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Gel Chromatography or Gel Permeation Chromatography

38.1 Introduction

Gel permeation chromatography (GPC), or exclusion chromatography, uses a porous material as the stationary phase and a liquid as a mobile phase. The diameters of the pores of the porous material are of the order of 50–3000 Å, which is similar to the size of many molecules. The latter penetrate the pores according to their size. Small molecules penetrate more rapidly than larger molecules, which frequently are excluded from the smaller pores present. This results in a difference in the rates at which the molecules pass down the column, the smaller molecules travelling faster than the larger molecules.

Selectivity based on steric factors between the adsorbate and the adsorbent is utilised in achieving specific separations through gel chromatography. The technique is known as *gel filtration, molecular sieve filtration,* and *exclusion chromatography.* All these names essentially refer to the same process.

Gel chromatography is a simple and reliable method for separating molecules according to size. Its versatility makes it generally applicable to the purification of all classes of biological substances, including giant macromolecules not readily fractionated by other technique. Good separations and high activity yields are easily obtained.

In gel permeation, separation takes place according to size of the solute molecule. **Exclusion** takes place according to molecular size. **In molecular filtration** separations are carried out on natural and synthetic zeolites (metal–alumino silicates), called **molecular sieve.** A typical zeolite or molecular sieve has a general formula $M_{2/n}$. Al_2O_3 x. $SiO_2 . yH_2O$, where M is a cation of valency *n*. The molecular sieve contains permanent cavities and channels in their structure. The sieve properties of the zeolite is determined by the size of these cavities, while adsorptive properties of the zeolite are determined by the large surface area and presence of M, Al, Si and O in the zeolite structure.

38.2 Principle

The separation of molecules on the basis of their molecular size and shapes utilises the *molecular sieve* properties of a variety of porous materials. Probably the most commonly used of such materials are a group of polymeric organic compounds which possess a three–dimensional network of pores which confer gel properties upon them. The term *gel filtration* is used to describe the separation of molecules of varying molecular size utilising these gel materials. Porous glass granules have been used as molecular sieves and the term *controlled–pore glass chromatography* introduced to describe this separation technique. The terms *exclusion or permeation chromatography* describe all molecular separation processes using molecule sieves. This section is mainly devoted to gel filtration since its principles and applications are best documented, but it must be appreciated that controlled–pore glass chromatography has much in common with it.

The general principle of exclusion chromatography is quite simple. A column of gel particles or porous glass granules is in equilibrium with a suitable solvent for the molecules to be separated. Large

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molecules which are completely excluded from the pores will pass through the interstitial spaces, while smaller molecules will be distributed between the solvent inside and outside the molecular sieve and will then pass through the column at a lower rate. Three stages in such a column are represented diagrammatically in Fig. 38.1.

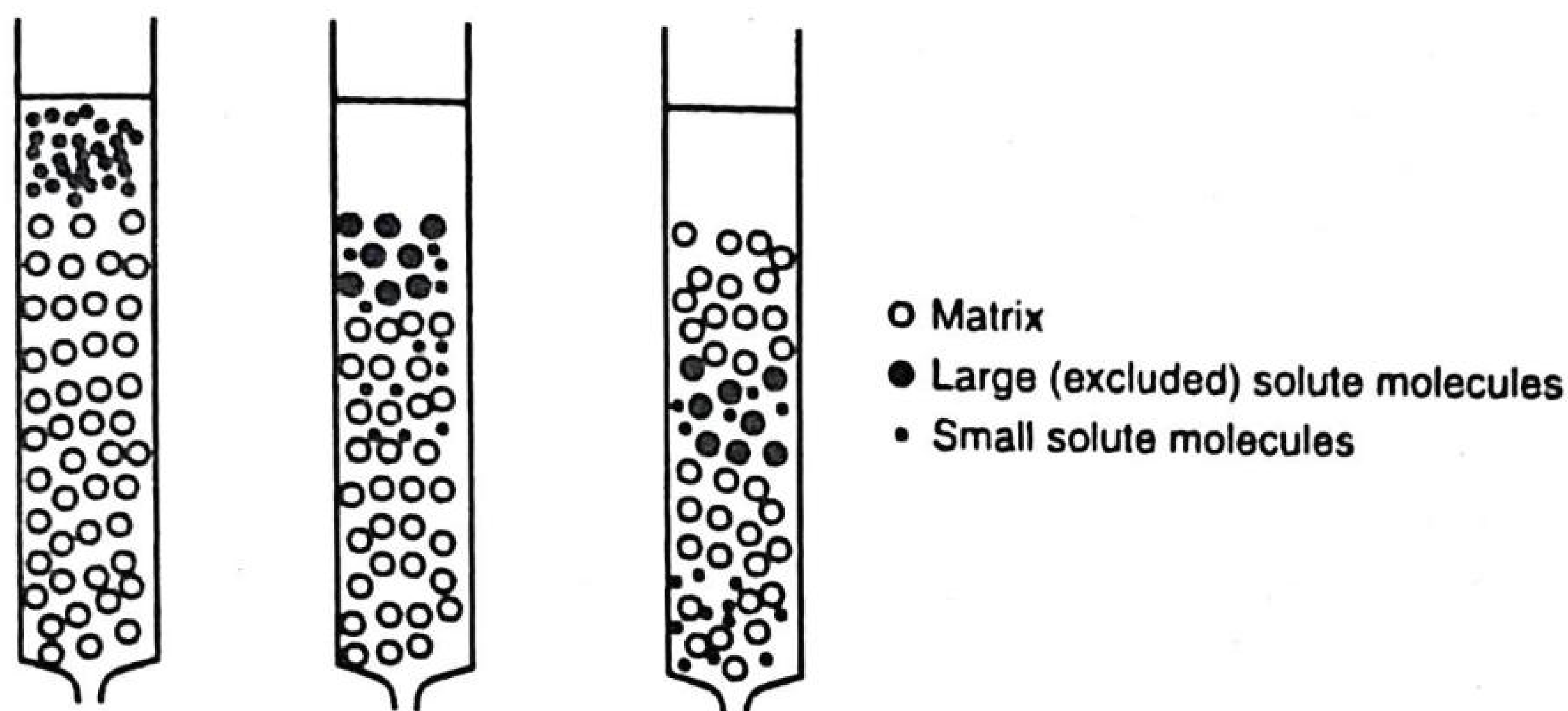

Fig. 38.1 : Diagrammatic Representation of Separation by Exclusion Chromatography.

The solvent absorbed by a swollen gel is available to a solute to an extent which is dependent upon the porosity of the gel particle and the size of the solute molecules. Thus the distribution of a solute in a column of a swollen gel is determined solely by the total volume of solvent, both inside and outside the gel particles, which is available on it.

For a given type of gel, the distribution coefficient, K_d, of a particular solute between the inner and outer solvent is a function of its molecular size. If the solute is large and completely excluded from the solvent within the gel, $K_d = 0$, whereas if the solute is sufficiently small to gain complete accessibility to the inner solvent, $K_d = 1$. Due to variation in pore size for a given gel, there is some inner solvent which will be available and some which will not be available to solutes of intermediate size, hence K_d values vary between 0 and 1. It is this complete variation of K_d between these two limits which makes possible separation of solutes within a narrow molecular size range on a given gel.

The elution volume, V_e of a given solute depends on the void volume, V_0, the distribution coefficient, and the volume inside the gel matrix itself, V_i.

Thus,

$$V_e = V_o + K_d V_i \tag{38.1}$$

The inner volume, V_i can be calculated from the known dry weight of the gel, a, and the *water regain* value, W_r, since :

$$V_i = aW_r \tag{38.2}$$

The numerical value of V_e for a given solute will vary with the size of the column, whereas K_d is a characteristic value for this solute and is independent of the geometry of the gel bed.

For two substances of different molecular weight and K_d values, K_d' and K_d'', the difference in their effluent volumes, V_s, is given by :

$$V_s = V_c' \times V_e'' = (V_0 + K_{d'}V_i) \times (V_0 + K_d'' v_i)$$

therefore,

$$V_s = (K_d' - K_d'')V_i \tag{38.3}$$

Thus, for complete separation of the two substances, the sample volume must not be larger than V_s. In practice, deviations from ideal behaviour, for example due to poor packing of the column, makes it advisable to reduce the sample volume below the value of V_s since the ratio between sample volume and inside gel volume affects both the sharpness of the separation and the degree of dilution of the sample. Equations 38.2 and 38.3 can be used to calculate the optimum bed volume for a given purification.

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It is also possible to undertake gel filtration using the thin layer mode. *Thin layer gel filtration* (TLG) and TLC have much in common, but there are some important distinctions. In TLG a layer of swollen gel is spread onto a glass plate. The gel beads adhere to the plate without the addition of a fixative, and form the stationary phase; the interstitial fluid forms the mobile phase. In contrast to TLC, the layer must not be dried, hence in TLG there is no solvent front. The TLG plate is placed in an airtight container and connected to reservoirs at either end by means of filter paper bridges. The plate is inclined at an angle of 20° to the horizontal, thus facilitating transport of the mobile phase through the layer. Equilibration must be carried out for a minimum of 12 hours. The main function of this equilibration is to normalise the ratio between the stationary and mobile phase volumes. It is possible to use a horizontal plate and produce solvent flow by having the two reservoirs at different levels. The sample is applied as a spots or band and the plate developed for a suitable period. The separated spots are detected by an appropriate method.

Whereas TLC is mainly used for the separation of amino acids, sugars and oligosaccharides, alkaloids, steroids, and lipophilic substances in general TLG is used for the separation of hydrophilic substances requiring mild conditions, such as proteins, peptides, nucleic acids. *i.e.* high molecular weight biological material.

The great advantage of TLG over column gel filtration is that a number of samples can be chromatographed at the same time, under identical conditions. In addition, very small amounts of the sample can be used; that it is ideal for clinical samples.

38.3 Materials

Gels which are commonly used include cross–linked dextrants (trade name Sepahdex), agarose (Sepharose, Bio–Gel A, Sagavac), polyacrylamide (Bio–Gel P), polyacryloylmorphine (Enzocryl Gel) and polystyrenes (Bio–Beads S).

The dextran gels are obtained by cross–linking the polysaccharide dextran with epichlorhydrin. In this way the water soluble dextran is made water insoluble, but it retains its hydrophilic character and swells rapidly in aqueous media, forming gel particles suitable for gel filtration. By varying the degree of cross–linking several types of Sephadex have been obtained. They differ in porosity and consequently are useful over different molecular size ranges. Due to the random distribution of cross–linking there is also a wide distribution of pore sizes in each gel type. This means that molecules of a size below the limit where complete exclusion occurs are either partly or fully able to enter the gel. Each type of Sephadex is characterised by its water regain, *i.e.*, the amount of water taken up in the completely swollen gel granules by one gramme of Sephadex.

Agarose gels, which are produced from agar, are linear polysaccharides consisting of altering residues of D–galactose and 3,6–anhydro L–galactose units. Their gelling properties are attributed to hydrogen bonding of both inter–and intra–molecular type. Due to their hydrophilic nature and the nearly complete absence of charged groups, agarose gels, like dextran gels, cause very little denaturation and adsorption of sensitive biochemical substances. By virtue of their greater porosity they complement the dextran gels. Whereas the latter allow fractionation of spherical molecules such as globular proteins, of dimensions corresponding to molecular weights of up to 800,000 daltons, or randomly coiled polymers like dextran of molecular weights up to 200,000 daltons, the agarose gels may be used to separate molecules and particles up to a molecular weight of several million daltons. They have, therefore, been widely used in the study of viruses, nucleic acids and polysaccharides.

Polyacrylamide gels are prepared by the polymerisation of acrylamide and methylene bisacrylamide. By varying the relative proportions of the two monomers, a range of gels with differing porosities may be obtained. They have characteristics very similar to the dextran and agarose gels. They have a molecular weight exclusion limit ranging from 1800 to 400,000 daltons.

Some commonly used gels are listed in Table 38.1. The Sephadex and polyacrylamide gels must be converted to their swollen form before use whereas the Sephacryl and agarose gels are supplied in the pre–swollen form. Many gels are available in a range of sizes : superfine, fine, medium and coarse. The

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coarser the bead, the better column flow rate but poorer resolution it gives. Thus the fine and superfine beads are preferred for analytical work and the coarse ones for preparative purpose. The *capacity* of a particular gel is a measure of the weight of a solute that can penetrate a particular weight of gel. It therefore gives an indication of the amount of solute that can be separated by a particular column or gel.

Table 38.1 : Some Commonly used Gels for Exclusion Chromatography

Polymer	*Trade Name*		*Fractionation range (Daltons)*	*Bed volume ($cm^3\ g^{-1}$ dry gel)*
Dextran	Sephadex	G10	< 700	2–3
		G25	1×10^3 to 5×10^3	2–3
		G50	1.5×10^3 to 3×10^4	4–6
		G100	4×10^3 to 1.5×10^5	15–20
		G200	5×10^3 to 6×10^5	30–40
	Sephacryl	S200	5×10^3 to 2.5×10^5	...
		S300	1×10^4 to 1.5×10^6	...
		S400	2×10^4 to 8×10^6	...
Agarose	Sepharose	2B	1×10^4 to 4×10^6	...
		4B	6×10^4 to 2×10^7	...
		6B	7×10^4 to 4×10^7	...
	Bio–Gel	A5m	1×10^4 to 5×10^6	...
		A15m	4×10^4 to 1.5×10^7	...
		A50m	1×10^5 to 5×10^7	...
		A150m	1×10^6 to 1.5×10^8	...
Polyacry–lamide	Bio–Gel;	P2	1×10^2 to 1.8×10^3	3–4
		P6	1×10^3 to 6×10^3	7
		P30	2.5×10^3 to 4×10^4	11
		P100	5×10^3 to 1×10^5	15
		P300	6×10^4 to 4×10^5	30

38.4 Gel Preparation, Column Packing and Detectors

The methods which are generally used for the preparation of gel are :

1. The dry powder of the gel is allowed to swell in the solvent which is to be used as eluant. In actual practice a weighed amount of the dry powder of the gel is mixed with solvents and allowed to swell. The mixture is kept until the equilibrium is established.
2. In the second method gel slurry is warmed at about 100°C in a water bath. As a result the gel swells in few days. The slurry is cooled and packed in the column.

There are generally two types of columns packings; porous glasses or silicas and porous crosslinked organic gels such as dextrans, methacrylate–based gels, polyvinyl alcohol–based gels, and hydroxethyl cellulose gels. The detectors used are based on UV fluorescence, UV absorption, or changes in refractive index.

38.5 Applications

Gel chromatography has been used with great success in the separation of the sugars, polypeptides, proteins, liquids, asphalts, butyl rubbers, polyethylenes, polystyrenes, silicon polymers and others. The extensive analytical applications of GPC cover both organic and inorganic materials. Gel permeation chromatography (GPC) has been mainly applied to studies of complex, biochemical or highly polymerised molecules.

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The main application of gel chromatography is the separation and characterization of molecules of different molecular weights. Very often it becomes possible to separate molecules of similar molecular weights by a proper selection of the appropriate gel and column length. Another important application is the separation of large molecules of biological origin from inorganic and ionizable species. This is termed as *desalting*. For example, employing a column of Sephadex gel, haemoglobin can be separated from sodium chloride. Fractionation of mixtures of biopolymers is the most widespread application of gel chromatography. The method can also be used for preparative purposes. However, the main applications of the gel chromatography are as follows :

(*a*) **Purification.** The main application of exclusion chromatography is in the purification of biological macromolecules. Viruses, proteins, enzymes, hormones, antibodies, nucleic acids and polysaccharides have all been separated and purified by the use of appropriate gels or glass granules. Mixtures of lower molecular weight compounds may also be separated. Thus amino acids can be separated from peptides, and peptides obtained from the partial hydrolyses of a protein can be fractioned, as can oligonucleotides from a nucleic acid hydrolysate. Low molecular weight dextrans, in such mixtures as corn syrup oil, can also be separated.

(*b*) **Molecular Weight Determination.** The effluent volumes of globular proteins are largely determined by their molecular weight. It has been shown, that over a considerable molecular weight range, the effluent volume is approximately a linear function of the logarithm of the molecular weight. Hence, the construction of a calibration curve, with proteins of a similar shape and known molecular weight, enables the molecular weight of other proteins, even in crude preparations, to be estimated.

(*c*) **Solution Concentration.** Solutions of high molecular weight substances can be concentrated by the addition of dry Sephadex G–25 (coarse). Water and low molecular weight substances remain in solution. After ten minutes the gel is removed by centrifugation, leaving the high molecular weight material in a solution whose concentration has increased but whose pH and ionic strength are unaltered.

(*d*) **Desalting.** By use of a column of Sephadex G–25, solutions of high molecular weight compounds may be desalted. The high molecular weight substances move with the void volume while the low molecular weight components are distributed between the mobile and stationary phases and hence move slowly. This method of desalting is faster and more efficient than dialysis. Applications include removal of phenol from nucleic acid preparations, ammonium sulphate from protein preparations as well as monosaccharides from polysaccharides and amino acids from proteins.

(*e*) **Protein–Building Studies.** Exclusion chromatography is one of a number of methods commonly used to study the reversible binding of a ligand to a macromolecule such as protein including receptor proteins. A sample of the protein/ligand mixture is applied to a column of a suitable gel (*e.g.* G–25) which has previously been equilibrated with a solution of the ligand of the same concentration as that in the mixture. The sample is eluted with the buffer in the standard way and concentration of ligand and protein in the effluent determined. The early fractions will contain unbound ligand, but the subsequent appearance of the protein will result in an increase in the total amount of ligand (bound plus unbound). By repeating the experiment at a series of ligand concentrations the appropriate binding constants can be calculated.

The chromatogram obtained results from a separation based on the sizes of the sample molecules, which are directly related to the molecular weights of the components. If the sample is single polymer, the chromatogram represents the molecular weight distribution. This method is very valuable for determining the molecular weight distributions of polymers up to very high molecular weights. The actual calculation is usually based on a comparison with a standard polymer compounds, such as proteins. Gel permeation chromatography is also capable of separating different polymers from each other and, under the correct conditions, mixtures of polymers can be characterized with respect to the percentage and weight range of each polymer present.

Care must be taken in interpreting the results, since the separation is based on the size of the molecule rather than the actual molecular weight. This means that the shape of molecule has a significant

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effect on the results. For example, two molecules of the same molecular weight, one straight chained and the other highly branched, will give somewhat different results because each has a different penetrating power through the column. This must be taken into account when selecting standards for calibrating the results.

38.6 Advantage of Gel Chromatography

An advantage of gel permeation chromatography is that it can be carried out at room temperature and the sample are not decomposed because of exposure to high temperature. This is especially important when dealing with very high molecular weight compounds, which are easily broken into two or three fragments, resulting in great changes in apparent molecular weight.

TEST YOUR KNOWLEDGE

Q.1. What is gel permeation chromatography?

Q.2. What are different names of gel chromatography?

Q.3. Discuss the principle of gel permeation chromatography.

Q.4. Which gels are most commonly used?

Q.5. Write a short note on gel preparation.

Q.6. What are the main applications of gel chromatography?

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Ion Exchange Chromatography

39.1 Introduction

For many years it has been known that clays include metal ions in their composition and that these ions can change places with other metal ions in their vicinity. This observation was important to the understanding of soil chemistry. Zeolites (sodium aluminum silicates) have some properties similar to those of clays. Using this phenomenon, natural and artificial zeolites became useful in removing Ca^{2+} and Mg^{2+} from water. The Ca^{2+} and Mg^{2+} ions dissolved in the water are exchanged for Na^{+} ions from the zeolite, thereby providing soft water. Ion exchangers have been created by combining a polymer and a functional group. Such ion exchanges have properties very similar to those of zeolites. Two types are available, cationic and anionic exchanges. Both use a resin as the insoluble inert support, but they use different functional groups in order to provide the type of exchanger necessary.

The phenomenon of ion exchange was first reported by two British agricultural chemists in 1850 who proved that soil can remove potassium or ammonium salts from water with release of an equivalent amount of the calcium salt. After the work of *Gans* in 1913, natural and synthetic inorganic cation exchangers were used for softening hard water. Modern ion exchange resins were first used in 1935 by *Adams* and *Holms*. These resins consist of three–dimensional net work of polymeric chains cross–linked with short chains containing ionisable functional groups. The fundamental work on chromatographic separations by the use of ion exchange resins is mainly derived from the studies carried out in connection with the work on atomic energy in the USA.

39.2 Definition

Ion exchange may be defined as a reversible reaction in which free mobile ions of a solid called ion exchange are exchanged for different ions of similar charge present in solution.

Most ion exchangers for practical use consist of an insoluble organic polymer into which a charged group has been introduced in some suitable manner The backbone is generally a styrene–divinyl–benzene copolymer. Copolymers of acrylic acid derivatives diviny–benzene are also frequently used. For use with biological macromolecules, it is usual to introduce charged groups into celluose fibres. For several years, cross–linked dextran (Sephadex) has been also used as carrier material.

The most common properties of all ion exchangers as follows:

(i) They are almost insoluble in water and organic solvents such as benzene, carbon tetrachloride, ethe etc.,

(ii) They are complex in nature, *i.e.,* infact they are polymeric.

(iii) They have active or counter ions that will exchange reversibly with other ions in a surroundin solution without any substantial change in the material.

39.3 Principle

The principal feature underlying this form of chromatography is the attraction between opposite charged particles. Many biological materials, for example amino acids and proteins, have ionisable grou

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and the fact that they may carry a net positive or negative charge can be utilised in separating mixtures of such compounds. The net charge exhibited by such compounds is dependent on their pK_a and on the pH of the solution in accordance with the Henderson–Hasselbalch equation.

Ion–exchange separations are mainly carried out in columns packed with an ion–exchanger. There are two types of ion–exchanger, namely *cation and anion exchangers*. Cation exchangers posses negatively charged groups and these will attract positively charged molecules. These exchangers are also called *acidic ion–exchange materials* since their negative charges result from the protolysis of acidic groups. Anion exchangers have positively charged groups which will attract negatively charged molecules. The term *basic ion exchange materials* is also used to describe these exchangers since positive charges generally result from the association of protons with basic groups.

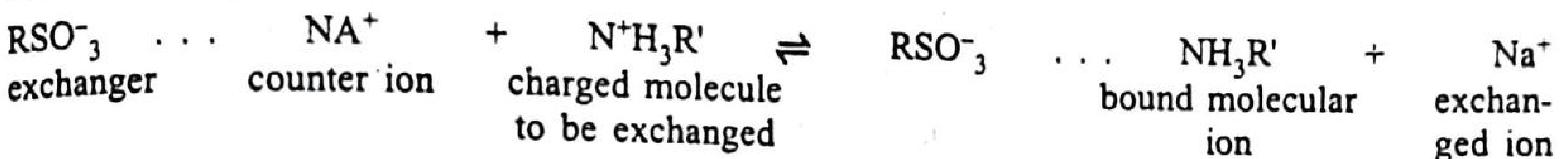
The actual ion–exchange mechanism is thought to be composed of five distinct steps :

(i) diffusion of the ion to the exchanger surface. This occurs very quickly in homogeneous solutions;

(ii) diffusion of the ion through the matrix structure of the exchanger to the exchange site. This is dependent upon the degree of crosslinkage of the exchanger and the concentration of the solution. This process is thought to be the feature which controls the rate of the whole ion–exchange process;

(iii) exchange of ions at the exchange site. This is thought to occur instantaneously and is an equilibrium process:

Cation exchanger :



$$\underset{\text{exchanger}}{RSO^-_3} \;\ldots\; \underset{\text{counter ion}}{NA^+} + \underset{\text{charged molecule to be exchanged}}{N^+H_3R'} \rightleftharpoons RSO^-_3 \;\ldots\; \underset{\text{bound molecular ion}}{NH_3R'} + \underset{\text{exchanged ion}}{Na^+}$$

Anion exchanger :

$$(R)_4N^+ \;\ldots\; Cl^- + {}^-OOCR' \rightleftharpoons (R)_4N^+ \;\ldots\; {}^-OOCR' + Cl^-$$

The more highly charged the molecule to be exchanged, the tighter it binds to the exchanger and the less readily it is displaced by other ions;

(iv) diffusion of the exchanged ion through the exchanger to the surface;

(v) selective desorption by the eluant and diffusion of the molecule into the external solution. The selective desorption of the bound molecule is achieved by changes in pH and/or ionic concentration or by affinity elution, in which case an ion which has greater affinity for the exchanger than has the bound molecule is introduced into the system.

The rate of ion exchange is controlled by the law of mass action. For example, in the case illustrated above, Na^+ from solution will replace H^+ from the resin. However, if every strong acid is washed through the resin, the high concentration of H^+ in the acid will by mass action reverse the process and replace the Na^+ from the resin.

At all times the metal ions present are in competition for the functional groups. In general, at equal concentrations the functional group sites are taken by the metal with the greatest affinity for that group. The affinity is controlled as follows.

1. The ion with the highest charge has the highest affinity, that is, $Na^+ < Ca^{2+} < Al^{3+}$
2. The ion with the greatest size (and charge) has the highest affinity, that is, $Li^+ < Na^+ < K^+ < Cs^+ < Be^{2+} < Mg^{2+} < Cu^{2+} < Sn^{2+}$

If the ion becomes too large, distortion of the resin takes place and the rule breaks down.

39.4 Cation Exchangers

A cation exchanger is a high molecular weight, cross linked polymer having sulphonic, carboxylic, phenolic etc., groups as an integral part of the resin and an equivalent amount of cations. Thus, a cation exchanger is nothing but a polymeric anion to which active cations are attached. In these cation exchangers,

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the hydrogen ions are mobile and exchangeable with other cations. The anions ($-COO^-$, SO^{2-}_3 and $-O^-$) remain attached to the resin network.

When a cation exchanger is kept in a solution of a salt, some of the H^+ ions of the resin enter the solution and in equivalent amount of the cations of the salt get attached to the resin. This reaction may be represented as follows :

$$\underset{\text{(Resin)}}{H_nR} + \underset{\text{(Solution)}}{nNa^+} \rightleftharpoons \underset{\text{(Resin)}}{NanR} + \underset{\text{(Solution)}}{nH^+}$$

Some commercially available cation ion exchangers are given in Table 39.1.

The resin having solium ions, produced in the above reaction, can exchange these ions with other cations. The reaction with calcium ions may be represented as follows :

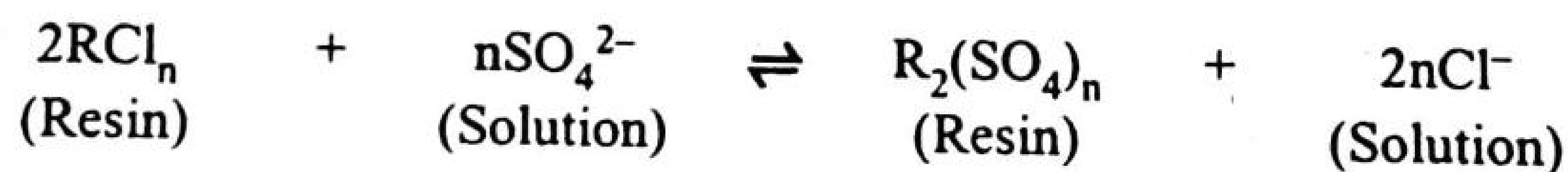
$$2Na_nR + nCa^{2+} \rightleftharpoons Ca_n R_2 + 2nNa^+$$

Table 39.1 : Some Commercial Available Cation Exchangers

Trade Name	*Functional Group*	*Framework Material*
1. Amberlite IR–120	$-SO_3H$	Styrene/divinylbenzene (copolymers homoporous)
2. Dowex	$-SO_3H$	—do—
3. Zerolit	$-SO_3H$	—do—
4. Amberlite–200	$-SO_3H$	Same framework material as above but heteroporus
5. SE cellulose	$-C_2H_4-SO_3H$	Cellulose
6. Amberlite IRC–50	$-COOH$	Methacrylic acid/divinyl
7. CM	$-CH_2\ COOH$	cellulose, fibrous

39.5 Anion Exchangers

An anion exchanger is a polymer having amine or quaternary ammonium groups as integral parts of the resin and an equivalent amount of anions such as Cl^-, SO_4^{2-}, OH^- ions, etc. These anions are mobile and exchangeable. The anions exchange behaviour of these materials may be represented as follows :



$$\underset{\text{(Resin)}}{2RCl_n} + \underset{\text{(Solution)}}{nSO_4^{2-}} \rightleftharpoons \underset{\text{(Resin)}}{R_2(SO_4)_n} + \underset{\text{(Solution)}}{2nCl^-}$$

$$\underset{\text{(Resin)}}{RCl_n} + \underset{\text{(Solution)}}{nOH^-} \rightleftharpoons \underset{\text{(Resin)}}{R(OH)_n} + \underset{\text{(Solution)}}{nCl^-}$$

Some commercially available anion exchangers are given in Table 39.2.

Table 39.2

Trade Name	*Functional Group*	*Framework Material*
1. Amberlite IRA–400	$-CH_2-N^+(CH_3)_3$	Styrene/divinyl benzene copolymers (homoporous)
2. Zerolit FF–IP	—do—	Same as above but (heteroporous)
3. Amberlite IRA–410	$-CH_2-N^+(CH_3)(CH_3)-CH_2-CH_2$ OH^-	Styrene/divinyl benzene copolymers (homoporous)
4. Zerolit N–IP	—do—	Same as above but isoporous
5. QAE Sephadex A–25	$-\overset{\vert}{\underset{\vert}{N}}-$	Dextran

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39.6 Regeneration

Anion exchangers are generally supplied in the form of salts, amines, in particular, are stable only in this form. On the other hand, cation exchangers can be converted into the H^+ form by treatment with aqueous acid followed by washing the ammonium base having hydroxyl groups from the strongly basic anion exchanger on treatment with sodium hydroxide while the weakly basic anion exchangers are converted into free amines. Whatever the form is in which the resin is to be used, it should always be submitted to the full cycle of charging and discharging before use. Carrying out this step enhances its effective capacity.

39.7 Ion Exchange Column Used in Chromatographic Separations

The principle of ion–exchange chromatography is based upon the simple fact that different cations (or anions) have different capacity to undergo exchange reaction on the surface of a given exchanger. The capacity of an ion to undergo exchange reaction has been found to depend upon the charge and the size of the hydrated ion in solution. Under similar conditions the capacity has been found to increase in the charge on the ion (*i.e.*, the valency of the ion) but has been found to decrease with the increase in the size of the hydrated ion.

If we compare other hydrated ions of the same size, it is observed that the ionic charge plays an important role to determine their capacity to undergo exchange reaction. According to this view, the capacity amongst cations has been found to decrease in the following order.

$$Th^{4+} > Al^{3+} > Ca^{2+} > Na^+$$

Similarly, the capacity amongst anions of same size has been found to decrease in the following order :

$$PO_4^{3-} > SO_4^{2-} > I^-$$

If we compare the ions carrying the same charge the size of the hydrated ion plays an important role to determine their capacity to undergo exchange reactions. Thus amongst the univalent cations, the capacity has been found to decrease in the following order.

$$H^+ > C_S^+ > Rb^+ > K^+ > NH_4^+ > Na^+ > Li^+$$

Amongst the doubly charged cations, the capacity has been found to decrease in the following order :

$$Ba^{2+} > Pb^{2+} > Sr^{2+} > Ca^{2+} > Ni^{2+} > Cu^{2+} > Co^{2+} > Zn^{2+} > Mg^{2+}$$

Amongst the univalent anions, the capacity has been found to decrease in the following order :

$$I^- > NO_3^- > Br^- > CN^- > Cl^- > OH^- > F^-$$

If the active polyvalent ion in a resin is to be exchanged with an ion of lower valency, the exchange has been found to be favourable by using much higher concentration of the solution.

The quality of an ion exchange resin is determined by its capacity which in turn depends upon the total number of ion active groups per unit weight of the material. Greater the number of ions, the greater the capacity of the resin for the exchange process.

The efficiency of the resin has been found to depend upon the degree of cross–linking, *i.e.*, the greater the cross linking, the higher the efficiency of the resin.

39.8 Selection of Suitable Systems

It is useful to classify ion exchangers according to the pK values of their ion groups. However, Table 39.3 gives a simplified survey of their working ranges.

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Table 39.3 : Operating Range of Commonly Used Ion Exchangers

Specification of Ion Exchanger	*Ionic Groups*	*Suitable Chromatographic Medium*
Strong acid	$—SO^-_3$	Acidic and alkaline
Weak acid	$—COO^-$	Only alkaline
Strong base	$—N^+R_3$	Acidic and alkaline
Weak base	$—NHR_2$	Only acidic

For practical use the dissolved material must be present also in the dissociated form if it is to be retained by the resin. It is most convenient to work at a pH where one of the components will be retained while the other does not interact with the ion exchanger.

The concentration of the participating ions of opposite charge also plays an important role. The retained ions can be eluted with the aid of a high concentration of cations or anions without changing the pH and hence, the dissociation relationships.

39.9 Ion Exchange Capacity

An ion exchange resin may be characterised by the number of ion active groups. This is called the *total ion exchange capacity*. It is expressed in terms of milliequivalents per gram of ion exchange resin. The exchange capacity of a cation exchange resin is usually found in the laboratory by determining the number of milligram equivalents of sodium ion which are adsorbed by 1g of the dry resin in the hydrogen form. A solution of sodium chloride of known concentration is passed through a column containing a known weight of the resin and the acid eluting from the column is collected and titrated with a standard solution of an alkali. In V ml of an alkali solution of strength N in milliequivalents per litre is required to titrate all the acid eluted from the resin weighting W g, then,

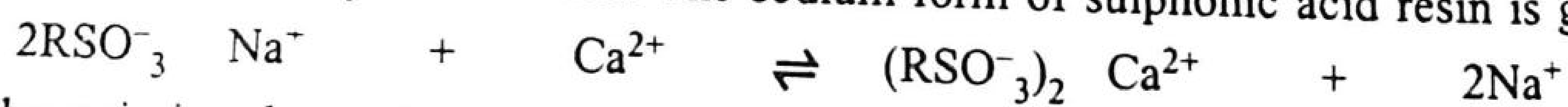
$$\text{Capacity} = \frac{V \times N}{W}$$

Similarly, the exchange capacity of an anion exchanger is determined by passing a solution of sodium nitrate of known concentration through a column. The concentration ions or the hydroxide ions in the solution eluting from the column is found by titration with a standard solution of silver nitrate or acid respectively.

39.10 Ion Exchange Techniques

Two techniques are generally used to bring solutions in contact with ion exchange resins. They are (*i*) batch method and (*ii*) column method.

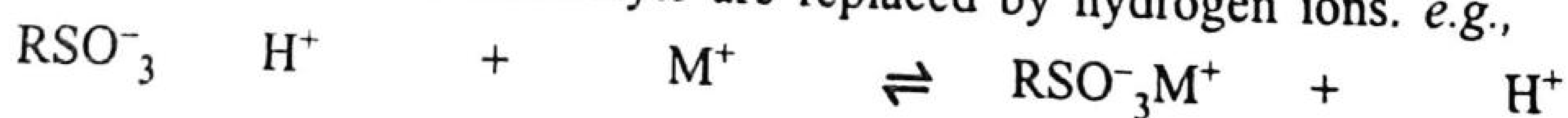
(a) **Batch Method.** This involves a single step equilibrium. The resin and the solution are mixed in a vessel until equilibrium is attained. The solution is then filtered off. The extent to which ions from the solution are exchanged for those on the resin depends upon the selectivity coefficient. In a single step equilibrium of this type, only a small portion of the exchange capacity of the resin is utilised. The batch method is used for softening of water and the production of deionised or demineralised water. Softening of water involves an exchange of calcium and magnesium ions, which cause hardness, by sodium ions. The sodium form of sulphonic acid resin is generally used.



$$2RSO^-_3 \;\; Na^+ \quad + \quad Ca^{2+} \quad \rightleftharpoons \quad (RSO^-_3)_2 \;\; Ca^{2+} \quad + \quad 2Na^+$$

When the resin is exhausted, a 15% solution of sodium chloride is added to the resin. This removes the calcium or the magnesium ions from the resin by exchange with sodium ions.

Deionised or demineralised water is water from which all cations and anions of electrolytes have been removed. It is prepared by treating water with a cation exchanger in the acid or the hydrogen form. All the cations of the electrolyte are replaced by hydrogen ions. *e.g.*,



$$RSO^-_3 \;\; H^+ \quad + \quad M^+ \quad \rightleftharpoons \quad RSO^-_3M^+ \quad + \quad H^+$$

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The water is then treated with an anion exchanger in the basic or the hydroxide form.

$$R^+OH^- + Cl^- \rightleftharpoons R^+Cl^- + OH^-$$

Combination of H^+ and OH^- ions forms water almost completely free from ions of any dissolved electro-lytes. Deionised water finds a large number of applications in the laboratory. Its conductivity equals that of conductivity water prepared by distillation, which is a time consuming process. Deionised water can be used in conductance experiments and for preparing solutions of silver nitrate. Deionised water is also used in biological studies. Biologists have very often to prepare culture media of precise composition. If the solutions are prepared even in distilled water, the ions present can introduce sources of uncertainty into the experiments. Use of deionised water eliminates this uncertainty and errors. Deionised water is also used in lead storage batteries and industrial boilers. Commercial units to prepare deionised water for use in laboratories are now available.

(b) **Column Method : Ion Exchange Chromatography.** Essentially, ion exchange chromatography is a technique in which separation of the components of a mixture is brought about by taking advantage of the different selectivity coefficients for the resin. The differences in the selectivity coefficient leads to different migration rates on an ion exchange column. As explained in chromatography, there are three important methods, *viz.*, frontal analysis, elution analysis and displacement development. The principles of these techniques are also applicable to ion exchange chromatography.

The apparatus used in the column method, consists of a glass column fitted with a glass wool plug or a sintered glass disc at the lower end. An ordinary burette can also be used. The column is shown in Fig. 39.1. The resin to be used should have a small particle size. This provides a large surface area for contact between the solution and the resin. In general, the diameter of the resin beads should be less than one–tenth of that of the column. Dry resin should never be used to pack a column. A slurry of the resin is made in distilled water and any fine particles are removed by decantation. The slurry is then slowly poured into the column containing some water. Care being taken that no air bubbles are formed in the packed column. To ensure that no air bubbles remain in the column and that the resin is uniformly distributed, the column is 'backwashed' with distilled water or deionised water, Backwashing is done by running water up from the bottom of the column. This removes air bubbles trapped in the column and also removes any fine particles of the resin that might have remained in the column. The flow of water is stopped and the resin allowed to settle. The excess water is then drained off.

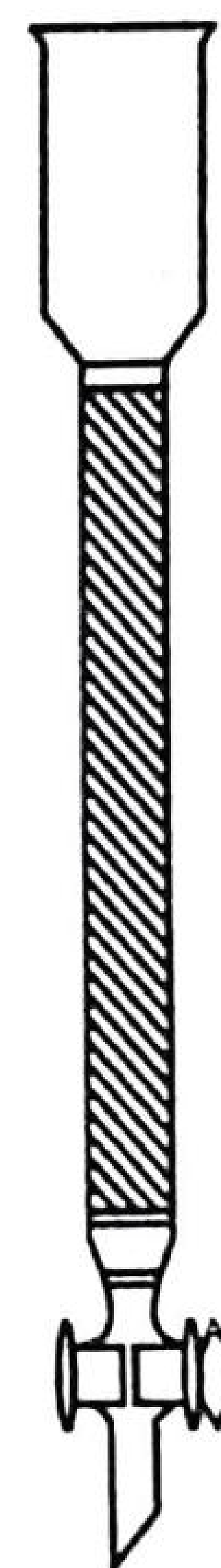

Fig. 39.1 : Ion Exchange Column.

However, the level of water must never be allowed to fall below that of the surface of the resin; as otherwise, the resin may dry up and channels may be formed in the resin bed. In such a case, there will be incomplete contact between the solution and the resin when the column is subsequently used.

39.11 Applications of Ion Exchangers

Because of the particular selectivity forces of material, separation with ion exchange is used mostly in inorganic chemistry. Organic ions which form salts with the oppositely charged ions in another phase can be separated too. Almost all ion exchanger reactions require aqueous solvents. Some application of ion exchangers are as follows :

(a) **Separations of Similar Ions from One Another.** Ion exchange chromatography is being used to separate similar ions from one another because the different ions undergo exchange reactions to different extents. For instance, a mixture of Li^+, Na^+ and K^+ ion can be separated by passing their solution through a cation exchanger. Subsequently, 0.1 N HCI has been used as an eluant.

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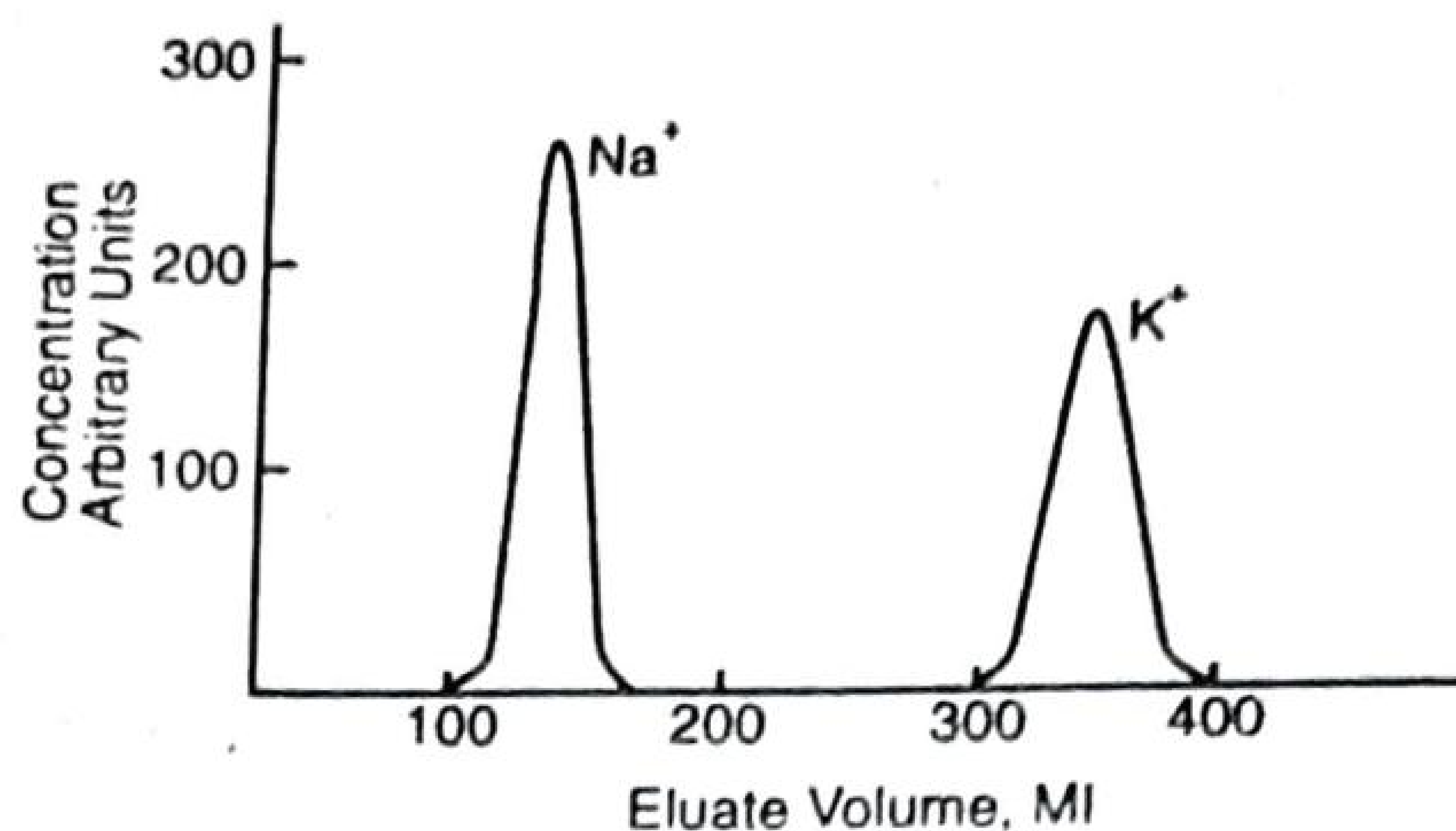


Fig. 39.2 : Separation of potassium from sodium on Dowex 50 × 12 (in the hydrogen form) by elution with 0.6 M HCl

Similarly, ion exchange chromatography has been used to separate a mixture containing Cl^-, Br and I^-. The mixture solution is passed through a basic anion exchanger. Sodium nitrate solution has been used as an eluant. When 0.5 N sodium nitrate is used, Cl^- ion will come first in the eluate. If the concentration of sodium nitrate is raised, Br^- will be eluted next and by further increasing the concentration of sodium nitrate, I^- ions will be eluted.

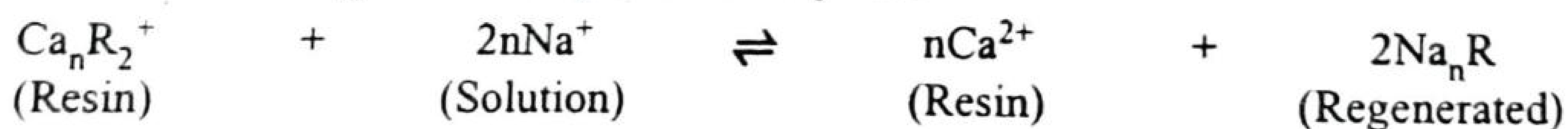
Fig. 39.2 gives a typical chromatographic separation of alkali metal ions with an acid eluant.

(b) **Removal of Interfering Radicals.** In the estimation of Ca^{2+} or Ba^{2+} ions by the oxalate or sulphate method, phosphate ion is found to interfere. Therefore, its removal becomes necessary which is achieved by passing a solution of Ca^{2+} or Ba^{2+} ions having phosphate ions through a sulphonic acid cation exchanger. The Ca^{2+} or Ba^{2+} ions get exchanged with H^+ ions while the phosphate ions will pass as such through the column. The process has to be repeated so that the phosphate ions are completely removed. Now, the Ca^{2+} and Ba^{2+} ions held by resin will be removed by using suitable eluant. Finally, these ions are estimated by the usual methods.

(c) **Softening of Hard Water.** We are aware of the fact that the hardness of water is due to the presence of Ca^{2+}, Mg^{2+} and other divalentions. These ions may be removed by passing hard water through cation exchangers charged with Na^+ when the following exchange reaction takes place

$$\underset{\text{(Resin)}}{2Na_nR} + \underset{\text{(Hard water)}}{n\,Ca^{2+}} \rightleftharpoons \underset{\text{(Resin)}}{Ca_nR^{2+}} + \underset{\text{(Solution)}}{2nNa^+}$$

The Ca^{2+} and Mg^{2+} ions are retained in the column whereas the Na^+ ions pass into solution. These Na^+ ions are harmless for washing purposes. After using the ions exchanger for a long time, it becomes inactive. Its activity can be revived by percolating through it a concentrated solution of NaCl when the following reverse reaction takes place.



$$\underset{\text{(Resin)}}{Ca_nR_2^+} + \underset{\text{(Solution)}}{2nNa^+} \rightleftharpoons \underset{\text{(Resin)}}{nCa^{2+}} + \underset{\text{(Regenerated)}}{2Na_nR}$$

(d) **Complete Demineralisation of Water.** This requires the removal of cations as well as anions. For their removal, the water is first passed through an acidic cation exchanger when the metallic cations (Na^+, Ca^{2+}, Mg^{2+} etc.) are exchanged by H^+ ions. The water obtained from cation exchanger is then passed through a basic anion exchanger when the anions commonly present in water (Cl^-, NO^-_2, SO^{2-}_4 etc.) are exchanged by OH^- ions of the exchanger. The H^+ and OH^- ions which pass into solution in exchange for cations, and anions respectively combine to form unionised water. Generally sulphonic acid resin is employed as the cation exchanger while a strong basic resin is employed as the anion exchanger.

Regeneration of the mixed bed has been done in an elegant manner by making one resin of lower specific gravity than the other. Flotation can then be used to separate them and each resin is given its specific regeneration treatment.

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(e) **Separation of Lanthanides.** During the second world war the separation of fission products presented a major problem to analytical chemistry, especially the mixture La, Sm, Eu, Y, Ce, Pr, Nd and Pm as well as the mixtures of Cs, and Rb, Sr and Ba and Zr and Nb in tracer amounts. To separate these synthetic resins were employed in combination with the technique of chromatographic elution. When the solution having a mixture of lanthanides is passed through a column packed with particles of a suitable ion exchange resin, the cations present in solution undergo exchange with the hydrogen or any other cation that may be present in the ion exchanger. The cation having the maximum capacity to undergo exchange reaction gets held near the top while the other cations get held further down in the column in the order of their decreasing capacities to undergo the exchange reaction. When elution is done with a citrate buffer solution, the La^{3+} citrate complex ion having maximum preference for eluant comes out first while the La^{3+} citrate complex ion having minimum preference comes out last. A typical elution curve of lanthanide ions using buffered ammonium citrate solution as the eluant has been shown in Fig. 39.3.

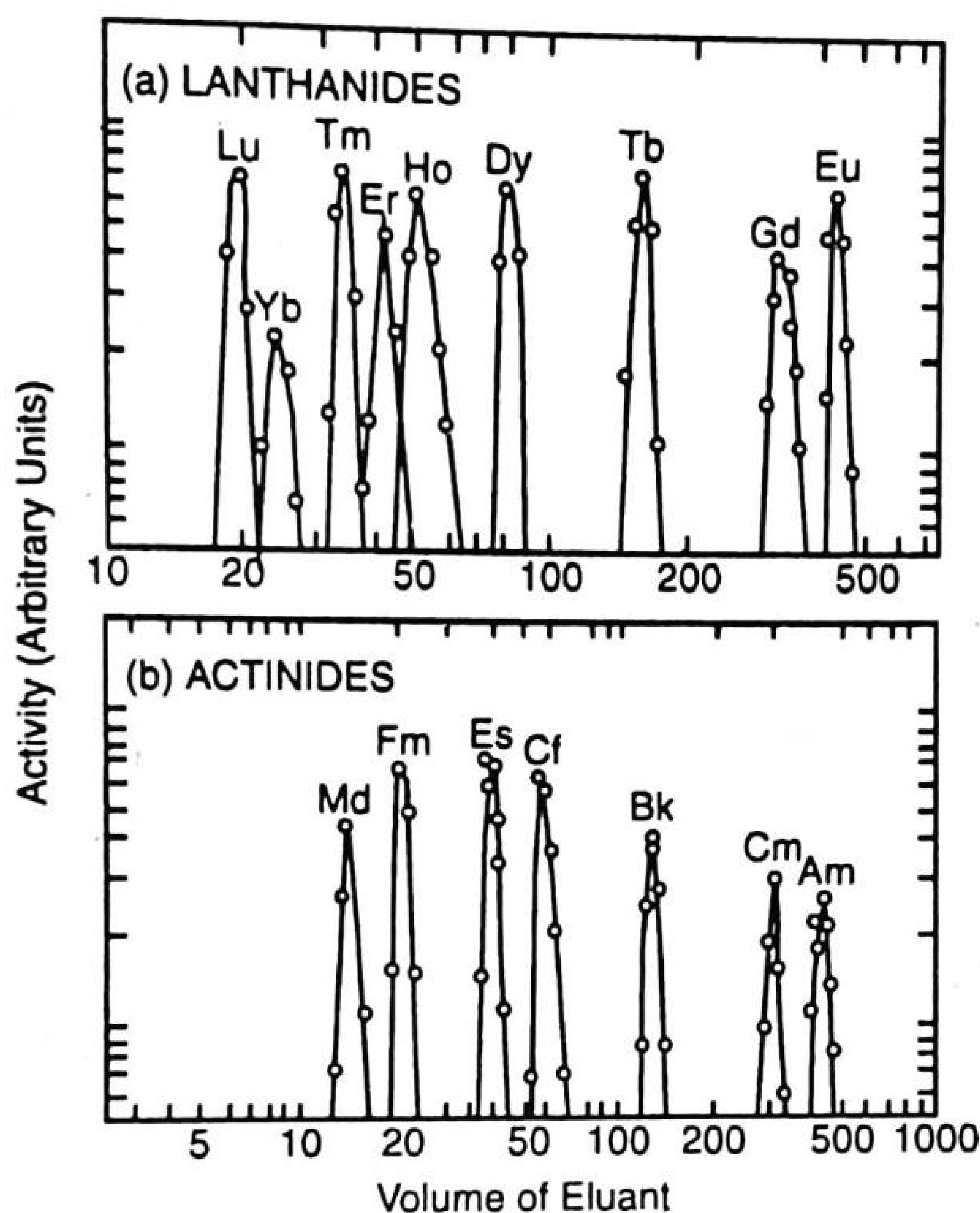

Fig. 39.3 : Elution of tripositive lanthanide and actinide ions on Dowed 50 cation exchange resin with ammonium citrate and hydroxyisobutyrate as in eluant. The order and the spacing of elution which led to the discovery of transcurium elements can be seen.

Improved separations employing other complexing agents like H_4 EDTA and a variety of hydroxy and other amino acids have led to the facile separation of kilogram quantities of the lanthanides in the highest degree of purity.

(f) **Separation of Actinides.** The ion exchange chromatographic technique has played a unique role in the discovery of the transplutonium elements in the actinide series. The power of the method can be judged from Fig. 39.3. which compares the order of elution of the lanthanide (4f) and actinide (5f) ions in the +3 oxidation state from a cation exchange resin column with an aqueous solution of ammonium hydroxyisobutyrate. In the actinide series also the elution occurs in the reverse order of the atomic number due to *actinide contraction* and this proved to be the only way for identifying these elements, some of them having been produced only in atom quantities. It may be said that their isolation would have been impossible by any other separation method.

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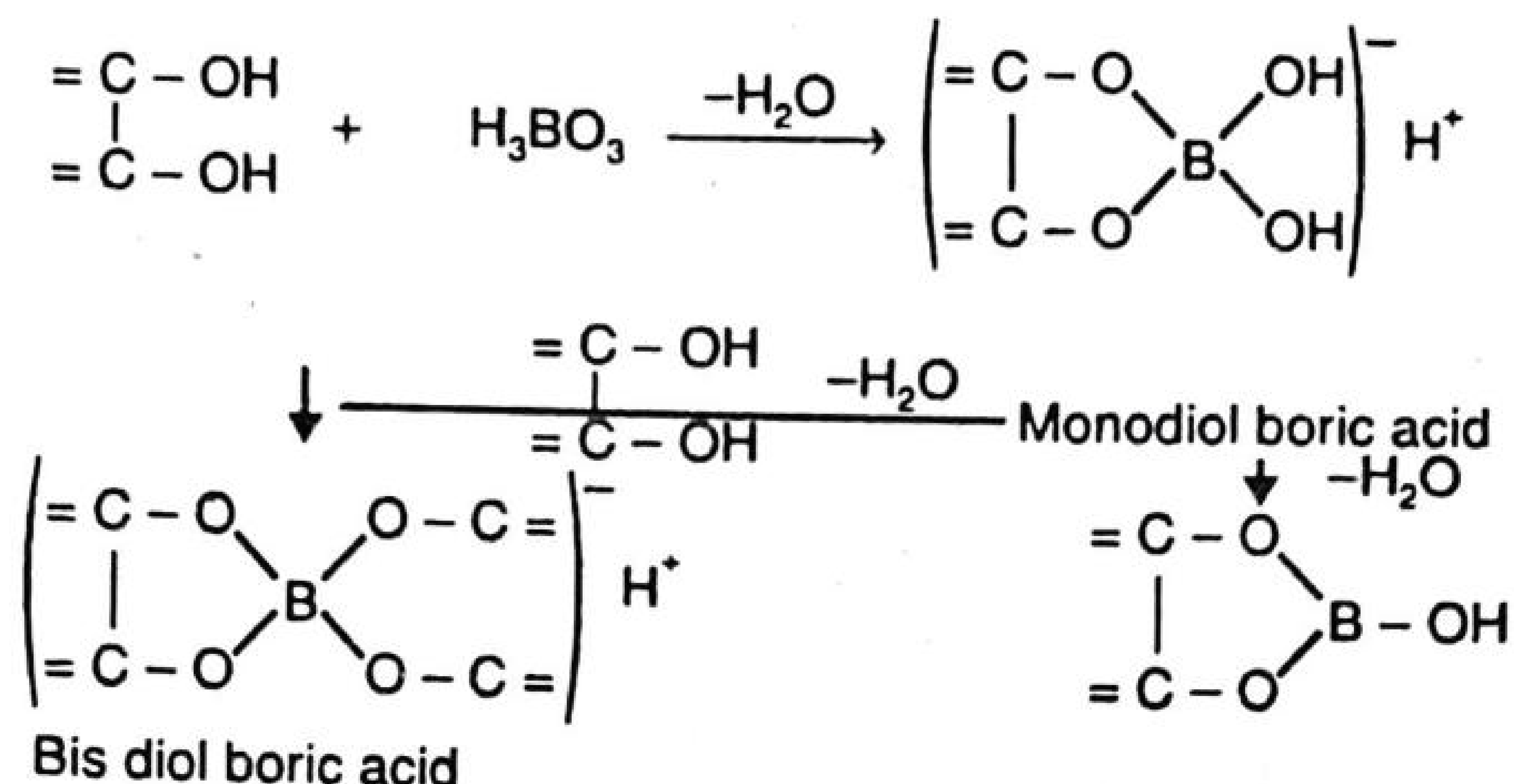
(g) **Purification of Organic Compounds Extracted in Water.** Many natural products extracted in water have been found to be contaminated with ions originally present in water. Those ions can be removed by using ion–exchange processes.

(h) **Separation of Sugars.** This method was developed by *Khym* and *Zill* (1951). These sugar are first of all converted into borate complexes.



Separations of borate complexes of sugars have been achieved on 11 × 0.9 cm columns of 200–400 mesh Dowex 1 resin, using a loading of 5–10mg of borate complex and flow rates of 0.5–1 ml/min. Quantitative recovery of sugars is possible after separation of the borate complexes. Similarly, disaccharides can be separated from the monosacharides and the individual compounds of hexose and pentose mixtures resolved.

(i) **Separation of Amino Acids.** Ion exchange chromatography has been used to separate the complex mixture of 18 aminoacids obtained by the acid hydrolysis of proteins. Fig. 39.4, shows the analysis of a test mixture having all amino acids in molar ratios. The area under the peaks is a measure of the amount of material used which can be employed for calibration purposes.

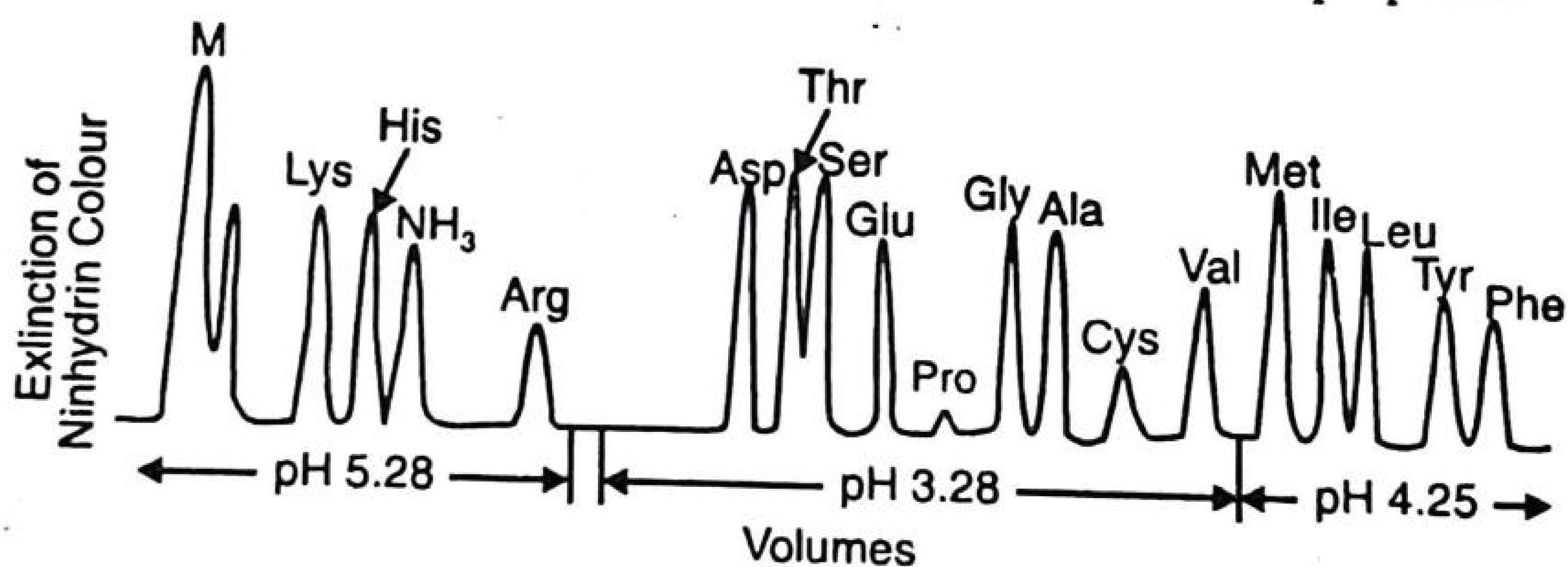


Fig. 39.4 : Mixed amino acids in an amino acid analyser chromatographed on special polystryene sulphonic acid resin.

The mixture of amino acids is first introduced into very short column at *p*H2 and eluted with 0.35 N sodium citrate buffer at *p*H 5.25, with which the column has been equilibrated. Acidic and neutral amino acids at first leave the column unseparated as is shown by the first peaks (M). They are followed by lysine, histidine, NH_3 and arginine. Now a second sample is chromatographed into a longer column with 0.2 N sodium citrate buffer at *p*H 3.28 and subsequently eluted with 0.88 N citrate buffer at *p*H 4.25.

A diagrammatic representation of such an analyser is shown in Fig. 39.5. The effluent from the column is mixed with ninhydrin colour reagent and nitrogen is also introduced to break the effluent stream into discrete bubbles. The mixture is heated to 105°C to develop the colour, the intensity of which is then determined by two colorimeters, one set at 570 nm to monitor the majority of the

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amino acids and a second set at 440 nm to specifically monitor the colour produced by proline and hydroxyproline. Alternatively, the amino acids many be detected by conversion to derivatives which fluoresce. Whilst this dispenses with the need for two detectors, the method is more tedious and generally less reproducible than the ninhydrin method. Many amino acid analysers use two separate columns, the second one containing an anion exchanger to separate the basic amino acids and ammonia faster and more effectively.

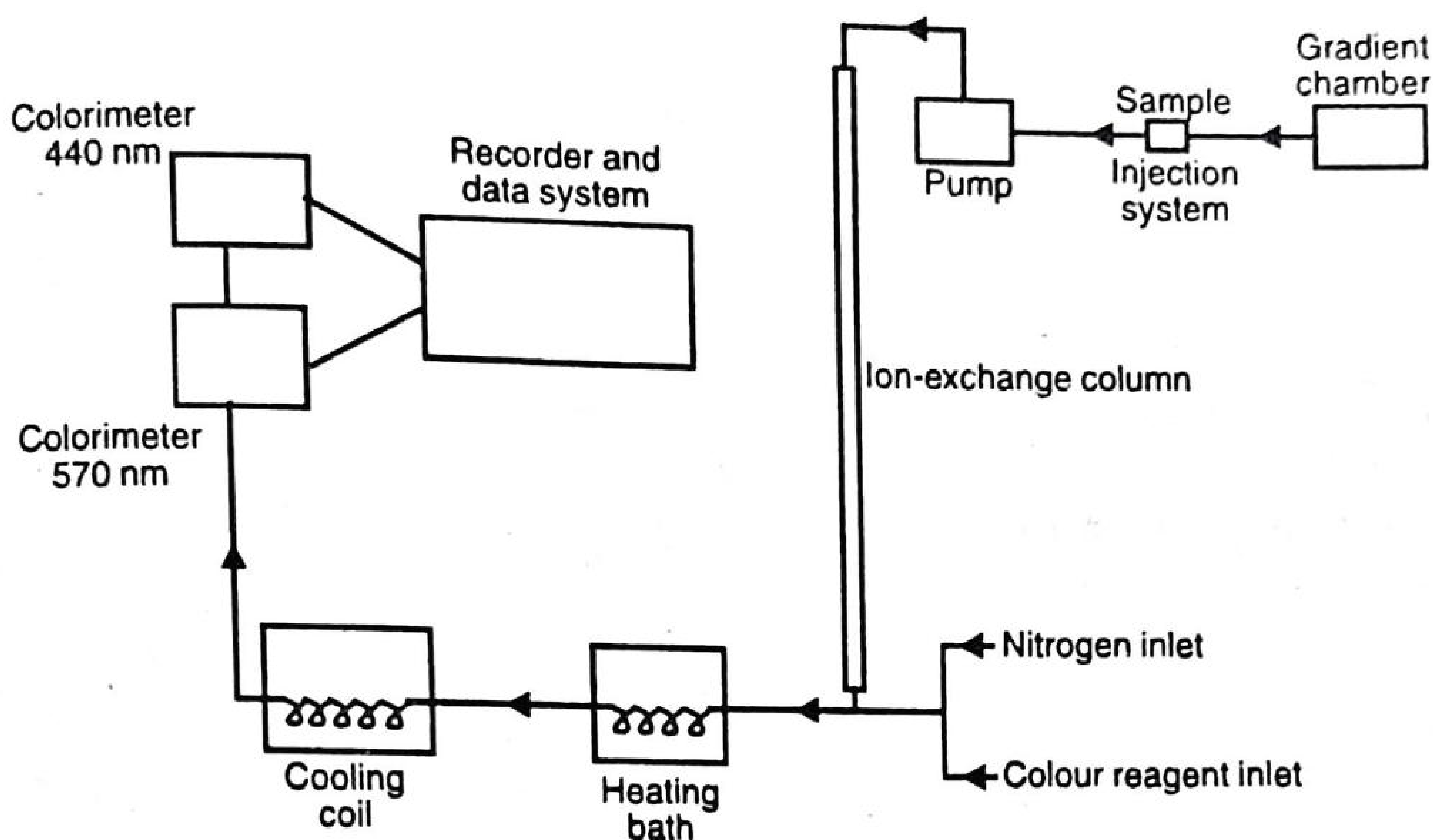

Fig. 39.5 : Diagrammatic representation of an amino acid analyser.

(j) **Preparation of Pure Reagents.** Carbonate is invariably present in solutions of sodium hydroxide used for volumetric determinations. This gives rise to errors in acid–base titrations. The simplest way is to remove carbonate by allowing the solutions to pass through a column of a strongly basic anion exchange resin in the hydroxide form when the carbonate is absorbed releasing an equivalent concentration of hydroxide.

The preparation of tetramethyl ammonium hydroxide which is not available in the required degree of purity, can be undertaken by passing a solution of tetraethyl ammonium chloride through a bed of a cation exchanger which will be converted to the tetraethyl ammonium form. The bed is then washed with water followed by passing sodium hydroxide. The tetramethyl ammonium hydroxide is obtained in the effluent ready for use.

Another example of commercial importance involves the preparation of silicic acid from sodium silicate. During the passage of the silicate through the hydrogen form of a cation exchanger, the sodium ions of the silicate get replaced by hydrogen ions from the hydrogen form of the cation exchanger to yield silicic acid. Compared with the silicic acid formed by treatment of a silicate with mineral acids, silicic acid produced by ion exchange is non–flocculent.

(k) **llydrometallurgy.** Many metals are recovered and purified commercially by ion exchange. Examples include, uranium, thorium, lanthanides, actinides, gold, silver and platinum. In some cases, the scale of operation is relatively small *e.g.*, lanthanides, But the intrinsic value of these metals is very high. In addition, the separation and recovery of trace amounts of toxic metals from effluent and waste streams is an important application from environmental considerations. Some examples include the recovery of chromium from spent metal–plating solutions, and copper and zinc from effluents in rayon and synthetic fibre industry.

Ion exchange is especially used for treatment of low–grade uranium deposits. The ore in Rossing uranium mine in Namibia is having about 0.035% U_3O_8. Continuous cation exchange process is

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used to upgrade the low concentration feed 0.19 g/litre U_3O_8 and produce an cluate feed of 3.49g/litre. This is then fed into a liquid–liquid extraction plant that performs the effective separation of uranium from trace impurities. The final strip solution contains about 10g/litre of U_3O_8 which after precipitation produces a produce containing approximately 97% U_3O_8 by weight.

The extraction of uranium from sea water occurring in concentrations as low as 10^{-8}% by the ion exchange technique is being studied extensively. Resins incorporating the chelating amido oxime group show specificity towards uranium.

TEST YOUR KNOWLEDGE

Q.1. Explain why it is difficult to assess the activity of an ionic species held in the pores of an ion exchanger.

Q.2. Suggest reason for the observation that species are eluted in the order of decreasing molecular size.

Q.3. "Ion exchange materials may be classified in terms of acidic or basic strength of the functional groups attached to the polymer matrix". Explain this statement.

Q.4. Write an essay on the analytical applications of ion exchangers, including discussion on the use of these materials to remove interferants, purify chemicals, and concentrate trace components in solution.

Q.5. Briefly outline the advantages which may be gained by preparing specific ion exchange resins which contain functional groups known to react selectively with particular groups of cations.

Q.6. Explain the term "selectivity coefficient" and indicate its relationship to the equilibrium constant for an exchange reaction.

Q.7. Indicate the significance of molecular adsorption in non–ionic retention.

Q.8. Explain how the pore size in synthetic polymers is influenced by the degree of cross–linking and the degree of swelling.

Q.9. Outline the role of diffusion in ion exchange chromatography.

Q.10. Ion exchange resins charged in particular forms have been proposed as catalysis for a range of reactions. Suggest some advantages and disadvantages which might arise from this approach.

Q.11. Describe the role of ion exchanges in demineralisation of water.

Q.12. Explain the separation of lanthanides by ion exchange method.

Q.13. What are the principles of separation of metals by ion exchange chromatography?

Q.14. What is meant by cation exchangers and amino exchangers?

Q.15. Discuss the application of ion exchange chromatography in :

(a) separation of lanthanides

(b) softening of hard waters

(c) complete demineralisation of water

(d) purification of organic compounds extracted in water

(e) removal of interfering radicals in quantitative analysis.

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