IONEXCHANGECHROMATOGRAPHY

constructionandworkingofIonexchangechromatography

Ion exchange means exchange of ions from a medium. It has typical application in watersofteningbyexchangeof alkalinemetalionslikeCa^{2}+,Mg^{2}+by Na^{+}.Othercommonapplicationsaresugarprocessing,hydrometallurgicalapplication ,proteinfractionation,biologicalseparation,etc.

Fundamentals:

Ion from a solution is removed when it is passed through a bed of exchangeable ions, calledresins.

$$A^{+} + R^{-}B^{+} + X^{-} = R^{-}A^{+} + B^{+} + X^{-}$$

In this reaction, R^{-} is fixednegative charge on the resin. A^{+} and B^{+} are called counter-ionsand$X^{-}$is calledcoioninresinphase

IonExchangeResin

Most popular base for ion exchange resin is polystyrene. Cross linking with divinylbenzene(DVB)isdonewithresintomakeitinsoluble.About2-10%DVBisused.Both(i)macrosporeand (ii) geltyperesin beads are used. Macro porousbeadshaveporesinsidethebeadswhereionscangoinorgetout. Typical external porosity is about0.40. Gel type resin havevarious degrees of swelling.These may be polystyrene-sulfuric acid resin with various % of DVB, polyacrylic acid resin,etc.

Acidicresinshavenegativefixedchargesandcan exchangecations.Basicresinshavepositive fixed charges and can exchange anions. Exchangers can also weak or strong. Strongresins are fully ionized and all the fixed groups are available to exchange cations. Strong baseresins can degrade at higher pH and temperature. On the other

hand, weak resins are only partially ionized at most pH values. Theyhave lower exchange capacity but they are easier to regenerate. Weak resins require lessregenerantthanstrongresins.

But weak resins swell or contract when ions are exchanged. They can rupture due to improperstress distribution of during expansion/contraction cycle. In weak resin also the ions diffuseslowly.So,masstransferresistanceisvery highandtimerequirementislong.

TechniquesofIonExchangeChromatography:

1. PreparationofColumn

The ion exchange chromatography is carried out in a chromatographic column which usuallyconsists of a burette provided with a glass wool plug at the lower end. Generally a ratio of 10:1 or 100:1 between height and diameter is maintained in most of the experiment. Too narrowortoowidecolumngiveunevenflowofliquidandsometimespoorseparation.

2. PreparationofIonExchange

Ion exchange materials are first allowed to swell in buffer or in HCl or NaOH solution for 2-3hours or sometimes overnight. Almost all ion exchange resin swells when placed in buffer ordistilled water and this is due to hydration of their ions. In dry condition, the pore of resins isrestricted so in order to swell the pore of resin. Resins are suspended in buffer solution or indistilledwater.

3. WashingofIonExchangers

The ion exchange material is obtained in required ionic form by washing with appropriatesolution. For e.g. the H^+ form of cation exchange resins is obtained by washing the materialwithHClthenwithwateruntilthewashings areneutral. Anionicexchangersaregenerallysuppliedintheformofsaltandamines.Similarly,Na^+ formispreparedbywashingtheresinswithNaClorNaOHsolutionandthenwithwater.

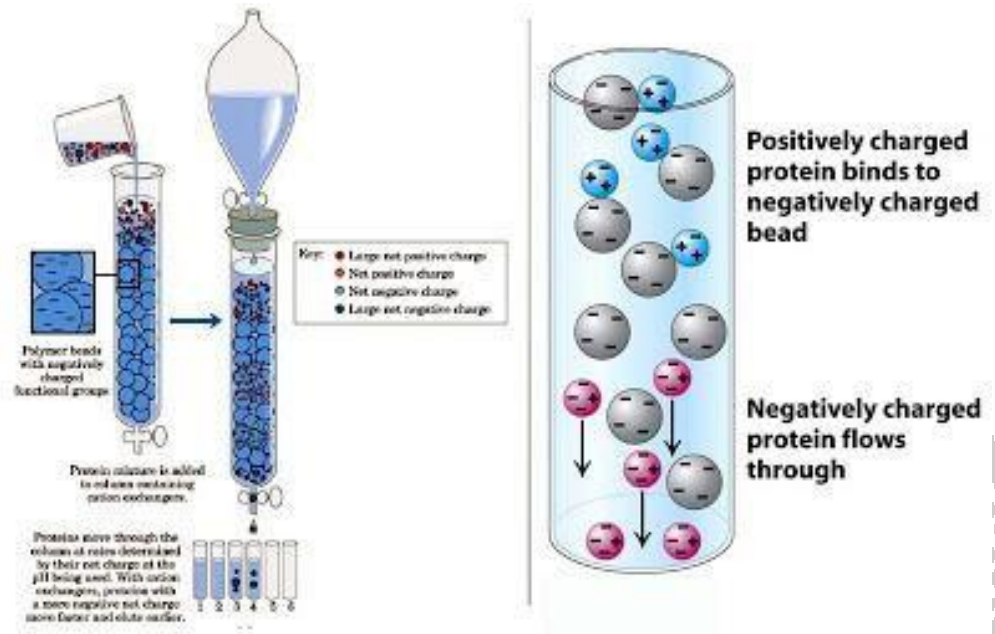

Figure2: Ionexchangechromatography

4. PackingofColumn

This is one of the most critical factors in achieving a successful separation. The column isheld in vertical position and the slurry of resins is poured into the column that has its outletclosed. The column is gently tapped to ensure that no air bubbles are trapped and that packingmaterialsettles evenly.

5. SampleApplication

Sample can be loaded by using pipette or syringe. The amount of sample that can be appliedto a column is dependentupon the size of the column and the capacity of resins, If thestartingbufferistobeusedthroughoutthedevelopmentofcolumn,thesamplevolumebe1 %to5%ofbedvolume.

6. DevelopmentanElution ofboundions

Bound ions can be removed by changing the pH of buffer. E.g. separation of amino acid isusually achieved by using a strong acidic cation exchanger. The

sample is introduced onto thecolumnatpHof1-2,thusensuringcomplete bindingofallofthe aminoacids.

Gradientelution usedin increasing pH andionic concentration resultsin the sequentialelution of amino acid. Then acidic amino acid such as aspartic acid and glutamic acid areeluted first. The neutral amino acid such as glycine and valine are eluted. The basic aminoacid such as lysine and arginine retain their net positive charge at pH value of 9 to 11 and areelutedatlast.

7. Analysisofeluate

Equal fraction of each elute are collected at different test tube keeping the flow rate at 1 mlper minute. The eluate collected in each fraction is mixed with ninhydrin color reagent. Themixture is then heated to 105 C to develop the color and intensity of color is determined bycolorimetermethodorspectrophotometermethodat540to570nm.