or batch format (where the sample extract serves to keep the gel beads in suspension). The former allows for adjustment of flow rates, and therefore for extending the time of antigen-antibody interaction. Generally, a higher flow rate will reduce the binding

efficiency, especially when the antibody-target interaction is weak, and/or the mass-transfer rate in the column is slow. In batch purification, the resin and sample are constantly mixed, thus promoting a maximum contact between the target and immobilized antibody. It often saves time, especially when dealing with large sample volumes, but requires optimization of the amount of resin used. Because excess resin can result in an increase in nonspecific binding, as well as reduced target recovery due to re adsorption during the elution step, it is preferable to saturate the resin with bound target. Binding is performed at or slightly above the neutral pH in a phosphate or borate buffer containing NaCl and a nonionic detergent. This facilitates only the binding of analyte of interest and the non-specific absorption on the stationary phase is minimized. Presence of salt in buffer is essential to reduce the ionic interactions of the charged surface groups of immobilized MAb. Detergents reduce

hydrophobic interactions. Use of detergents for potential therapeutic products is irrational owing to the inherent difficulties in removing detergents from proteins. Inclusion of polyethylene glycol (PEG) in the binding buffer helps reduce nonspecific binding.

Pre-clearing step

To remove proteins that bind nonspecifically to the affinity matrix, the extract can be pre incubated with the support matrix, or pre-cleared by incubation with an irrelevant antibody of the same species or with normal serum. This pre-clearing step will result in a lower background and an improved signal-to-noise ratio.

Washing (removal of extraneous matter)

A head of purification, particulate matter and contaminants must be removed from the extract by centrifugation and/or filtration, in order to avoid clogging of the chromatographic column. It may also be necessary to perform a desalting and buffer exchange step in order to transfer the sample to the correct buffer conditions (pH and salt concentration), and to remove unwanted small molecules. If the sample is reasonably clean after centrifugation, this last step can be omitted and replaced with a mere adjustment of the sample pH and ionic strength to that of the application buffer. Finally, if the sample extract represents a diluted protein solution, concentration of the sample before

purification may be necessary to enhance the probability of quantitative recovery of the target molecule. Contaminants with weak affinity to the ligand or to the support matrix itself can be removed by application of low amounts of competitive reagents. It is crucial to determine the appropriate flow rate and volume (e.g., 5–10 column bed volumes) of the wash buffer that will maximally remove contaminants while minimizing loss of target.

Elution

Elution of the antigen, often viewed as the most delicate step of an IAC protocol, should ideally be carried out in a way that keeps the antibody on the immune adsorbent intact and maintains antigen activity (e.g. enzymatic or hormonal activity), if present, while still allowing later regeneration of the column. This is especially important if the column is to be used for a large number of samples. The sample can always be desorbed from the antibody because the four forces that stabilize the antigen-antibody complex (ionic, hydrogen bonding, van der Waals interactions, and hydrophobic bonds) are all reversible. Thus, the antigen-antibody complex can be destabilized by counteracting the forces at work in a particular antibody-epitope interaction. Desorption is thus essentially the reverse process of binding, where conditions are optimized to weaken the antibody-target interaction. The objective of the elution step is to recover the specifically bound

protein at a high yield, purity, and stability. Elution conditions, which might denature the protein product, have to be avoided. Examination of the literature suggests a wide variety of elution conditions and the choice of an eluant seems empirical. However, a logical sequence of available elution strategies can be considered when selecting an appropriate elution protocol. The various elution strategies include –

a. Specific elution: Certain antibodies bind to their respective antigens under high pH or in the presence of metals like calcium or magnesium or in the presence of chelating agent like EDTA. Antigens bound to such antibodies can be eluted under gentle conditions where lowering the pH or adding EDTA to the elution buffer or adding divalent metals to the elution buffer causes the Ag-Ab complex to dissociate.

b. Use of extreme pH:

Acid elution:

This is the most widely used method of desorption and is normally very effective. The commonly used acid eluants are glycine-HCl, pH 2.5; 0.02 M HCl and sodium citrate, pH 2.5. Upon elution, the pH of the eluant sample is quickly neutralized to 7.0 with 2 M Tris base, pH 8.5, to avoid acid-induced denaturation. In some cases increased hydrophobic interactions between antigen and antibody gives low recovery with acid elution. Incorporation of 1 M propionic acid, or adding 10% dioxane or addition of ethylene glycol to the acid

eluant, is effective in dissociating such complexes.

Base elution:

It is less frequently employed than acid elution. Typically, 1 M NH₄OH, or 0.05 M diethylamine, pH 11.5 have been employed to elute membrane proteins (i.e., hydrophobic character) and other antigens that precipitate in acid but are stable in basic conditions.

Use of Chaotropic agents:

These agents disrupt the tertiary structure of proteins and, therefore, can be used to disrupt the Ag:Ab complexes. Chaotropic salts are particularly useful as they disrupt ionic interactions, hydrogen bonding, and sometimes hydrophobic interactions. The relative order of the effectiveness of chaotropic anions is SCN⁻ > ClO₄⁻ > I⁻ > Br⁻ > Cl⁻. Chaotropic cations are effective in the order of Mg > K > Na. Eluants such as 8 M urea, 6 M guanidine-HCl, and 4 M NaSCN are effective in disrupting most Ag-Ab interactions. To avoid and minimize chaotropic salt-induced protein denaturation, rapid desalting or dialysis of the eluant is essential.

Use of denaturants:

The denaturants most frequently used in IAC are 6-8M urea and 3- 4M guanidine hydrochloride. These reagents were more commonly used in conjunction with polyclonal antibodies with a very high

affinity for the antigen and in cases where the antigen is stable to the treatment.

Use of organic solvents:

Organic solvents have found favor as eluants in some circumstances but with the possible exception of ethylene glycol are infrequently employed in IAC.

Changes in ionic strength:

Raising the ionic strength of the solvent can be a mild method of elution but is rarely effective when using NaCl. Of more use are the salts of divalent cations such as Mg²⁺ and Ca²⁺. The ionic strength of a 4.5M MgCl₂ solution is 13.5M and the chloride concentration is 9M. This may in some way explain the effectiveness of such solutions as eluants in terms of very high concentrations of a weakly chaotropic ion in combination with an ability to disrupt ionic interactions. In addition Ca²⁺ and Mg²⁺ are more chaotropic than either Na⁺ or K⁺.

Advantages

This method is more commonly performed for the identification, quantification, or purification of antigens. IAC serves a promising approach for selective analysis of very dilute analyte samples and also bioanalysis of drugs. IAC is commonly used for the selective purification of target compounds (proteins, glycoproteins, carbohydrates, lipids, bacteria, viral particles, drugs) from complex samples. IAC methods are extensively used for sample clean-up prior to analysis of foods for

mycotoxins, veterinary drug residues, pesticides, and environmental contaminants.

Disadvantages

This approach suffers from some disadvantages such as loss of Ab activity upon coupling (because of poor control over protein orientation and conformation), low surface loading, potentially low mechanical stability (that prevents the on-line coupling of IAC columns with separation methods), difficulties in the loading of beads into narrow columns, difficulties in miniaturizing to very narrow columns, poor flexibility with certain proteins, long preparation time, low regenerability, and-most importantly- high cost. However, one serious disadvantage with monoclonal antibodies is their high cost. Compared to other immunochemical methods such as ELISA (Enzyme linked immune sorbent assay), the amount of antibody necessary to prepare an IS much higher, increasing thus the cost of the technique.

APPLICATIONS

Immuno affinity chromatography is a versatile, powerful purification method based on well characterized antibody-target interactions, making it amenable for use in many applications, including sample cleanup, and clinical and diagnostic assays for drugs, toxins, and biomarkers. IAC has been widely applied for over four decades for pharmaceutical and biomedical trace

analysis and, in the more recent decades, for analysis of environmental contaminants and pesticide residues in occupational and environmental health monitoring, in forensic examination and in food safety analysis. The varied and complex matrices that serve as sources for analyte monitoring. To date, IAC has been successfully utilized for monitoring pesticides and other trace organics in environmental and food samples as well as for detecting drug metabolites and endogenous compounds in biological fluids in occupational exposure and clinical trials. Interestingly, the applications where the high potential of IAC for class-selective extractions, has been clearly shown, belong to the environmental field of analysis. Antibodies isolated using these techniques have proven highly efficient in applications ranging from clinical diagnostics to environmental monitoring. Immunoaffinity chromatography has many uses, including isolation of specific proteins to determine their physical properties and capture protein complexes. A popular use of immunoaffinity chromatography is in the identification of novel proteins that interact and complex with a known protein. During the chromatography procedure associated proteins are isolated with its interacting antigen and these can be identified by electrophoresis and other downstream applications, such as mass spectroscopy. IAC offers a great potential for the selective purification of a wide variety of environmental contaminants, especially of those polar compounds which are very

difficulty to isolate by other commonly used supports. However besides its demonstrated versatility and specificity, IAC is still seldom used in routine environmental analyses. IAC, due to its high specificity is widely used for protein purification. Its application has also been extended for analytical purposes. These applications of IAC are discussed below. Owing to its high selectivity IAC has also been used for analysis. The analyzed substances include various proteins, hormones, toxins, drugs and even the antibodies employing the various IAC techniques from a range of matrices. The various IAC techniques are

- a) IAC with direct detection
- b) Immunoextraction
- c) Chromatographic immunoassays

a) IAC with direct detection: This is the simplest format for IAC in analytical applications. It involves the adsorption of the test solutes in an immobilized antibody column, followed by elution and detection of the analytes.

b) Immunoextraction

Immunoextraction involves the use of IAC for the removal of a specific solute or group of solutes from a sample prior to its determination by a second analytical method. Operating scheme is same as that of IAC, but involves combining the immunoaffinity column either off-line or on-line with some other method for quantitation of analytes.

c) Chromatographic immunoassays

The use of immobilized antibody (or immobilized antigen) columns to perform various types of immunoassays is known as chromatographic immunoassay. The use of IAC to perform immunoassays is particularly

valuable in determining trace analytes that, by themselves, may not produce a readily detectable signal. In chromatographic immunoassays the use of labeled antibody or labeled analyte analog for indirect analyte detection overcomes this problem.

Table No1: List of Proteins Purified by IAC

Purified constituent	Sorbent	Eluant
Human parainfluenza virus type 3 glycoproteins ⁴⁶	Affi-Gel HZ Mab	0.1M glycine pH 2.5
Human insulin receptor ⁴⁷	CNBr-Sepharose MAb	HEPES + NaCl + TRITON X-100 + 15-mer peptide
<i>E. coli</i> RNA Polymerase ⁴⁸	CNBr-Sepharose Mab	50mM Tris + 0.1M EDTA + 0.7M NaCl + 30% ethylene glycol
<i>Shewanella oneidensis</i> RNA Polymerase ⁴⁹	CNBr-Sepharose Mab	50 mM Tris-HCl, pH 7.9 + 0.1 mM EDTA + 40% propylene glycol + 0.75 M ammonium sulfate
Flavocytochrome b (Cyt b) ⁵⁰	Protein A-Sepharose beads	0.25% DDM (Dodecylmaltoside) containing 1 mM elution peptide AC-PQVRPI-CONH2 (AC-, acetylation at the N-terminus of the elution peptide; -CONH2, amidation at the C-terminus of the elution peptide)
DNase II ⁵¹	Formyl-cellulofine resin conjugated with a murine monoclonal anti-human DNase II antibody	PBS + 50% v/v ethylene glycol

RECENT ADVANCES IN IMMUNO AFFINITY CHROMATOGRAPHY

Immuno affinity chromatography is one of the most powerful fractionation steps available for protein purification¹³. In recent years, IAC has been integrated with other analytical methods such as CE and MS. CE immunoassays (where antibodies are immobilized in CE capillaries) are utilized

because they are easily automated, require small amounts of sample and reagents and still maintain a good detection limit, and offer relatively fast separation.

CONCLUSION

Purification techniques for antibodies have a long history of highly qualified attempts to obtain them in an active and high purity. So there are various methods of enriching or

purifying a protein of interest from a complex mixture of other proteins and components. In this review, We have discussed the most powerful purification and versatile method, that is immuno affinity chromatography, this method mainly based on well characterized antibody- target interactions, making it amenable for use in many applications, including sample cleanup, and clinical and diagnostic assays for drugs, toxins, and biomarkers. The efficiency of the IAC process is dependent on the efficiency of the coupling of antibodies to support and the activation of the coupling as they affect the avidity of the antibodies. IAC serves a promising approach for selective analysis of very dilute analyte samples and also bioanalysis of drugs.

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