Review Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

https://doi.org/10.36673/AJRCPS.2020.v08.i01.A05

IMMUNOAFFINITY CHROMATOGRAPHY: A REVIEW

E. Swaroopa*[1] and E. Pushpa Latha[1]

[1*]Department of Pharmaceutical Analysis, CMR College of Pharmacy, Kandlakoya, Medchal- 501401, India.

ABSTRACT

Immunoaffinity chromatography also called immunoadsorption chromatography is a type of affinity chromatography used for the separation of antibodies and identification, purification and quantification of antigens. It is based on the specific interaction of antigen to its antibody. This review discusses the basic components of immunoaffinity chromatography which includes structure, properties, production of antibodies, chromatographic conditions, immobilisation and elution techniques, applications and recent developments of IAC.

KEYWORDS

Immunoaffinity chromatography, Antibodies and Identification.

Author for Correspondence:

Swaroopa E,

Department of Pharmaceutical Analysis,

CMR College of Pharmacy,

Kandlakoya, Medchal, India.

Email: latha.pushpa999@gmail.com

INTRODUCTON

Affinity chromatography is a high resolution, high capacity and one of the most powerful and diverse methods for separating proteins and other biological molecules of interest on the basis of a highly specific, reversible biological interaction between two molecules: an affinity ligand attached to a solid matrix to create a stationary phase and a target molecule in a mobile phase. Specifically, immunoaffinity chromatography (IAC) relies on a solid stationary phase consisting of an antibody coupled to a chromatographic matrix and the selective and strong binding of antibodies to their targets. Accordingly, any molecule that can be bound effectively by an antibody can be purified

using IAC. Purified antibodies are coupled to the inert solid phase and mixed with the antigen solution under conditions that favor adsorption. Following antigen capture, unwanted antigens are removed by washing, and the purified antigen is released by switching to conditions that favor desorption. Purification (often greater than 1000-fold) and simultaneous concentration of the target protein are thus achieved. One of the first uses of IAC was reported in 1951 by Campbell et al. who used immobilized bovine serum albumin on p-aminobenzyl cellulose to purify antialbumin antibodies. Since then, there has been a great expansion in the applications of IAC for analytical, clinical, and diagnostic purposes[1].

Basic components of IAC

Structure and properties of antibodies

The basis for IAC relies on the selective binding of antibodies. This binding is a result a large variety of noncovalent interactions that can occur between an antibody and an antigen and can result in association equilibrium constants in the range of 10^{5}–10^{12} M^{-1}. It has been estimated that the human body is able to produce between 10^{7} and 10^{8} different types of antibodies, with each capable of binding to a separate antigen. The typical structure of, an antibody, using IgG as an example, consists of four polypeptide chains. These four chains consist of two identical heavy chains and two identical light chains that are linked by disulfide bonds to from a Y-shaped structure .The lower stem region of an antibody is referred to as the F_c region and is highly conserved from one antibody class to the next. The upper arms of the antibody are called the F_{ab} regions. The amino acid sequences in the F_{ab} regions are identical within a single type of antibody but are highly variable between different antibodies. It is this variability that allows antibodies to bind a wide range of antigens. A foreign agent that is capable of initiating antibody production is called an antigen. Common antigens include viruses, bacteria and foreign proteins from animals and plants that are capable of producing an immune response. Due to the large size of naturally occurring antigens, antibodies that bind to several different regions of the antigen with a range of binding affinities are often generated. Each individual location on an antigen that can bind to a given antibody is called an epitope. In order for a substance to be recognized by the body's immune system and to lead to the production of antibodies, this substance must have a size that corresponds to a mass of several thousand Daltons Antibodies can also be produced against smaller substances, but these substances first must be coupled to a larger species (e.g., a carrier protein before antibody production can occur. A small substance that is used to produce antibodies after being linked to a carrier agent is known as a hapten[2].

IgG - class antibodies consist of four polypeptide chains that are linked by disulphide bonds

The two main types of antibodies that are used in IAC are polyclonal antibodies and monoclonal antibodies. Polyclonal antibodies are produced from multiple cell lines within the body and as a population can bind a variety of epitopes on a single antigen with a range of binding strengths. Monoclonal antibodies are produced by the fusion of a myeloma cell line with spleen cells obtained from an animal that has been immunized with the desired antigen. Because monoclonal antibodies are generated from a single cell line, they bind to a single epitope with identical binding affinities. Two other types of antibodies that can be used in IAC are autoantibodies, which are polyclonal antibodies obtained from patients with auto immune diseases, and anti-idiotypic antibodies, which are antibodies that can mimic the interactions of antigens, hormones or substrates for cell receptors[3].

Pr0duction of Antibody

Polyclonal antibodies can be produced against a given target by injecting the antigen or hapten–carrier a conjugate into a laboratory animal (e.g., a mouse, rabbit, goat or sheep). Often this solution of the antigen or hapten–carrier conjugate contains an enhancing agent called an adjuvant. After this initial injection, blood samples from the animal are collected after approximately a month (e.g., 3–4

weeks, although the exact times used in this sequence may vary) and tested for the presence of antibodies that are specific for the desired target. Another injection of the antigen or hapten conjugate (called a 'booster') is then made into the animal. The animal's blood is then retested later (e.g., after 10 days) for the presence of antibodies. Based on the antibody levels that are detected, the animal can be allowed to rest for a period of time (e.g., a few weeks) before being administered another booster injection. This booster/bleed routine can be repeated several times until the antibody concentrations for the required antigen reach the desired level (i.e., as determined by an assay of the blood). At this point, antibody-containing serum can be collected from the animal and stored for later use[2].

Process for Production of Monoclonal Antibodies

Monoclonal antibodies can be produced by isolating a single antibody-producing cell and combining this cell with a carcinoma or myeloma cell, the resulting hybrid cell line, called a hybridoma, is relatively easy to culture and grow for long-term antibody production[1.] This approach for monoclonal antibody production was first reported by Kohler and Milstein in 1975. In this method, a solution of the antigen or the hapten–carrier conjugate is mixed with adjuvant and injected subcutaneously into an animal. The animal is later given a booster shot, killed and the spleen harvested. The lymphocyte cells are mixed with myeloma cells in the presence of polyethylene glycol, which is added to promote cell fusion. The cells are then grown in the presence of drugs that kill myeloma cells and unfused lymphocytes, but permit the growth of hybridoma cells. Individual cultures of hybridomas are examined for the production of specific antibodies and those that make the desired antibody are cloned to produce a homogenous culture of cells making a monoclonal antibody[3].

CHROMATOGRAPHIC CONDITIONS

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[4].

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. The ideal support should be.

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.

Uniform size

Narrow size distribution is essential to prevent the blockage of column.

Nontoxic

The matrix should be safe to handle as well as nontoxic.

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. These may elute in the elution stage along with the analyte and affect the detection of analyte or purity of extracted product.

Macro porous

The matrix used should have pores large enough to allow diffusion of large protein molecules (or antigen) and provide high capacity support. Also the high surface area to volume ratio ensures better separation.

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. **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[4].

There are several types of supports that can be utilized to place antibodies within columns for use in IAC. Traditional immunoaffinity supports have been based on low-performance materials such as carbohydrate-related media (e.g., agarose and cellulose) or synthetic organic supports (e.g., acrylamide polymers, copolymers or derivatives, polymethacrylate derivatives and polyether sulfone matrixes. The low cost of these materials has made these supports popular for IAC applications involving target purification or offline immunoextraction. However, most of these materials can be used at only relatively low back pressures and are best suited for work under gravity flow or with a peristaltic pump. These supports also can have slow mass-transfer properties

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.

IMM0BILIZATION TECHINQUE

Antibodies can be immobilized onto supports by using a variety of techniques that range from covalent attachment to adsorption-based methods. Of these techniques, those that make use of covalent attachment are the most common, but even these methods can range from the use of random attachment via amino or carboxyl groups to more site-selective immobilization approaches that make use of modified carbohydrate residues or thiol groups. The ideal situation in any of these immobilization methods is to have antibodies attached to the support in a way that does not affect the activity of the binding sites or the accessibility of these sites to their target compound[5].

Antibodies can be immobilized through free amine groups by using supports that have been activated with agents such as *N,N′*-carbonyldiimidazole, cyanogen bromide, *N*-hydroxy succinimide . Antibodies can also be immobilized through amine groups using a support that has been treated to produce reactive epoxy or aldehyde groups on its surface. The use of amine groups is one of the easiest ways to immobilize antibodies but can cause a decrease in activity if the antibodies have some of these amine groups in their antigen-binding sites[3].

Antibodies or Fab fragments can be covalently linked to IAC supports through more site selective methods. This can be achieved by utilizing the free sulfhydryl groups that are created when Fab fragments are generated. These groups can be used for immobilization by using techniques such as the divinylsulfone, epoxy, iodoacetyl/bromoacetyl, maleimide or tresylchloride methods. In addition, site-selective immobilization can be accomplished by coupling antibodies through the carbohydrate residues that are located in their Fc regions. This process is carried out by first oxidizing the carbohydrate residues under mild conditions to generate aldehyde residues. These aldehyde groups are then reacted with a hydrazideor amine-containing support[4].

APPLICATION AND ELUTION CONDITIONS

The application and elution conditions are another important set of factors to consider in the design and use of an IAC method. The application buffer used in IAC is generally chosen for its ability to promote fast and efficient binding of the desired analyte or target compound to the immobilized antibodies. Optimum binding for antibodies typically occurs under physiological conditions, so IAC generally makes use of a neutral pH buffer

(i.e., pH 7.0–7.4). Under these conditions, the equilibrium constants for antibody binding is usually in the range of 10^6–10^{12} M^{-1}. Due to this strong binding between the antibody and its target, isocratic elution is often not feasible unless the IAC method is using low-affinity antibodies (i.e., those with association equilibrium constants of less than 10^6 M^{-1}).

The elution conditions for IAC need to allow for fast elution of the analyte while still allowing later regeneration of the immobilized antibodies. The need for fast but reversible binding and regeneration is especially important when an IAC column is to be used for a large number of samples. Elution is often carried out by temporarily lowering the effective strength of antibody binding to the target. The most-common approaches for elution in IAC include changing the mobile phase pH or adding a chaotropic agent to the mobile phase. Other, less-common IAC elution methods include adding a competing agent, organic modifier or denaturing agent to the mobile phase, or changing the temperature of the column during elution. Usually the elution buffer is applied in a step gradient, but gradual or nonlinear gradients can be used as well [1, 3]. Shows a common scheme by which an IAC column can be used to selectively bind and elute analytes from a sample[3].

The analyte is later eluted by disrupting the antibody -antigen interactions with an appropriate elution buffer. This on/off mode can be used for direct detection and /or purification of the analyte.

Changing the mobile phase pH is the most popular method for eluting retained compounds from IAC columns. This approach is usually conducted by applying an acidic buffer (pH 1–3) to the column. Alkaline elution conditions have been used in conjunction with low-performance IAC supports, but cannot be used with common HPIAC supports, such as silica or glass beads, due to the instability of these supports at a pH greater than 8.0[2]. One difficulty with changing the pH of the mobile phase is the possibility of denaturing the immobilized antibodies or any retained compounds that are susceptible to variations in pH. However, many IAC columns have been shown to be quite stable when moderate pH changes in the range of 7.0–7.5 to 2.5–3.0 are used for elution[1,3].

To avoid denaturing effects that are caused by lowering the pH, the elution of retained compounds in IAC can alternatively be performed by adding chaotropic agents such as thiocyanate (SCN^-), trifluoroacetate (CF_3COO^-), perchlorate (ClO_4^-), iodide (I^-) or chloride (Cl^-) to the elution buffer. The elution strength of these agents follows the approximate order $SCN^- > CF_3COO^- > ClO_4^- > I^- > Cl^-$. These agents are typically used at concentrations of 1.5–8 M and have been shown to be effective in dissociating high-affinity antibody–antigen complexes. When an organic modifier is used in the mobile phase, care must be taken to ensure that the concentration of the organic additive does not permanently denature the antibodies. When methanol or other organic modifiers are used, the capacity of the immunoaffinity column has been shown to decrease. Immunoaffinity column was regenerated 20-times after offline elution with 2 ml of ethanol 80% in water. However, this column was shown to lose half its activity over these 20 regenerations. Therefore, if harsh elution conditions are required, the capacity of the immunoaffinity column should be much larger than the actual amounts of analyte that are to be measured or isolated.

FORMATS AND APPLICATION OF IAC

Immunoaffinity chromatography is a powerful technique that can selectively isolated a given compound from complex samples. As a result, many formats utilizing IAC have involved preparative applications or selective analyses. IAC has also been used with both direct and indirect detections methods and has been coupled with others methods such as HPLC, GC MS, and CE.

On/off Elution Direct Detection

In this method, the sample of interest is first injected onto the affinity column under conditions in which the analyte will bind strongly to the immobilized ligand. This is usually done at a PH and ionic strength that mimic the natural environment of the ligand and analyte .Because of the specificity of the analyte -ligand interaction,

other solutes in the sample tend to have little or no binding to the ligand and quickly wash from the column. After these nonretained solutes have been removed an elution buffer is applied to dissociate the retained analyte; this commonly involved changing the PH of the buffer composition of the mobile phase (to decrease the strength of the analyte -ligand interaction) or adding competing agent to the mobile phase (to displace analyte from ligand). As the analyte elutes, it is then detected or collected for further use. Later initial buffer is reapplied to the system, and the column is allowed to regenerate before the next sample is injected. The overall result is separation that is selective and easy to perform.

The on/off mode of IAC is commonly used in biochemistry and other fields for the selective purification of target compounds from complex samples. Compounds that have been isolated by this approach include proteins, glycoproteins, carbohydrates, lipids, bacteria, viral particles, drugs and environmental agents. This mode can also be used for the direct detection of an analyte by placing a suitable detector after the IAC column[1,2]. For this type of application, the analyte must be present at relatively high concentration and be eluted in a sharp, well-defined peak that allows a good detection limit. Depending on the desired level of detection, UV/visible absorbance, fluorescence and MS have all been used for detecting analytes in the on/off mode of IAC. Specific examples of analytes that have been measured by this approach include human serum albumin recombinant antithrombin III, IgG, *Escherichia coli*, phenyl urea herbicides, benzidine, dichlorobenzidine, aminoazobenzene, azo dyes, triazine, and diethylstilboestro.

Immunoextraction and Immunodepletion

When IAC is used to remove a specific analyte or group of analytes from a sample prior to analysis by a second analytical method, this approach is referred to as immunoextraction. Immunoextraction is coupled with a second analytical method such as LC. Immunoextraction can be carried out either offline or online with the second analytical method. In the offline mode, antibodies are typically immobilized onto a low-performance support and packed into a small disposable syringe or SPE cartridge[4]. Samples are then applied through the affinity support, which binds the analytes of interest while other sample components are washed away. An elution buffer is then passed through the affinity support to elute the extracted analytes. In addition to removing undesirable sample components, immunoextraction can allow for analyte concentration. In fact, by applying a larger sample volume to the immunoaffinity support (as long as column capacity is not exceeded), more analyte can be made available for detection by the second analytical method due to the essentially irreversible binding of the analyte to the antibodies under typical application conditions . Just as in traditional SPE methods, in offline IAC the eluted fraction can be collected, dried down, and dissolved in a solvent more suitable for analysis. If necessary, the sample can also be derivatized prior to analysis. Offline immunoextraction coupled with other methods has been used in the analysis of urine, food, water and soil extracts. Examples of analytes that have been examined by this approach include α_1-anti-trypsin, atrazine benzylpenicilloyl-peptides, bovine serum albumin, carbendazim, chloramphenicol, cortisol, clenbuterol and phenytoin, among others[3].

A related method that uses IAC is immuno depletion. In immunodepletion, an antibody column is used to remove abundant analytes from a complex sample prior to using a second method of analysis for the minor sample components. Typically, this method is used to remove high- and mid-abundance proteins from serum samples prior to the analysis of low-abundance proteins, as is often required in proteomics. In contrast to other methods that can be used to remove high- and mid-abundance proteins (e.g., precipitation, SPE, ultracentrifugation, molecular-weight separation and pI separation), immunodepletion can provide highly selective depletion of multiple high-abundance proteins simultaneously.

Competitive Binding Assay

Another way in which IAC can be used as an analytical tool is in an immunoassay format. This approach is known as a chromatographic immunoassay or flow-injection immunoassay. One

general type of chromatographic immunoassay is a competitive binding assay, in which a signal is generated as the analyte competes with some labelled species for antibody binding sites.

The most common type of competitive binding immunoassay in IAC is the simultaneous injection immunoassay. A schematic diagram of this format is shown in. In this method, a sample is mixed with a labelled analogue of the analyte and applied to a column that contains a limited amount of immobilized antibodies. The limited amount of antibodies causes the labelled analogue (*A**) and the analyte (*A*) to compete for binding sites. Due to the presence of this competition, the amount of labelled analogue that is detected in the bound and/or retained fractions is affected by the presence of analyte[7]. Typical calibration curves are prepared by plotting the relative response of the labelled analogue, B/B_o, versus the concentration of analyte in the sample, where *B* is the amount of labelled analogue bound in the presence of a given amount of analyte and B_o is the amount of labelled analogue bound in the absence of any analyte. This type of calibration curve will have a maximum value of 1 when no analyte is present and should approach 0 at high analyte concentration, assuming there is no non-specific binding between the labelled analogue and the column. A large variety of analytes have been measured by using simultaneous injection competitive binding immunoassay.

In this type of competitive immunoassay, the sample and a labelled analogue of the analyte are mixed and injected onto an immunoaffinity column. The labelled analogue can bind to this column while others sample components pass through non retained .the analyte and a labelled analogue are then eluted with an appropriate mobile phase .In this method the analyte concentration in the sample is inversely related the to the amount of retained labelled analogue that is detected.

Non-Competitive Immunoassay

Non-competitive immunoassays are another group of immunoassays that use indirect detection. These assays are often referred to as immunometric methods. In these methods, there is no competition between the analyte and other substances. Two types of non-competitive immunoassays have been used in IAC: sandwich immunoassays and one-site immunometric assays.

Sandwich immunoassays utilize two different antibodies that bind the same analyte. One of the antibodies is immobilized onto a solid support and is used to extract the analyte from samples. A second, labelled, antibody is either mixed with the sample prior to application or applied to the column directly after the sample is applied to the IAC column. Once the analyte is 'sandwiched' between the two antibodies, an elution buffer is applied to elute the analyte and labelled antibodies. The labelled antibodies can then be detected and give a response that is directly proportional to the amount of retained analyte. When the sample and labelled antibodies are mixed prior to application onto the column, better detection limits can be obtained than when sequential injection of the sample and labelled antibodies is employed, because there is more effective binding between the labelled antibody and analyte. A calibration curve for sandwich immunoassays is constructed by plotting the relative response of the eluted labelled antibody against the amount of analyte in a sample and can give a linear response over a broad range of analyte concentrations[7].

In this type of non-competitive binding immunoassay, the sample is injected onto an immuno affinity column and the analyte allowed to bind to the immobilized antibodies. A labelled antibody that is specific for the analyte is then injected onto the same column and also allowed to bind, creating a sandwich complex for the analyte. An elution buffer is applied to disrupt the antigen - antibody and regenerate the column. The amount of retained, labelled antibody that is eluted during this step is directly proportional to the amount of analyte that was present in the original sample[8].

One-Site Immunoassay

In this noncompetitive immunoassay format, the analyte and the labelled antibodies are mixed and incubated prior to injection onto an immobilized analyte column. The analyte column binds any excess antibodies and the analyte-bound antibodies are eluted in nonretained, fraction, providing a

signal that is directly related to the analyte's concentration.

The column is later regenerated by eluting off the excess labelled antibodies.

RECENT DEVELOPMENTS IN IAC

The development of IAC is ongoing and continues to be integrated with other analytical techniques, including CE and MS. Other new developments include ultrafast immunoaffinity CE and the use of antibodies in microanalytical systems.

When immobilized ligands are used in CE, the method is referred to as affinity electrophoresis. Affinity ligands, such as antibodies, can be immobilized in CE capillaries by several methods, including physical entanglement in gels and covalently binding the ligand to the capillary wall, or polymers, frits or beads inside the capillary. CE can also be used with antibodies/antibody fragments to quantitatively measure analytes by allowing CE to separate free analytes from analyte–antibody complexes. These formats can be either competitive or non-competitive, with non-competitive formats giving better LOD. CE immunoassays are utilized due to their ease of automation and their relatively fast separation of antibodies, analytes and/or antibody–analyte complexes] Another advantage of CE immunoassays is that only small amounts of sample and reagents are used while still maintaining good LOD. The best LOD in CE immunoassays are generally achieved when using laser-induced fluorescence detection or MS[3].

By combining IAC with MS (IAC–MS), a technique is produced that utilizes the selectively of antigen–antibody interactions and the sensitivity of MS. When performing online IAC– MS, the IAC elution buffer should contain only volatile buffer salts (e.g., ammonium acetate and ammonium formate) to avoid lowering ionization efficiency. In addition, methanol or acetonitrile is often added to the eluant prior to ionization to increase sensitivity. MALDI can use immobilized antibodies on the target to help extract desired compounds from a sample prior to analysis by MS[9].

Due to the speed and specificity of antibody–antigen interactions, IAC can also be used for very fast immunoextractions that often take less than a second to perform. Ultrafast immunoextraction has been used to quantify the free fraction for drugs and hormones in clinically related samples. Measuring the free fractions of drugs and hormones in serum is often difficult for other methods, because any removal of the free fraction can perturb protein binding and cause additional drug or hormone to dissociate in the sample. In recent work, ultrafast immunoextraction conducted in less than a few hundred milliseconds has been used with both direct detection based on fluorescence and chromatographic immunoassays using a displacement format for detection of warfarin, phenytoin and thyroxine in protein and serum samples[3].

Owing to recent advances in micro fabrication technologies, micro and ultra-micro (Nano) analytical systems can also be developed to utilize the specificity of antibodies. These micro total analysis systems can be engineered in a variety of formats, including the construction of microarrays which utilize several channels that can each separate multiple analytes all at once.

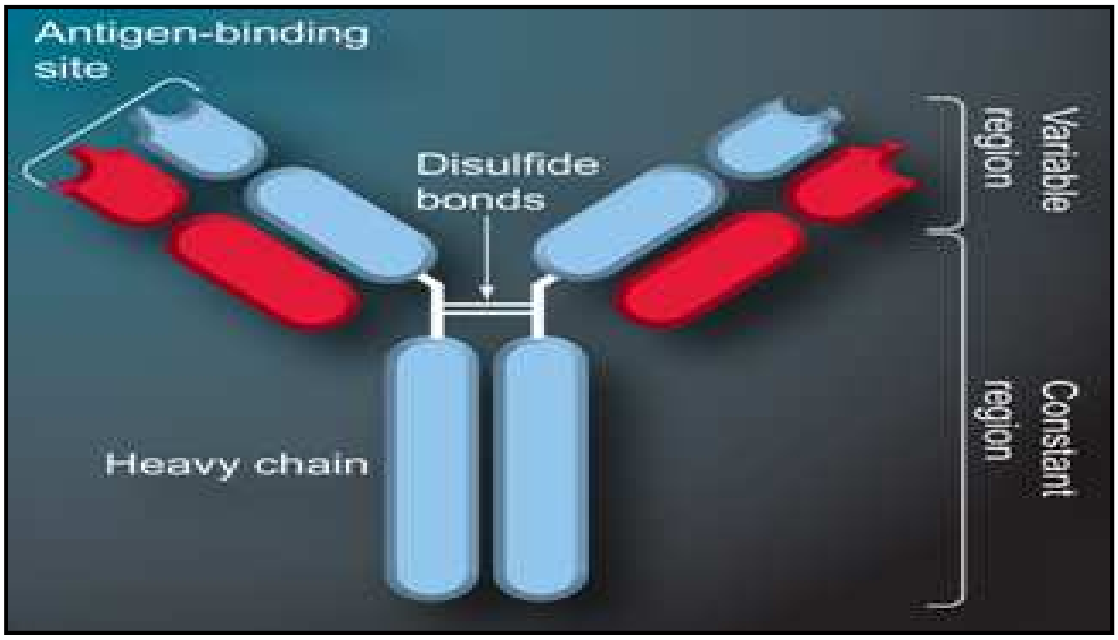

Figure No.1: Structure of Antibody

Process for Production of Polyclonal Antibiodies

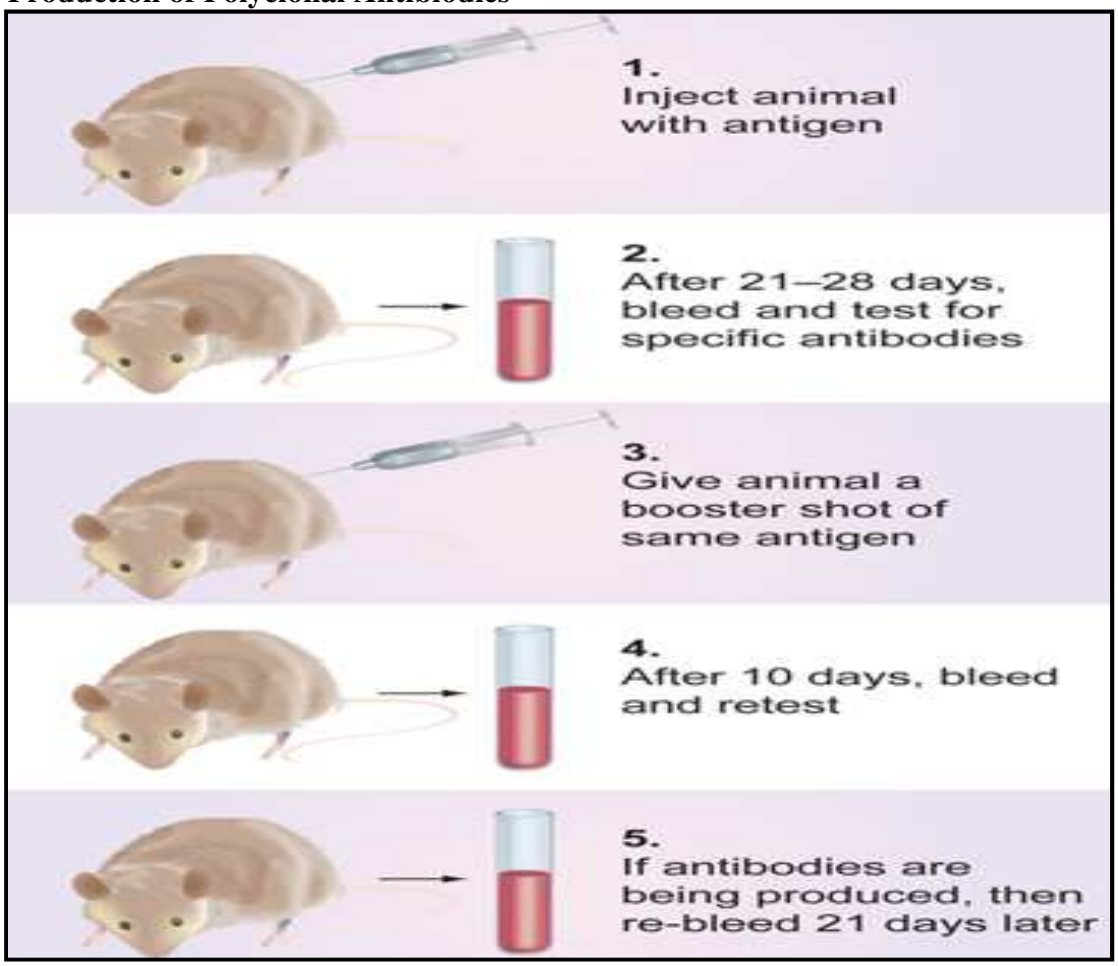

Figure No.2: Production of Polyclonal Antibiodies

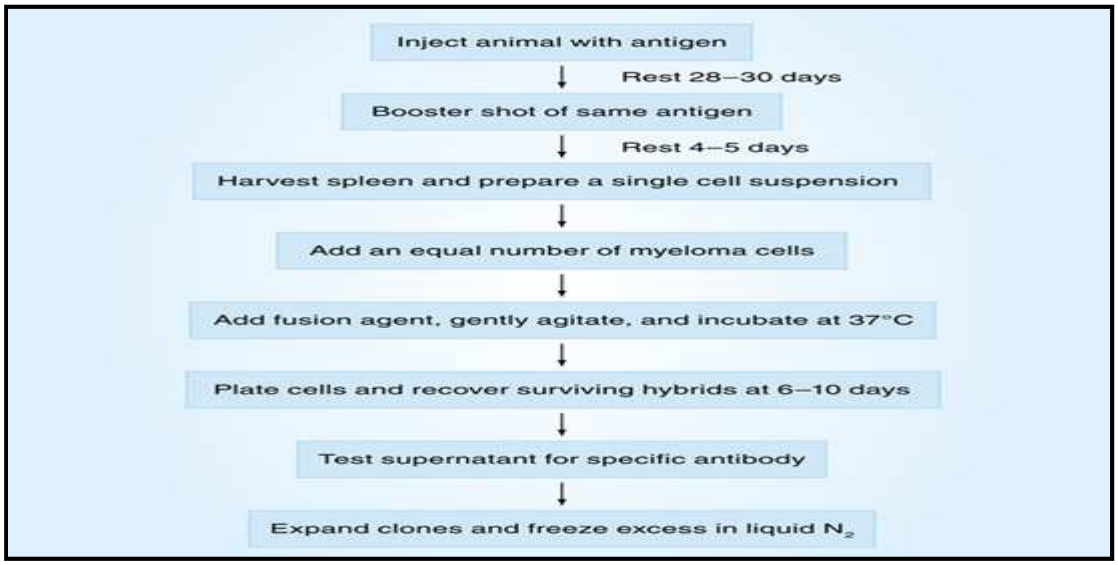

Figure No.3: Production of Monoclonal Antibodies

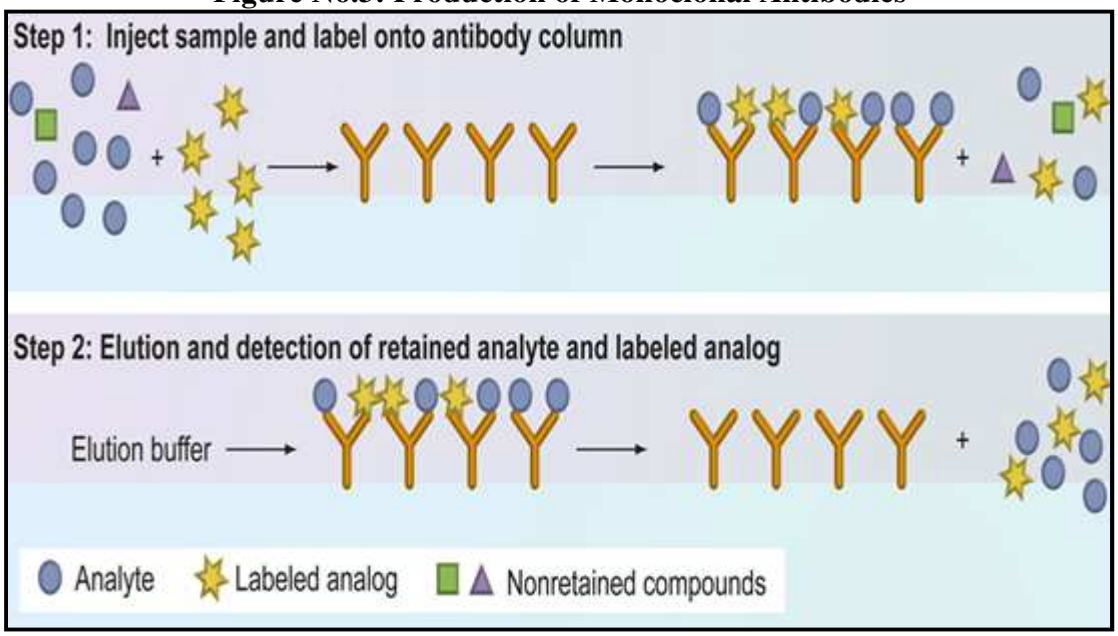

Figure No.4: Application of sample to Immunoaffinity column and elution

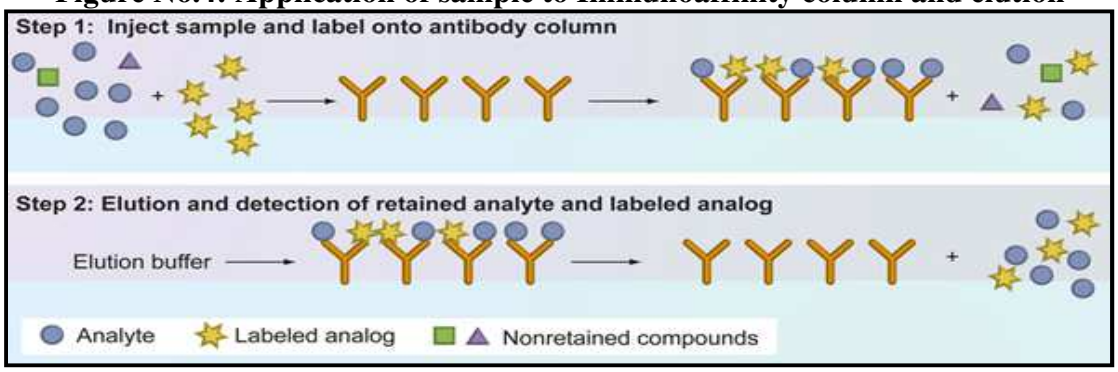

Figure No.5: Competitive Immunoassay

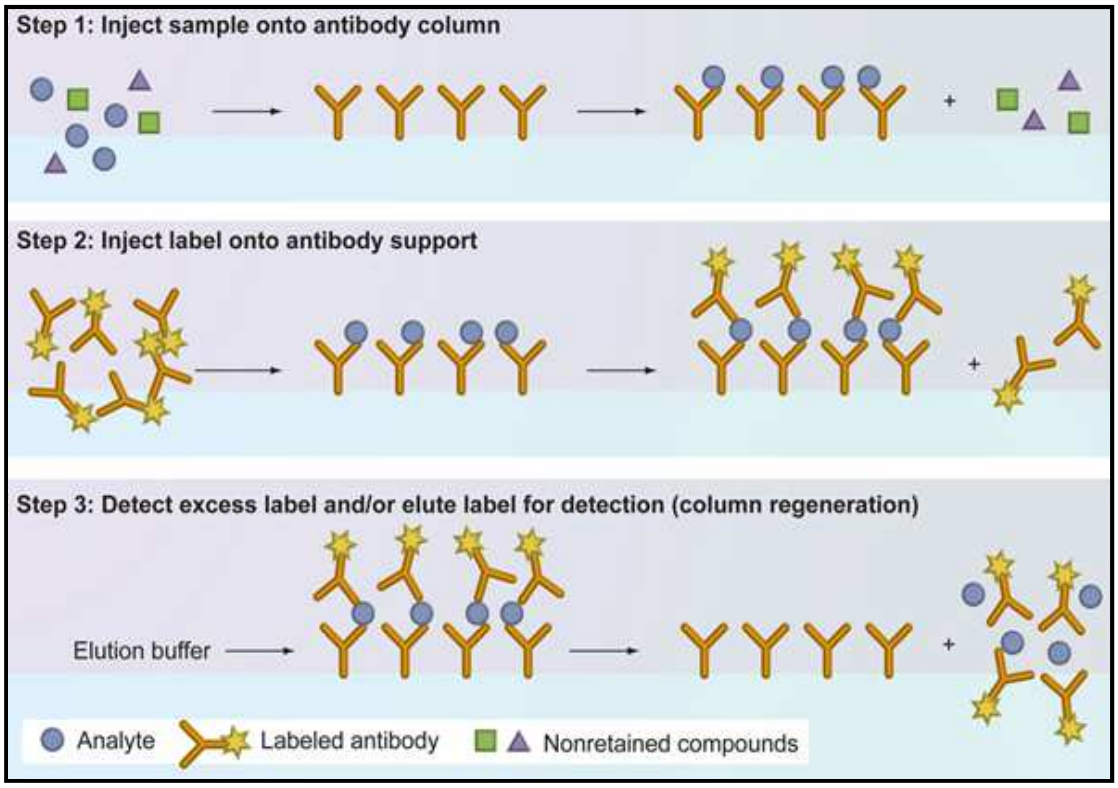

Figure No.6: Sandwich Immunoassay

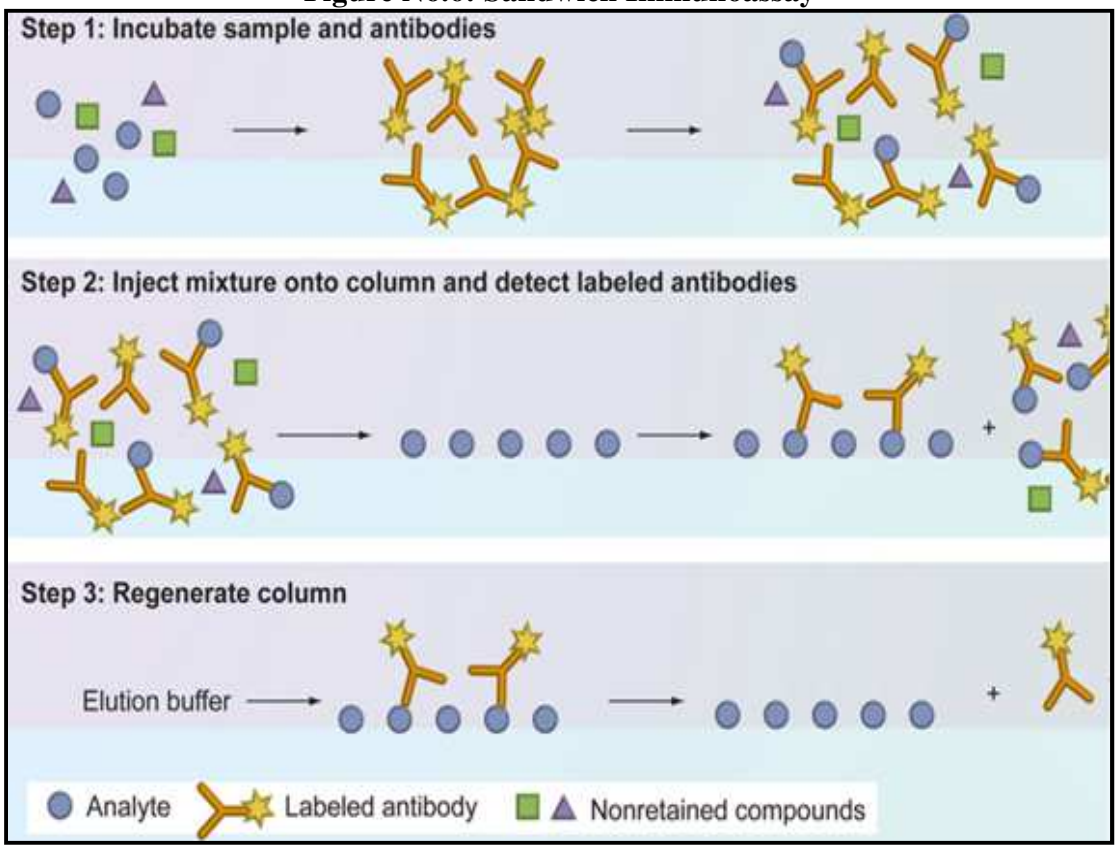

Figure No.7: Onesite Immunoassay

CONCLUSION

Immunoaffinity chromatography can be used to extract compounds of interest from samples either for purification or quantification. The general parameters of antibodies and antibody production were discussed, along with various immobilization methods. Support materials and the characteristics needed for various types of analyses were also discussed. Multiple applications of IAC were presented including immunoextraction, immunodepletion and chromatographic immunoassays. In addition, combining other analysis methods with IAC was discussed including CE, MS and microanalytical systems.

ACKNOWLEDGMENT

The authors wish to express their sincere gratitude to Department of Pharmaceutical Analysis, CMR College of Pharmacy, Kandlakoya, Medchal-501401, India for providing necessary facilities to carry out this review work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Daad Sameh Magdeldin A, Abi-Ghanem and Luc Berghman R. Immunoaffinity Chromatography: A Review, *Affinity Chromatography, Intech Open,* 2012, 92-106.
2. Hage D S, Phillips T M. Immunoaffinity chromatography, Handbook of Affinity Chromatography, *NY, USA: Taylor and Francis,* 2006.
3. Moser Annette C and Hage David S. Immunoaffinity chromatography: an introduction to applications and recent developments, *Bioanalysis,* 2(4), 2010, 769-790.
4. Kotadia Bhargav V, Khanvilkar Vineeta V, Kadam Vilasrao. Immunoaffinity chromatography, *International journal of research in pharmacy and chemistry and pharmaceutical Analysis, India,* 2(2), 2012, 346-364.
5. Kim H, Hage D S. Immobilization methods for affinity chromatography, Handbook of Affinity Chromatography, *NY, USA: Taylor and Francis,* 2006.
6. Williams B A R, Diehnelt C W, Belcher P, *et al.* Creating protein affinity reagents by combining peptide ligands on synthetic DNA scaffolds, *J. Am. Chem. Soc,* 131(47), 2009, 17233-17241.
7. Moser A C, Hage D S. Chromatographic immunoassays, Handbook of Affinity Chromatography, *NY, USA: Taylor and Francis,* 2006.
8. Bouzige M, Legeay P, Pichon V, Hennion M C. Selective on-line immunoextraction coupled to liquid chromatography for the trace determination of benzidine, congeners and related azo dyes in surface water and industrial effluents, *J. Chromatogr. A,* 846(1-2), 1999, 317-329.
9. Briscoe C J, Clarke W, Hage D S. Affinity mass spectrometry, Handbook of Affinity Chromatography, *NY, USA: Taylor and Francis,* 2006.
10. Hage D S. Survey of recent advances in analytical applications of immunoaffinity chromatography, *J of Chromatogr B,* 715(1), 1998, 3-28.

Please cite this article in press as: Swaroopa E and Pushpa Latha E. Immunoaffinity chromatography: a review, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 8(1), 2020, 22-33.