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REVIEW ARTICLE

RECENT TRENDS IN ION-EXCHANGE CHROMATO-GRAPHY

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Abstract: Ion exchange chromatography involves the separation of ionizable molecules based on their total charge. This technique enables the separation of similar types of molecules that would be difficult to separate by other techniques because the charge carried by the molecule of interest can be readily manipulated by changing buffer pH. Ion exchange chromatography is commonly used to separate charged biological molecules such as proteins, peptides, amino acids, or nucleotides. The amino acids that make up proteins are zwitterionic compounds that contain both positively and negatively charged chemical groups. Depending on the pH of their environment, proteins may carry a net positive charge, a net negative charge, orno charge. Ion-exchange chromatography is a relatively mature area of chromatographic separation yet advances in this technique continue unabated. This review highlight the basic principle, techniques, selection criteria of resin, factors affecting, applications of in pharmaceutical industry and also provides a summary of the latest in new ion-exchange phases for ion chromatography. It starts by focusing on general aspects of phase design and then reviews anion-exchange and cation-exchange columns introduced in the past few years.

Key words: Ion Exchange Chromatograph, Ion-Exchange Phases, Resin selection, Factors affecting, Application

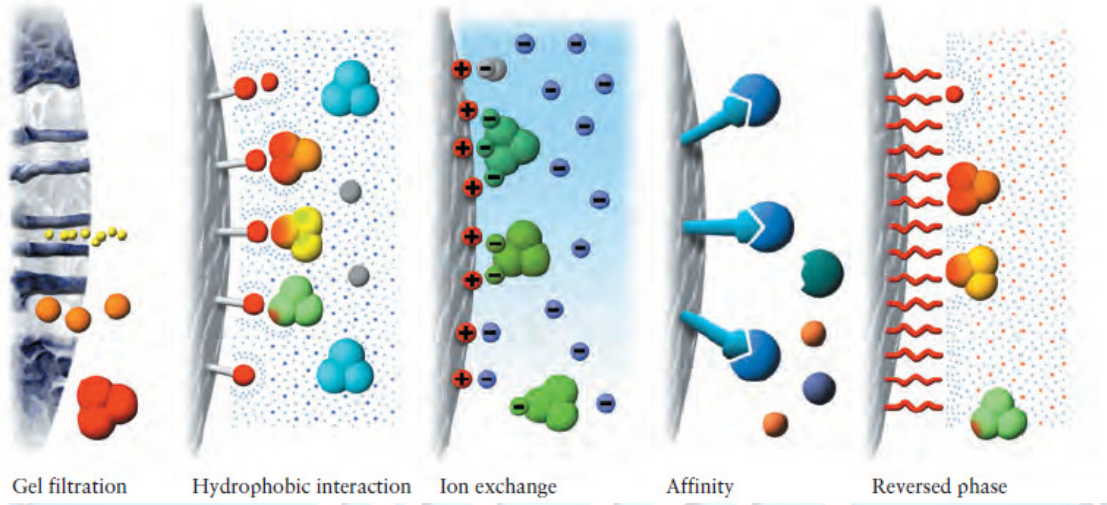
INTRODUCTION:

Ion exchange chromatography is the process by which a mixture of similar charged ions can be separated by using an ion exchange resin which exchanges ions according to their relative affinity is uses. There is reversible exchange of similar charged ions. Mostly like cations and anions can be conventionally separated by this technique. Many drugs and pharmaceutical agents are weakly or strongly acidic or basic in nature. Hence a mixture of similar charged substances can also be separated into pure components.

Biomolecules are purified using chromatography techniques that separate them according to differences in their specific properties, as shown in Figure 1. Ion exchange chromatography (IEX) separates biomolecules according to differences in their net surface charge. Property Technique Charge Ion exchange chromatography (IEX), chromatofocusing (CF) Size Gel filtration (GF), also called size exclusion Hydrophobicity Hydrophobic interaction chromatography (HIC) Reversed phase chromatography (RPC) Biorecognition (ligand specificity) Affinity chromatography (AC) Fig. 1. Separation principles in chromatographic purification. IEX for the separation of biomolecules was introduced in the 1960s and continues to play a major role in the separation and purification of biomolecules. Today, IEX is one of the most frequently used techniques for purification of proteins, peptides, nucleic acids and other charged biomolecules, offering high resolution and group separations with high loading capacity. The technique is capable of separating molecular species that have only minor differences in their charge properties, for example two proteins differing by one charged amino acid. These features make IEX well suited for capture, intermediate purification or polishing steps in a purification protocol and the technique is used from microscale purification and analysis through to purification of kilograms of product.

Ion chromatography (IC) continues to be the chromatographic technique most widely used for the separation of ionic and ionizable compounds, with a special focus on the analysis of inorganic anions, inorganic cations, small hydrophilic organic acids, and aliphatic amines. Although a number of separation modes are included under the umbrella term of *ion chromatography*, ion exchange is by far the most widely used technique in IC. Although use of ion-pair techniques in conjunction with reversed-phase columns remains a viable alternative to ion exchange for IC applications, ion exchange continues to be the focus of development when it comes to new stationary phases designed for specific applications involving the separation of ionic compounds. There are a number of reasons why ion exchange has proven to be the preferred separation technique in IC. These include a broad range of available selectivities, the ability to tailor selectivity for specific applications, the exceptional chemical stability of poly-

meric ion-exchange materials, the ability to separate ions of similar size, and rapid equilibration when operated in the gradient mode.



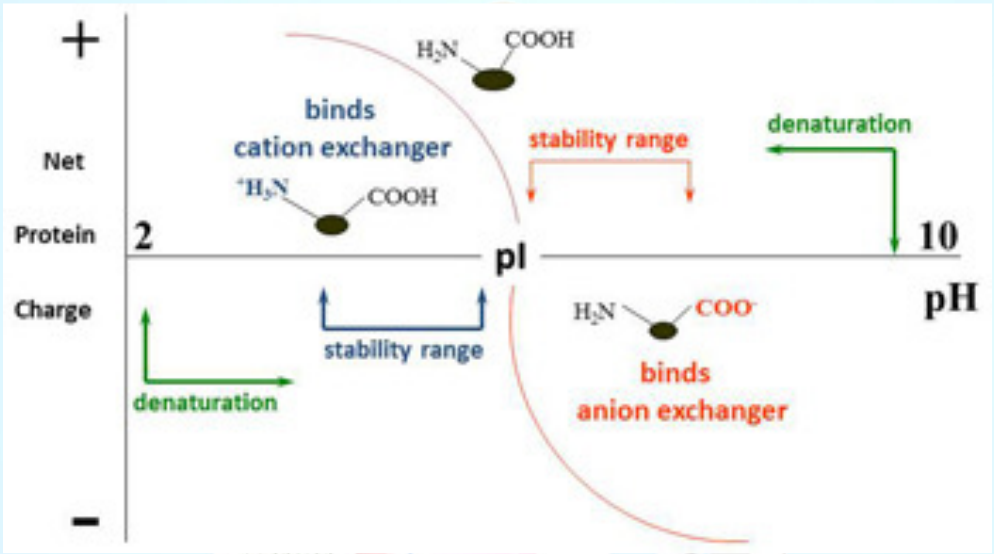


Fig. 1. Protein charge vs. pH. Protein stability and ion exchange media binding vary with total protein charge, which depends on pH.

PRINCIPLES OF ION EXCHANGE CHROMATOGRAPHY

Ion exchange chromatography is commonly used to separate charged biological molecules such as proteins, peptides, amino acids, or nucleotides. The amino acids that make up proteins are zwitterionic compounds that contain both positively and negatively charged chemical groups. Depending on the pH of their environment, proteins may carry a net positive charge, a net negative charge, orno charge. The pH at which a molecule has no net charge is called its isoelectric point, or pI.

Resin Type	Cation Exchanger	Anion Exchanger
Net charge of molecule of interest	+	−
Charge of resin	−	+
Running conditions	0.5–1.5 pH units below the pI of the molecule of interest	0.5–1.5 pH units above the pI of the molecule of interest

Fig. 2. Ion exchange resin selection.

The pI value can be calculated based on the primary se-

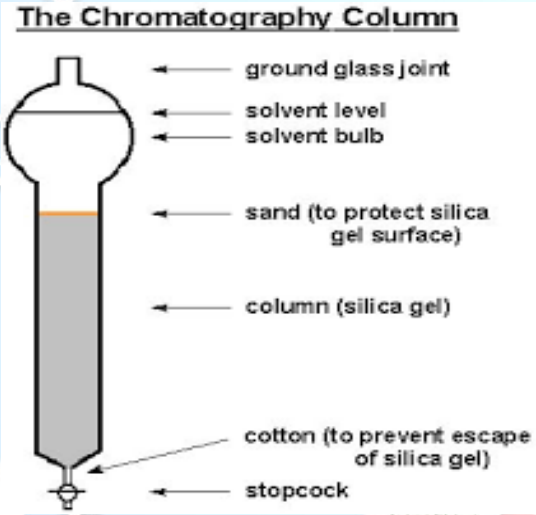
quence of the molecule. The choice of buffer pH then determines the net charge of the protein of interest.

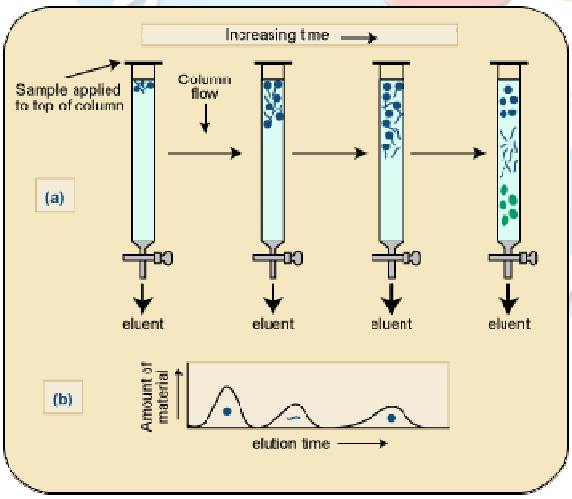
In a buffer with a pH greater than the pI of the protein of interest, the protein will carry a net negative charge; therefore, a positively charged anion exchange resin is chosen to capture this protein.

In a buffer with a pH lower than the pI of the protein of interest, the protein will carry a positive net charge; thus a negatively-charged cation exchange resin is chosen.

When an ion exchange chromatography column is loaded with a sample at a particular pH, all proteins that are appropriately charged will bind to the resin. For example, if an anion exchange resin is chosen, all proteins that are negatively charged at the loading buffer pH will bind to the positively charged column resin. A good rule of thumb for choosing a buffer pH is the following:

- Anion exchanger — 0.5–1.5 pH units greater than the pI of the protein of interest
- Cation exchanger — 0.5–1.5 pH units less than the pI of the protein of interest





The principle of separation is by reversible exchange of ions between the ions present in the solution and those present in the ion exchange resins.

The cation to be separated is present in the solution and exchanges for similar ions present in cation exchange resin, a solid matrix. The exchange can be represented by the following equation:

$$Solid - H^+ + M^+ \rightarrow solid - M^+ + H^+$$

The cation retained by the solid matrix of ion exchange resin can be eluted by using buffers of different strengths and hence the separations of cations can be effected.

2 Anion exchange:

Similarly, separation of anion using anion exchange resin can be carried out . the anions to be separated are present in the solution and exchanges for similar ions present in anion exchange resin, a solid matrix the exchange can be represented by the following equation:

$$Solid - OH^- + A^- \rightarrow solid - A^- + OH^-$$

The anions retained by the solid matrix of ion exchange resin can be eluted by using buffers of different strengths and hence separation of anions can be effected. The technique of ion exchange chromatography can be studied with respect to the following headlines:

1. Ion exchange resins – classification of resin
2. Practical requirements
3. Factors affecting ion exchange separation

CLASSIFICATION OF RESINS

According to the chemical nature they can be classified as

1. Strong cation exchange resin
2. Weak cation exchange resin
3. Strong anion exchange resin
4. Weak anion exchange resin

According to source they can be classified as

1 Natural: cation – zeolytes, clay etc.

Anion- dolomite

2 Synthetic: inorganic and organic resins

Of the above types, organic resins are the most widely used.

Organic ion resins are polymeric resin matrix containing exchange sites. The resin is composed of polystyrene and divinyl benzene. Polystyrenes contain sites for exchangeable functional groups. Divinyl benzene acts as a cross linking agent and offers adequate strength i.e., mechanical stability.

Functional groups present in different ion exchange resins:

Strong cation exchange resin – SO_3 H

Weak cation exchange resin –COOH, OH, SH, PO_3 H_2

Strong anion exchange resin – N^+ R_3, NR_2

Weak anion exchange resin – NHR, NH_2

Structural types of ion exchange resins:

a. **Pellicular type with ion exchange film:** the particles have size of 30-40μ with 1-2μ film thickness. These have very low exchange capacity separate the ions. Their ion exchange efficiency is 0.01 – 0.1 meq/ g of ion exchange resin.
b. **Porous resin coated with exchanger beads:** the size ranges from 5 – 10 μ. They are totally porous and highly efficient. The exchange capacity from o.5 – 2 meq/ g of ion exchange resin.
c. **Macroreticular resin bead**: A reticular network of the resin is seen superficially on the resin beads. They are not highly efficient and have very low exchange capacities.
d. **Surface sulfonated and bonded electrostatically with anion exchanger:** the particles are sulfonated and they are bonded electrostatically with anion exchanger resin. They are less efficient and have low exchange capacity. Their exchange capacity is 0.02 meq/g of exchange resin.

Physical properties of resins:

1. **Particle size:** they are available as fine powder of uniform particle size from 50 – 200 mesh. They should allow free and uniform flow of mobile phase. They should contain more exchangeable functional groups.
2. **Cross linking and swelling:** when more cross linking agent is present, they are more rigid, but swell less. When swelling is less, separation of ions of different sizes is difficult as they cannot pass though the pores present.

When less cross linking agent is present, they are less rigid, but swell more. When swelling is more, separation will not be efficient as exchange of functional group does not takes place due to wide pore. Hence an optimum quantity of cross linking agent should be added to the polymeric ion exchange resin for the separation to be effective.

Practical requirements:

1. Column material and dimentions: columns used in the laboratories are made up of glass. But those used in the industries are made up of either high quality stainless steel or polymers which are resistant to strong acids and alkalies. The column dimentions are also important and a length : diameter ratio of 20:1 to 100:1 for higher efficiency can be used.
2. Type of ion exchange resin and physical characteristics: the selection of ion exchange resin depends upon the following properties.
a. Type of the ions to be separated – cations or anions.
b. Nature of the ions to be separated – strong or weak
c. Efficiency of the resin – it is measured by ion exchange capacity. Ion exchange capacity is the total ion exchange capacity in terms of the exchangeable functional groups expressed as milli equivalents per gram of the ion exchange resin

M.eq/g = 1000/eq.wt.

d. particle size of the resin – 50–100 mesh or 100-200 mesh is used.
d. Structural type of the resin – porous, pellicular.
e. Amount of cross linking agent present – which decides swelling of the resin.

3. Packing of the column: wet packing method is used. The resin is mixed with the mobile phase and packed in the column uniformly, the sample tube to be separated is dissolved the mobile phase and introduced all at once into the column.
4. Mobile phase : organic solvents are less useful and they are not used at all. Only different strengths of acids, alkalies and buffers are used as eluting solvents. Eg. 0.1N HCl, 1N NaOH, phosphate buffer, acetate buffer, boric buffer, phthalate buffer.
5. Development of the chromatogram and elution : after introduction of the sample , development of the chromatogram is done by using different mobile phases as mentioned earlier, organic solvents are less useful and only acids, alkalies and buffers of different pH are used. There are two elution techniques. They are isocratic elution and gradient. In isocratic elution technique the same solvent composituin is used. That is same strength of acid or alkli or buffer. In gradient elution technique initially less acidic or basic character is used followed by increasing the acidity or basicity of the mobile phase. Gradient elution is usually used for complex mixture. The different fractions of the eluent are collect volume wise or time wise and analysed.
6. Analysis of the elute: different fractions collected with respect volume or time analysed for their contents. Several methods of analysis can be used which depends upon the nature and quantity of the sample. They are Spectrophotometric method, polar graphic methods, conductometric method, amperometric method, flame photometric method, radio chemical method etc. After analysing similar fractions are mixed in order to get pure ion or compound of each type.
7. Regeneration of ion exchange resin: the ion exchange resin after separation may not be useful for next separation as exchangeable groups are lost. But due to the cost of the ion exchange resins, they cannot be disposed off hence like reactivation, regeneration of the resin is most important. Regeneration makes the used ion exchange resin to be as efficient as a very in resin.

Regeneration refers to the replacement of the exchangeable cations or anions present in the original resin. Hence regeneration of the cation exchange resin is done by the

charging of the column with strong acid like HCl acid. Regeneration of the anion exchange resin is done by using strong alkali like NaOH or KOH.

ION EXCHANGE CHROMATOGRAPHY RESIN SELECTION

Ion exchange chromatography resins are composed of positively or negatively charged functional groups that are covalently bound to a solid matrix. Common matrices are cellulose, agarose, polymethacrylate, polystyrene, and polyacrylamide. The latter three matrices allow higher flow rates.

Several factors inform resin choice:

Anion exchanger or cation exchanger

Weak vs. strong ion exchanger

Ionic form of the resin

Resin particle size

Permissible flow rate

Dynamic binding capacity

Deciding between an Anion Exchanger and a Cation Exchanger

For many protein purification workflows, protein folding and stability is a concern. In these scenarios, the selection of an anion or a cation exchanger depends on the protein of interest's stability.

Some proteins are stable both above and below their pI. These proteins can be purified with either an anion or cation exchanger. Other proteins are stable only above or below their pI. For these proteins, stability dictates resin choice; if, for example, a protein is stable only above its pI, an anion exchange resin should be chosen. When protein stability is not of concern, either an anion or cation exchanger can be used.

Weak vs. Strong Ion Exchangers

Ion exchange resins come in two types: strong and weak.

The number of charges on a strong ion exchanger remains constant regardless of the buffer pH. These types of resins retain their selectivity and capacity over a wide pH range. Examples of strong ion exchangers are quaternary ammonium (Q), sulfonate (S), and sulfopropyl (SP) resins.

Weak ion exchangers, in contrast, display pH-dependent function and so deliver optimal performance over only a small pH range. When the pH of the buffer no longer matches the acid dissociation constant (pKa) of the resin functional group, these resins suffer significant capacity loss. Weak anion exchangers function poorly above a pH of 9 and weak cation exchangers begin to lose their ionization below pH 6. When working with weak ion exchange resins such as diethylaminoethyl (DEAE) or carboxymethyl (CM) resins, it is important to work within the supplier-provided working pH range.

Support	DEAE	High Q	CM	High S
Type of exchange	Weak anion	Strong anion	Weak cation	Strong cation
Functional group	$-N^+(C_2H_5)_2$	$-N^+(CH_3)_3$	$-SO_3^-$	$-COO^-$
pH Range*	5–9	0–14	5–9	0–14

* Some Proteins of interest may not be stable over the full pH range.

Strong ion exchangers are often preferred resins for many applications because their performance is unaffected by pH. However, weak ion exchangers can be powerful separation tools in cases where strong ion exchangers fail because the selectivities of weak and strong ion exchangers often differ.

Ionic Form of IEX Resin

The ionic form of a support refers to the counterion that is adsorbed onto the resin's functional groups. This ion can be changed by swapping the column equilibration buffer. Common counterions for anion and cation exchangers are Na^+ and Cl^-, respectively.

The strength of the interaction with a given resin varies for different counterions. The lower the selectivity of a counterion for the support, the more readily it can be exchanged for another ion of like charge (for example, the protein of interest). Similarly, elution buffer containing a counterion with a relatively lower selectivity for the support will displace proteins from the column resin less readily during elution. In some cases, this difference can be exploited, and counterions such as Li^+, Br^-, and SO_4^{2-} are often used to improve resin selectivity.

Resin Particle Size

Resin particle size refers to the size of the resin solid support. Particle size does not affect the selectivity of the resin but it does impact resolution.

Smaller particles provide higher resolution but typically also require lower flow rates. High-resolution media are commonly used for analytical and small-scale work as well as for the final polishing steps of preparative chromatography. Very viscous samples such as cleared *E. coli* lysates or samples containing glycerol often cannot be separated using small-particle IEX resins due to the in-

creased backpressure of small-particle resins, which can exceed the column's operating pressure limit.

Larger particles permit higher flow rates but yield lower resolution. A method that yields sharp, distinct peaks using a small-particle IEX resin will yield broader, less defined peaks when a larger-particle resin is used. Large-particle IEX resins are a great choice for large-scale and preparative work. Larger particle sizes are also the best choice when samples are viscous, such as when IEX is used as a first step in a protein purification workflow.

Flow Rate

Flow rate refers to how fast buffer is being passed over a resin. The flow rate therefore determines the amount of time in which proteins can interact with the column resin, which is called the residence time of a particular column at a given flow rate. Unlike particle size, flow rate affects both resolution and capacity: longer residence times increase both the capacity and the resolution of a resin.

Flow rates are not only limited by the loss of resolution and capacity at higher flow rates but also by the resin itself. As flow rates increase, pressure on the resin increases. If the backpressure is too high, it can crush the column resin. Manufacturers thus provide a pressure limit for all of their resins. Generally, the fastest flow rate that still renders the desired capacity and resolution is chosen. Although slower flow rates may provide even better resolution and capacity, this is often at the expense of protein activity, as many proteins lose activity with time under the conditions in the chromatography system.

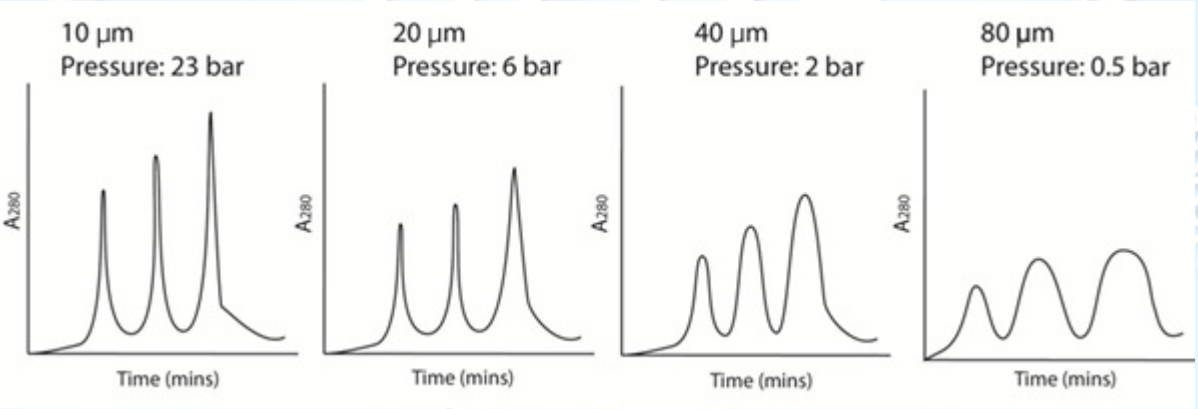


Fig. 6. Relationship between resin particle size, pressure, and resolution.

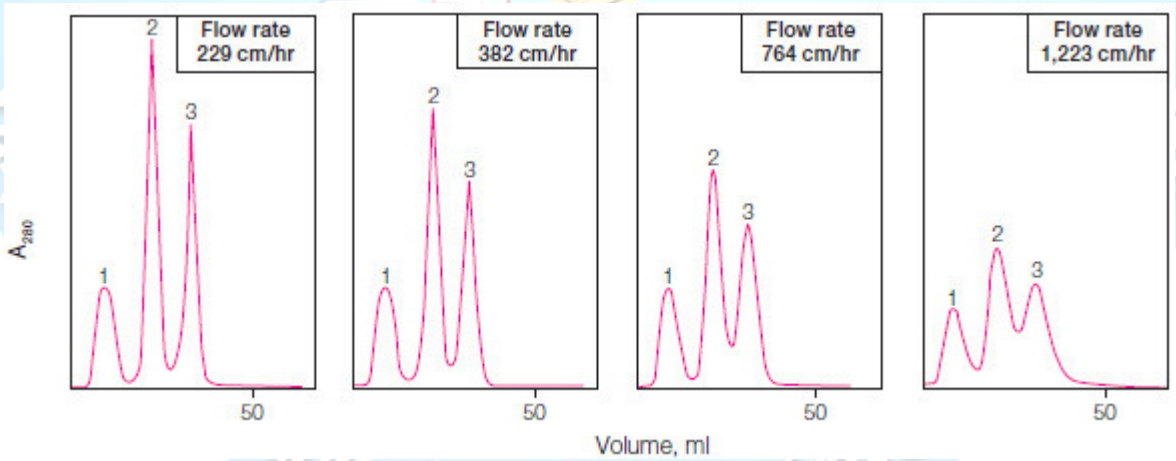


Fig. 7. Effect of flow rate on IEX resolution. Separation of a 5 ml sample of myoglobin (peak 1), ribonuclease A (peak 2), and cytochrome c (peak 3) on a 1 x 13 cm (8.7 ml)

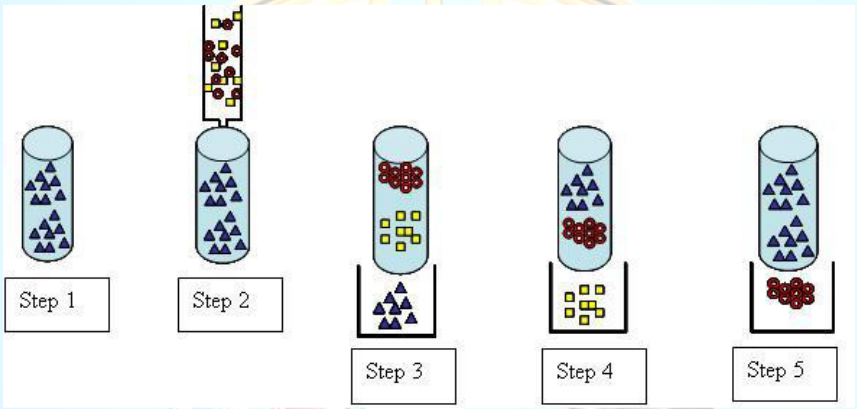
Dynamic Binding Capacity of Resin

The dynamic binding capacity of a resin refers to the amount of protein the resin can bind at a given flow rate; it is generally reported as mg/ml of protein bound at a certain flow rate. This value varies from resin to resin and can be important when fast flow rates are required to maintain protein activity.

THE BASIC PROCESS STEPS OF ION EXCHANGE CHROMATOGRAPHY

eluent loading, sample injection, separation of sample, elution of analytes A, and elution of analyte B, shown and explained below. Elution is the process where the compound of interest is moved through the column. This happens because the eluent, the solution used as the solvent in chromatography, is constantly pumped through the column. The chemical reactions below are for an anion exchange process.

1. ***Step 1***: The eluent loaded onto the column displaces any anions bonded to the resin and saturates the resin surface with the eluent anion.
   This process of the eluent ion (E^-) displacing an anion (X^-) bonded to the resin can be expressed by the following chemical reaction:
   $Resin^+\text{-}X^- + E^- <=> Resin^+\text{-}E^- + X^-$
2. ***Step 2***: A sample containing anion A and anion B are injected onto the column. This sample could contain many different ions, but for simplicity this example uses just two different ions ready to be injected onto the column.
3. ***Step 3***: After the sample has been injected, the continued addition of eluent causes a flow through the column. As the sample elutes (or moves through the column), anion A and anion B adhere to the column surface differently. The sample zones move through the column as eluent gradually displaces the analytes
   The continued addition of the eluent causes a flow through the column. As sample elutes, anion A and anion B adhere to the column surface differently. The sample zones move through the column as eluent gradually displaces the analytes.
4. **Step 4**: As the eluent continues to be added, the anion A moves through the column in a band and ultimately is eluted first.
   This process can be represented by the chemical reaction showing the displacement of the bound anion (A^-) by the eluent anion (E^-).
   $Resin^+\text{-}A^- + E^- <=> Resin^+\text{-}E^- + A^-$
5. **Step 5**: The eluent displaces anion B, and anion B is eluted off the column.



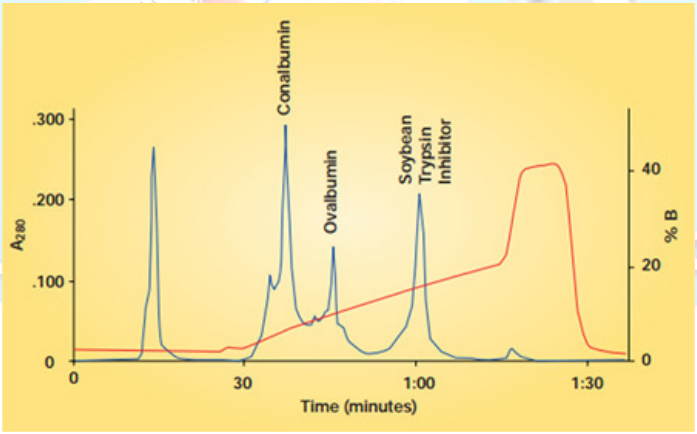


Fig. 3. Salt gradient elution. Elution of proteins (**blue trace**) with an increasing salt gradient (**red trace**).

ION EXCHANGE CHROMATOGRAPHY WORKFLOW

After loading an impure protein sample onto an ion exchange chromatography column, the column is washed to remove undesired proteins and other impurities, and then the protein(s) of interest is eluted using either a salt gradient or a change in pH.

The charged salt ions compete with bound proteins for the charged resin functional groups. Proteins with few charged groups will elute at low salt concentrations, whereas proteins with many charged groups will have greater retention times and elute at high salt concentrations.
Although less common, a pH gradient can also be used for elution. Here, a pH gradient is chosen that approaches the protein of interest's pI. Proteins will elute when the pH gradient reaches their pI, because they will no longer carry a net charge that allows them to interact with the column resin.

To elute proteins from an anion exchange resin, a decreasing pH gradient is chosen, while an increasing pH gradient is chosen for elution from cation exchangers.Since it is very difficult to generate reproducible and accurate linear pH gradients, a step-gradient is generally chosen when pH is used for elution. Lastly, pH can be used to refine elution when using a salt gradient. Altering the pH of the elution buffer can affect the resolution of the method.

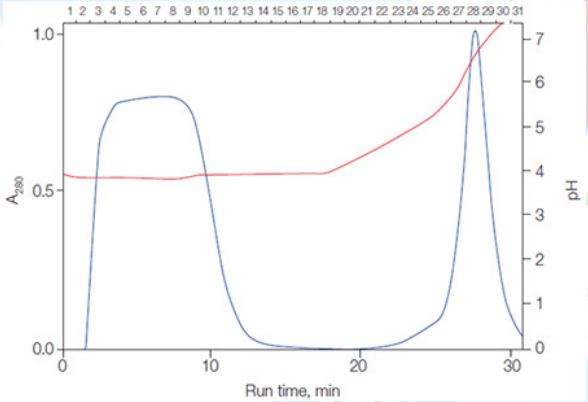


Fig. 4. Elution of protein (blue trace) with an increasing pH gradient (red trace).

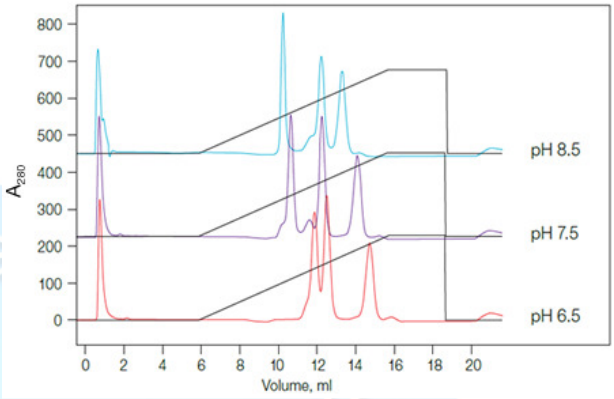


Fig. 5. pH can alter resolution of a method.

Three overlaid chromatograms showing how changing pH from 6.5 to 8.5 shifts the elution profile when eluting using a salt gradient. ***Note:*** *Some proteins fall out of solution at a pH equal to their pI. For these proteins, elution with a pH gradient may not be possible.*

FACTORS AFFECTING ION EXCHANGE SEPARATION

A. Nature and properties of ion exchange resin
B. Nature of exchanging ion

A. **Nature of ion exchange resin:** cross linking and swelling is important factor which depends on proportion of cross linking agent and polystyrene. When more cross linking agent is present they are more rigid but swells less. When swelling is less separation of ions of different sizes is difficult as they cannot pass through the pores present and it becomes selective to ions of different sizes. When less cross linking agent is present, the are less rigid, but swell more., separation may not be efficient as exchange of functional groups does not takes place due to wide pore. Hence and optimum quantity of cross linking agent should be added to the polymeric ion exchange resins for the separation to be effective.

B. **Nature of exchanging ions:**

1. Valency of ions: at low concentrations and at ordinary temperatures extend of exchange increases with increase in valency.

$$Na^{+} < ca^{++} < Al^{+3} < Th^{+4}$$

2. Size of ions : for similar charged ions, exchange increase with decrease in size of hydrated ion

$$Li^{+} < H^{+} < Na^{+} < NH^{4+} < K^{+} < Rb^{+} < Cs^{+}$$

3. Polarizability: Exchange is preferred for greater polarisable ion e.g.

$$I^{-} < Br^{-} < Cl^{-} < F^{-}$$

4. Concentration of solution: In dilute solution, polyvalent anions are generally adsorbed preferentially.
5. Concentration and charge of ions: If resin higher +ve charge and solution has lower +ve charge, exchange

is favoured at higher concentration . If the resin has lower +ve charge and solution has higher +ve charge, then exchange is favoured at low concentration.

RECENT TRENDS IN ION EXCHANGE CHROMATOGRAPHY

Stationary-Phase Architecture

Stationary-phase construction for IC columns comprises nine basic architectures: silane-based modification of porous silica substrates, electrostatic-agglomerated films on nonporous substrates, electrostatic-agglomerated films on ultrawide-pore substrates, polymer-grafted films on porous substrates, chemically derivatized polymeric substrates, polymer-encapsulated substrates, ionic molecules adsorbed onto chromatographic substrates, step-growth polymers on polymeric substrates, and hybrid materials based on a combination of a silane-modified silica substrate with a polymeric exterior surface coating. Five of these — electrostatic-agglomerated films on ultrawide-pore substrates, polymer-grafted films on porous substrates, chemically derivatized polymeric substrates, polymer-encapsulated substrates, and step-growth polymers on polymeric substrates

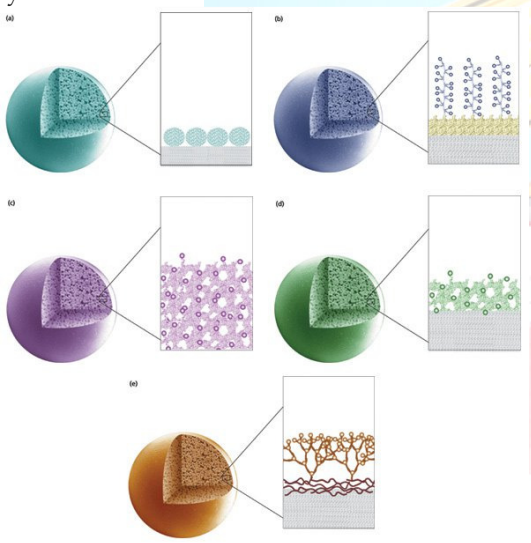


Figure: Ion chromatography stationary-phase architectures most widely used in recently introduced phases: (a) electrostatic agglomerated ultrawide-pore substrates, (b) polymer-grafted film on porous substrates(c) chemically derivatized polymeric substrates, (d) polymer-encapsulated substrates, and (e) step-growth polymers on polymeric substrates.

Electrostatic agglomerated films on ultrawide-pore substrates:

For the most part, electrostatic agglomerated films on nonporous substrates have been largely supplanted by higher capacity versions utilizing ultrawide-pore substrates (Figure 1a). By using an architecture similar to that based on nonporous substrates, but making use of substrates with pore sizes in the 100–300 nm range, it is possible to construct materials with substantially higher capacity (1). The pore size of the ultrawide-pore substrate and the particle size of the colloidal ion-exchange material are chosen such that the pore size is large enough to accommodate a coating of ion-exchange colloid on both the interior and the exterior surfaces of the porous substrate. With the optimal ratio of substrate pore size to colloidal particle size, the resulting material can exhibit 6–8 times the capacity achievable on an identical particle size nonporous substrate (that is, 30–150 μEq/mL for materials using an ultrawide pore format compared to 5–30 μEq/mL for materials using a nonporous format). Given the increasing importance of high capacity chromatographic materials in IC and the availability of high capacity suppressor devices, this stationary phase architecture has seen wide application in recent years.

Polymer-grafted films on porous substrates: This type of material (Figure 1b) is widely used to prepare high capacity packings where crosslinking is not required for selectivity control. Chromatographic materials of this sort are prepared through attachment of polymer strands to the surface of a substrate (2,3). To prepare such materials, the substrate is either prepared with polymerizable groups on the surface, the surface is modified to introduce polymerizable groups, or the surface is modified to introduce an initiator species. Resin, monomer (or monomers), and initiator are then allowed to react to produce a composite polymer graft with polymer strands projecting from the substrate surface. Because including a crosslinking monomer into the reaction mixture will cause the reaction mixture to form a gel with substrate particles suspended in the gel, this synthesis approach precludes the use of crosslinking monomers. The fact that no crosslinker can be used in grafted polymer films limits the ability to control selectivity in such grafted films. This architecture is mainly used in applications that require a stationary phase with relatively high capacity and high water content. Such materials can be prepared from either polymer-based or silica-based substrates, but in practice nearly all such materials are produced using polymeric substrates.

Chemically derivatized polymeric substrates: This type of material (Figure 1c) tends to involve proprietary synthesis techniques, so the actual chemistry used for the derivatization reaction is generally unknown in commercial products. In general, chromatographic materials of this sort have substantial capacity because functional groups are not necessarily limited to the surface of the substrate. Such materials have become popular in recent years as column capacities have shifted higher. The critical difficulty with this stationary-phase synthesis methodology is the requirement that the derivatization be con-

strained to the surface to achieve good chromatographic performance. Reactions that take place beneath the surface in the dense polymer matrix of the substrate will exhibit sluggish mass transport and relatively poor chromatographic performance. Early examples of this stationary-phase architecture exhibited relatively poor performance but newer materials such as the IC SI-52 4E column (Showa Denko) illustrate that high performance materials can indeed be constructed in this manner.

Polymer-encapsulated substrates: Professor Gerard Schomburg of the Max Planck Institute in Mulheim-Ruhr, Germany, pioneered this type of material (Figure 1d) as a means of preparing materials for reversed-phase chromatography using alumina as the base material. Synthesis of polymer-encapsulated materials is accomplished by combining the substrate, a preformed polymer with residual double bonds, and a suitable free radical initiator dissolved in an appropriate solvent, stripping off the solvent to leave a polymer film on the surface of the substrate, and then curing the film at elevated temperature to yield a crosslinked film permanently encapsulating the substrate. The advantage of this architecture is that chemical attachment to the surface of the substrate is not required, allowing it to be used with inorganic substrates not amenable to covalent modification. Although initially developed as a means of producing a reversed-phase material based on alumina, the technique was later adapted by Schomburg's group as a means of preparing a weak-cation-exchange phase using a preformed butadiene-maleic acid copolymer as the encapsulating polymer (4). The first commercial introduction of stationary phases based on this approach brought about a major shift in stationary-phase design as applied for the separation of inorganic cations. Before the introduction of this new synthesis method, nearly all separation products were based on strong-acid cation-exchange stationary phases. Since that time, nearly all stationary phases utilized for the separation of inorganic cations have used weak-cation-exchange carboxylic acid–based stationary phases. A disadvantage of this synthetic approach is the possibility of swelling and shrinking of the phase during gradients or temperature programming depending on the cure conditions of the film. In addition, even if the coating is free of surface defects, alkaline reagents can still attack the underlying silica by penetrating to the surface coating, resulting in bed collapse.

PROS AND CONS OF ION EXCHANGE CHROMATOGRAPHY

Ion exchange chromatography is a very powerful separation technique that is used not only for preparative chromatography but also for analytical chromatography. However, like all other chromatography modes, IEX does have some limitations.

One of the main disadvantages of ion exchange chromatography is its buffer requirement: because binding to IEX resins is dependent on electrostatic interactions between proteins of interest and the stationary phase, IEX columns must be loaded in low-salt buffers. For some applications, this restriction may require a buffer exchange step prior to ion exchange chromatography.

Conversely, its requirement for loading samples in buffers of low ionic strength makes ion exchange chromatography an excellent second purification step after hydrophobic interaction chromatography (HIC).

Ion exchange chromatography, unlike some other chromatography methods, also permits high flow rates, which in some cases can be crucial to the recovery of active protein. Finally, a limitation of weak ion exchangers is their pH dependence. When working outside of their optimal pH range, these resins rapidly lose capacity, and more importantly, resolution.

IEX Pros	IEX Cons
Permits high flow rate	Sample must be loaded at low ionic strength
Concentrates samples	Clusters of positively charged residues can cause a net-negatively charged protein to bind a cation exchanger, and vice versa
High yield	Small changes in pH can greatly alter binding profile of IEX resin
Buffers are nondenaturing	Particle size greatly influences resolution

APPLICATION OF ION EXCHANGE CHROMATOGRAPHY

Application in water Treatments

Water softening and demineralization are also described with chemical reactions in the IX basics page. And regeneration methods are in another page.

1 Softening

A strongly acidic Cation exchange resin is used here in the sodium form. The ions forming hardness, essentially calcium and magnesium, are exchanged for the sodium ions of the resin, and the softened water can be used for several purposes:

- Laundries
- Domestic water boilers
- Low pressure industrial boilers
- Textile

Resins used:

- Amberlite™ IR120 Na, Amberjet™ 1000 Na
- Amberlite SR1L Na for drinking water

2 De-alkalisation

In a water containing bicarbonates — most waters in Western and Central Europe do — calcium and magnesium associated with bicarbonate ions are exchanged for hydrogen ions from a weakly acidic cation exchange resin. This is called removal of temporary hardness. The treated water contains carbon dioxide that can be removed with a degasifier. The salinity of the treated water is lower than that of the feed water. Dealkalisation is used:

- To treat water used to make beverages in breweries and soft drink plants
- To soften drinking water supplies in municipalities
- At home, to filter, soften and partially demineralise the water you use to make tea or coffee
- As a first demineralization step
- For certain industrial processes

3 Demineralization

All ions must be removed from water. Therefore the water passes first through cation exchange resins in the hydrogen form, then through anion exchange resins in the hydroxyl or free base form. All cations are replaced by ions from the cation resin, and all anions for the ions of the anion resin. These H^+ and OH^- ions recombine to create new water molecules (H_2O). The treated water contains only traces of sodium and silica.

Resins used:

- Amberlite IRC86 (weakly acidic resin)
- Amberlite IR120 or Amberjet 1000 (strongly acidic resin)
- Amberlite IRA96 or IRA67 (weakly basic resin)
-

Application in sugar Industries-

1 Softening of beet sugar juices before evaporation

The hardness of beet sugar juices results in scaling of the heat exchanger in the evaporators. To prevent it, increase the thermal efficiency and save energy, it is usual to soften the sugar juice. The plant can then operate continuously, without frequent interruptions required for de-scaling the equipment. In this process, the type of resin used is the same as that for water softening, but the resins must be approved for use with food and resist specific stress due to the temperature and concentration of the juice.

The calcium and magnesium ions present in the sugar juice are exchanged for sodium ions from the resin. The process is applied to thin juice, i.e. after carbonation. In general, several

2 The Gryllus process

This is an older process in which the softening resin is regenerated with thick juice, which contains high concentrations of sodium. The salt consumption is thus reduced, and again, no waste is produced, since the spent regenerant is recycled.

3 Demineralisation of sugar juices before evaporation

In this process, "non-sugars" are removed from thin juice to increase the efficiency of crystalllisation, i.e. the sugar yield. In general, each kilogram of removed non-sugar produces 1.4 kg of additional sugar. Otherwise, the process is similar to water demineralization: a strongly acidic cation exchange resin and a weakly basic anion exchange resin are used, regenerated respectively with acid and caustic soda.

Resins used:

Amberlite FPC14 Na (strongly acidic)
Amberlite FPA53 (weakly basic)

4 Colour removal from cane sugar syrups after evaporation

Cane syrups usually contain many organic compounds imparting colour to the crystallised sugar and reducing the crystallisation yield. The colour removal process uses strongly basic anion exchange resins, regenerated with a sodium chloride solution. These resins are macroporous, so that high molecular mass compounds can be removed. The most efficient method uses two columns in series, the first one filled with acrylic resin, the second, polishing column with styrenic resin.

5 Sugar recovery from molasses

This process is based on ion exclusion, a kind of ion exchange chromatography using fine mesh, uniform particle size resins. It separates sugar from non-sugars and increases the recovery of sugar contained in the molasses.

6 Sucrose inversions

Sucrose (common sugar) is a di-saccharide. In an acidic environment, the sucrose molecule splits into two monosaccharides: glucose and fructose, in equal proportions. Invert sugar has a more powerful sweetening power than sucrose (1.15 vs. 1.0) , and a lower tendency to crystallise, an important feature for some industrial food products. Inversion is produced by passing sugar syrup through a low cross-linked strongly acidic cation exchange resin in the H^+ form.

Resins used:

Amberlite FPC12 H

7 Chromatographic separation

As fructose has a higher sweetening power than glucose (1.3 vs. 0.7), invert sugar syrups can be enriched with

fructose by passing the syrup through a fine mesh, very uniform strongly acidic cation exchange resin in the calcium form. As the syrup stream moves down the column, fructose moves more slowly than glucose. This results in separated bands of higher purity of each component within the column. The fructose fraction is recovered separately in view of its commercial value. The glucose fraction can be either sold as a glucose syrup, or isomerised enzymatically to produce more fructose.

Resins used:

Amberlite CR1320 Ca

8 Glucose demineralisation

Glucose syrups are demineralised to increase purity. The principle is identical to that of water or sugar demineralisation. In view of the high concentration and high temperature of the syrups, resins with a good resistance to these stresses must be used.

Resins used:

Dowex™ 88 (strongly acidic resin)

Dowex 66 (weakly basic resin)

Application in Food Industry-

1 Whey demineralisation

Whey, a by-product of cheese production, contains valuable proteins and is used in the food industry. It is demineralised to increase purity. Again, the principle is the same as that of water or sugar demineralisation.

Resins used:

- Amberlite FPC14 (strongly acidic resin)
- Amberlite FPA51 (weakly basic resin)

2 Beverages

There are several applications in this area:

Treatment of the water used to make beer or soft drinks (see chapter 1)

- De-acidification of beverages with Amberlite FPA51 (weakly basic anion resin)
- Removal of metals
- Removal of bad taste or smell
- Colour and turbidity removal with non-ionic adsorbents

3 Treatment of fruit juices

- Acid removal with Amberlite FPA51 (weakly basic anion resin)
- Removal of bitterness from orange juices with a non-ionic adsorbent resin, Amberlite FPX66
- Colour removal with an adsorbent resin

4 Recovery of polyphenols

Polyphenols are praised today for their anti-oxidant properties. They are found in many types of fruit, such as berries or red grape. Anthocyanins are polyphenols that can be recovered from grape must.

Resins used:

- Amberlite FPX68 (non-ionic adsorbent resin)

5 Citric acid

This acid is used as a preservative in many industrial food products. It is produced by fermentation. Its purification requires ion exchange demineralisation.

6 Aminoacids

L-lysine and other essential aminoacids (not produced by the human body) are produced by fermentation. Lysine is recovered from the fermentation broth with a cation exchange resin in ammonium form.

Resins used:

- Amberlite FPC14 (strongly acidic)

7 Sorbitol demineralisation

Sorbitol is a polyol, a powerful sweetener and emollient used for instance in chewing gum. It can be produced by hydrogenation of glucose or by enzymatic processes. The final product often requires demineralisation.

Resins used:

- Amberlite FPC22 (strongly acidic)
- Amberlite FPA51 (weakly basic)
- Amberlite FPC52 and FPA90 in a polishing mixed bed

8 Gelatine demineralization

Gelatine is produced from the collagen present in pig skin and bones. To produce high purity gelatine, demineralisation is required.

Resins used:

- Amberlite FPC14 or FPC22 (strongly acidic)
- Amberlite FPA53 (weakly basic acrylic)

Application in chemical industries

1 Recovery and removal of metals

In surface finishing and plating shops, metals can be recovered or removed:

- Gold recovery from industrial jewelleries as cyanide complexes, with Amberlite IRA402
- Recycling of various rinse water streams in plating shops, with Amberlite 252 (for cation removal), IRA96 (for chromate), and IRA410 (for cyanide)
- Copper and iron removal from chromium plating shops with Amberlyst 15Wet
- Chromic acid recovery in plating shops with Amberlite IR120 and Amberlite IRA96
- Removal of iron from zinc baths with Amberlite IRC748

- Purification of pickling baths, removing iron and zinc as chloride complexes with Amberlite IRA402. Elution is done simply with water.

2 Production of chlorine and caustic soda

These chemicals are produced by electrolysis of saturated brine. In the production process, the absence of divalent metals is critical. A selective chelating resin is thus used to remove them (principally calcium), which reduces the initial calcium concentration from 10 – 20 mg/L down to a very low level, smaller than 20 μg/L.

3 Phenol

Two applications:

- Removal of sulphuric acid and organic acids from process streams in phenol production. A special weak base resin with a phenol-formaldehyde matrix is used.
- Removal of phenol from industrial waste. Phenol is removed on a non-ionic adsorbent resin. Regeneration is done with acetone.

4 Hydrogen peroxide purification

Resins are used in two different processes:

- Removal of anthraquinone derivatives. These organic compounds can be removed on a non-ionic adsorbent. Regeneration is done with methanol.
- Removal of metal traces such as iron, with a strongly acidic resin. The treatment is done at a very high specific flow rate.

In both cases, the product quality is excellent, with residuals of just a few μg/L. Caution: hydrogen peroxide (H_2O_2) is a powerful oxidant, and serious steps must be taken in both processes to avoid accidents.

Resins used:

- Amberlite XAD4 for organic contaminants
- Amberlyst 15Wet for metat

There are various and complex applications. As the pharmaceutical industry is intrinsically secretive, few details are known. Nevertheless, let us mention a few examples:

Application in Pharmaceutical Industry

1 Extraction and purification of antibiotics

Various antibiotics use ion exchange and adsorbent resins in their production process. The objective is to purify them after extraction from fermentation broths. Examples: streptomycin, gentamycin, cephalosporin, tetracyclin.

Resins used:

- Amberlite XAD1600 (non-ionic styrenic adsorbent)
- Amberlite XAD7HP (non-ionic acrylic adsorbent)

2 Slow-release formulations

Powdered, highly purified ion exchange resins are used as excipients in pharmaceutical formulations. The active ingredient is adsorbed on the resin and is released more slowly in the body than it would if it were present in their original state.

Resins used:

- Amberlite IRP64 (weakly acidic)
- Amberlite IRP69 (strongly acidic)
- Amberlite IRP88 (weakly acidic in potassium form)
- Duolite™ AP143 (strongly basic)

3 Resins used as drugs

The same resin types can be used as active substances in the medicine. It is obvious that they must meet very stringent specifications and be approved by health authorities. Let us mention two examples:

- Cholestyramine, a drug used to reduce the cholesterol level, is a powder based on a strongly basic anion resin in the choride form.
- Polacrilin potassium, a medicine used to regulate the potassium level in the blood, is a powder based on a weakly acidic resin with a methacrylic matrix.

Resins used:

- Duolite AP143 (cholestyramine)
- Amberlite IRP88 (polacrilin potassium)

4 Taste-masking

Similar resins are used to mask the unpleasant taste or smell of a drug.

5 Production chromatography

The chromatographic separation of various molecules used as active ingredients can be done with very fine particle size resins instead of silica gels or other media.

Resins used:

A whole range of products available as Amberchrom resins.

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