Research & Reviews: Journal of Pharmacognosy and Phytochemistry

e-ISSN: 2321-6182
p-ISSN: 2347-2332

A Brief Note on Affinity Chromatography

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Perspective

Received: 1-Mar-2022, Manuscript No. JPRPC- 55727; **Editor assigned:** 3-Mar-2022, PreQC No. JPRPC- 22-55727(PQ); **Reviewed:** 17-Mar-2022, QC No JPRPC-22- 55727; **Revised:** 21-Mar-2022, Manuscript No. JPRPC-22-55727 (R); **Published:** 28-Mar-2022, DOI: 10.4172/ 2321-6182.10.2.002

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DESCRIPTION

Affinity chromatography is a technique for isolating a biomolecule from a mixture that relies on a highly specific macromolecular binding interaction between the biomolecule and another substance. The type of binding interaction depends on the biomolecule of interest for example antigen and antibody, enzyme and substrate, receptor and ligand or protein and nucleic acid. Binding interactions are frequently used to isolate different biomolecules when compared to other chromatographic methods affinity chromatography is advantageous due to its high selectivity and separation resolution.

The stationary phase consists of a support medium to which the substrate (ligand) is covalently bound exposing the reactive groups required for target molecule binding. As the crude mixture of substances passes through the chromatography column substances with binding sites for the immobilised substrate bind to the stationary phase

while all other substances elute in the column's void volume. After the other substances have been eluted, the bound target molecules can be eluted by including a competing ligand in the mobile phase or changing the pH, ionic strength or polarity conditions. Column chromatography in which the solid medium is packed onto a column the initial mixture is run through the column to allow settling a wash buffer is run through the column and the elution buffer is then applied to the column and collected can achieve solid phase binding. These procedures are typically carried out at atmospheric pressure. Binding can also be accomplished with a batch treatment such as 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.

A hybrid method is sometimes used in which the binding is done in batches but the solid phase with the target molecule bound is packed onto a column and washed and eluted on the column. Affinity chromatography ligands are derived from both organic and inorganic sources. Serum proteins, lectins and antibodies are examples of biological sources. Moronic acts, metal chelates and triazine dyes are examples of inorganic sources. Third method expanded bed absorption has also been developed to combine the benefits of the two methods mentioned above. The solid phase particles are placed in a column with liquid phase pumped in from the bottom and exiting at the top. The particles' gravity ensures that the solid phase does not exit the column with the liquid phase. To resolve the particles of interest affinity columns can be eluted by changing salt concentrations, pH, pI, charge and ionic strength directly or through a gradient. Previously, more than one column in series setups has been developed. When compared to single column setups the advantage is that the resin material can be fully loaded because the non-binding product is directly passed on to a subsequent column with fresh column material. Periodic Counter-Current Chromatography (PCC) refers to these chromatographic processes. As a result, the resin costs per unit of product produced can be drastically reduced. Because one column can always be eluted and regenerated while the other column is loaded two columns are already enough to take full advantage of the advantages. Additional columns can provide more elution and regeneration time flexibility but at the expense of additional equipment and resin costs.

There are numerous affinity media available for a wide range of applications. In a nutshell they are (generalised) activated/functionalized materials that act as a functional spacer, support matrix and eliminate the need for toxic reagents. A variety of serum proteins, proteins, peptides and enzymes as well as rRNA and dsDNA are used in amino acid media. Avidin biotin media is used in the purification of biotin and its derivatives. Carbohydrate bonding is most commonly associated with glycoproteins or any other carbohydrate-containing substance carbohydrate is associated with lectins, glycoproteins or any other carbohydrate metabolite protein. Nonspecific dye ligand media mimics biological substrates and proteins. Glutathione can be used to separate GST-tagged recombinant proteins. Heparin is a generalised affinity ligand that is particularly useful for the separation of plasma coagulation proteins as well as nucleic acid enzymes and lipases.

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Most commonly, hydrophobic interaction media are used to target free carboxyl groups and proteins. Immunoaffinity media uses the high specificity of antigens and antibodies to separate immobilised metal affinity chromatography uses interactions between metal ions and proteins (usually specially tagged) to separate and nucleotide/coenzyme that works to separate dehydrogenases, kinases and transaminases. Affinity purification of antibodies from blood serum is another application for the procedure. If the serum contains antibodies against a specific antigen (for example if it comes from an organism immunised against the antigen in question) it can be used for affinity purification of that antigen. Immunoaffinity chromatography is another name for this. When an organism is immunised against a GST-fusion protein it produces antibodies against the fusion protein as well as possibly antibodies against the GST tag. The protein is then covalently coupled to a solid support such as agarose and used as an affinity ligand in antibody purification from immune serum.