Chapter 1

General: Introduction and Literature Survey
1.1 Introduction to Chromatography

Analytical chemistry is primarily the chemistry of the identification and separation of substances. Analytical Chemistry deals with number of separation techniques such as solvent extraction, chromatography, electrophoresis etc. Chromatography is general term applied to a wide variety of separation technique of great effectiveness. Chromatography involves a sample being dissolved in a mobile phase. The mobile phase is then forced through an immobile, immiscible stationary phase. It depends upon the equilibrium distribution of the solute molecule between two phases, one of which is stationary phase and other is mobile phase. Chromatographic separation methods have caused revolutionary developments in the field of chemistry and several other related branches of science. Many complicated problems in chemical analysis have been completely solved by this amazingly simple analytical technique.

Chromatography and other similar separation methods were of limited use even from ancient times; through the principles underlying these remained unrecognized. An American geologist and mining engineer David Talbot Day and a Russian botanist Michael Tswett (Mikhail Semenovich Tswett, 1872-1919) [1] were the two pioneers in adsorption chromatography. In the beginning of 19th century, the investigation of Tswett on chloroplast pigments lead to the invention of chromatographic technique, the remarkable outcome of his experiment was the concept of an adsorption and elution sequence. Michael Tswett in 1906 first used the term chromatography (color-writing, derived from the Greek, for chroma-color and graphein-write) to describe his work on the separation of colored plant pigments into bands on a column of chalk and other materials such as the polysaccharides, sucrose and induline [2-4]. In spite of the clear demonstration by Day and Michael Tswett, the technique they invented remained practically ignored for a number of years. It was only in 1931 when Kuhn and Lederer separated the carotenoids and
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xanthophils on column of alumina and calcium carbonate. Rapid development of the field took place, owing to the work of Booskmann, Karrer, Winter Stein, Zechmeister and other. In 1938, Reichsten introduced liquid chromatography, thus extending the applicability of the technique colourless substances. During 1940-50s the development of ion exchange, partition and column chromatography and the initial studies on gas adsorption chromatography were carried out. In 1960s there was rapid rise in the use of chromatographic techniques. Now it is an accepted routine technique, particularly in chemistry, biology, medical, pharmaceutical and environmental studies and in quality control. During the development years three chromatographers received Nobel Prizes, Tiselius (Sweden) for his work on ‘Electrophoresis and Adsorption Analysis’ and Martin and Synge (UK) for the ‘Invention of Partition Chromatography’. Martin-Syng model provided a foundation for the development of quantitative theories of partition, ion exchange and gas chromatography. The history of chromatography and revolutionary developments in adsorption chromatography has been covered in great detail in various monographs [5-10]. The book worthwhile to mention in the field of extraction chromatography is by Braun and Ghersini [11]. Important aspects of extraction chromatography have also been covered in ‘Advances in Chromatography’ by Cerrai and Ghersini [12]. The important reviews in this field are published by Alimarin and Bilshova [13], Brinkman [14-16], Cerrai [17], Katykhin [18], Kiba [19-20], O’Laughlin [21]. Developments in engineering techniques, microelectronics and microcomputers and new materials, particularly over the last decade, have enabled manufacturers to produce reliable complex automated instruments. These incorporate sophisticated programmable features to set-up and control the instruments and collect and process data, producing chromatogram and analytical reports which lead to enormous use of HPLC, GC, HPLC-MS, GC-MS, HPTLC and Flash Chromatography in the pharmaceutical industries and research laboratories.
1.2 What is Chromatography?

Chromatography is a non-destructive process for resolving a multicomponent mixture into its individual fractions. Chromatographic separation process is governed by distribution of solute between two phases, i.e. the mobile phase and stationary phase.

1.2.1 Definition of Chromatography [22]

*Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary while the other moves in a definite direction.*

1.2.2 Classification of Chromatography

The Chromatography methods are classified as,

a) according to the nature of stationary phase

b) according to the nature of mobile phase.

The four main subdivisions of chromatographic processes are shown in Figure 1.1. The system is called as adsorption chromatography, if the stationary phase is solid and as partition chromatography, if the stationary phase is liquid.
<table>
<thead>
<tr>
<th>CHROMATOGRAPHIC PRINCIPLE</th>
<th>Type of mobile phase</th>
<th>Type of stationary phase</th>
<th>Type of chromatography</th>
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<tbody>
<tr>
<td>Adsorption chromatography</td>
<td>Gas</td>
<td>Column</td>
<td>Gas–solid chromatography (GC/GSC)</td>
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<td>Competition between a</td>
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<td>solid adsorbent and the</td>
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<td>Partition chromatography</td>
<td>Liquid</td>
<td>Column</td>
<td>Liquid column chromatography (LC)</td>
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<td>Competition between a</td>
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<td>High performance liquid chromatography (HPLC)</td>
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<td>liquid stationary phase and the</td>
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<td>mobile phase</td>
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<td>Ion exchange chromatography</td>
<td>Liquid</td>
<td>Column</td>
<td>Gas–liquid chromatography (GC/GLC)</td>
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<td>Competition between an</td>
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<td>Supercritical fluid chromatography (SFC)</td>
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<td>ion exchange resin stationary phase and</td>
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<td>Permeation chromatography</td>
<td>Liquid</td>
<td>Column</td>
<td>Liquid–liquid chromatography (LC)</td>
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<tr>
<td>Competition between a</td>
<td></td>
<td></td>
<td>High performance liquid chromatography (HPLC)</td>
</tr>
<tr>
<td>polymer matrix and liquid mobile phase</td>
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**Figure 1.1** Classification of chromatographic methods.
1.3 Reverse phase partition Chromatography

Extraction Chromatography, also known as reversed phase partition chromatography. As the name implies the phases used in liquid-liquid partition chromatography are reversed. This means that instead of hydrophilic stationary phase we use the hydrophobic phase as the stationary phase on a suitable stationary support. Similarly instead of using the hydrophobic phase as the mobile phase, hydrophilic phase is used as the mobile phase. Related to this method many articles [23-30], reviews [31-43], monographs [44-46] and bibliographies [47-49] are available.

The materials used as stationary support are Teflon (polytetrafluoroethylene) or Kieselghur, silica gel, alumina, kcl-f (polytrifluorochloroethylene), co-polymer, amberlyst, polyethylene etc.

The range of extractants used for impregnating the support is virtually unlimited. The most frequently used extractant for extraction chromatography are tributyl phosphate (TBP), methyl isobutyl ketene (MIBK), tri-n-octyl phosphine oxide (TOPO), di-(2-ethyl-hexyl) orthophosphoric acid and aliquat-336 (methyl tri-n-alkyl ammonium chloride).

1.4 Theory of Extraction Chromatography

According to Gibb’s phase rule, this relates distribution of two phases,

\[ P + V = C + 2 \]  \hspace{1cm} (1.1)

with usual notations, According to Nernst distribution law,

\[ K_d = \frac{C_o}{C_w} \]  \hspace{1cm} (1.2)

Where \( C_o \) represents concentration of organic phase and \( C_w \) concentration of aqueous phase. The constant \( K_d \) is independent of initial solute concentration. However, here the activity coefficient of the species in the organic and aqueous phase is not considered. Also if chemical reactions such as association, dissociation or polymerization are present, then it will modify the value of \( K_d \). Therefore the term distribution ratio (D) is favored in broader sense which relates the total concentration of species in the organic and aqueous phase by an expression,
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\[ D = \frac{\text{Total concentration of solute in organic phase}}{\text{Total concentration of solute in aqueous phase}} \]  

(1.3)

This is stoichiometric ratio, which includes all the species of solute in organic and aqueous phases. In the absence of any chemical reaction under idealized condition the magnitude of distribution ratio and partition coefficient is same. However, for all practical purposes the term percentage extraction (\(\% E\)) is preferred, which is related to distribution ratio (\(D\)) by relationship,

\[ D = \frac{(V_w / V_o)E}{100 - E} \]  

(1.4)

\[ E = \frac{100 \times D}{D + (V_w / V_o)} \]  

(1.5)

where '\(V_w\)' and '\(V_o\)' represents volume of an aqueous and organic phases respectively. In limiting of cases when the extraction is quantitative i.e. \(E = 100\), \(D\) becomes infinity. For the separation of the elements to be effectively carried out by solvent extraction, it is absolutely necessary that the magnitude of the distribution ratio of individual elements should be sufficiently different. For instance the separation factor (\(\alpha\)) is usually measured to ascertain the effectiveness of separation. This is related to the individual distribution of two species (1 and 2) as,

\[ \alpha = D_1 / D_2 \]  

(1.6)

The magnitude of separation factor gives an idea about feasibility of the separation. When the difference between distribution ratios of exceedingly small the multiple extraction method like continuous or counter current extraction must be resorted, for successful separation.

The technique of partition chromatography [50] is considered to be analogous to counter current extraction wherein the components are distributed
between two liquid phases, one of which is held on the stationary support and other is mobile phase. The basic phenomenon is the partition of species to be separated in between two phases. The usual liquid-liquid partition chromatography is either organic solvent or extractant immiscible with the aqueous phase. But in reversed phase extraction chromatography, the mobile phase is aqueous phase while stationary phase is the extractant.

1.4.1 The theoretical plate model of chromatography

Martin and Synge [9] put forth the plate theory to explain the efficiency of separation. The plate model supposes that the chromatographic column contains a large number of separate layers, called theoretical plates. Separate equilibrations of the sample between the stationary and mobile phase occur in these "plates". The analyte moves down the column by transfer of equilibrated mobile phase from one plate to the next.

It is important to remember that the plates do not really exist; they are a figment of the imagination that helps us to understand the processes at work in the column. They also serve as a way of measuring column efficiency, either by stating the number of theoretical plates in a column, \( N \) (the more plates the better), or by stating the plate height; the Height Equivalent of a Theoretical Plate (the smaller the better) (HETP).

In extraction chromatography it is considered that the column is composed of infinitesimally small plates which are kept upon one another. When the liquid travels down the column during separation, equilibrium is attained in a single plate which is similar to the attainment of equilibrium in a separating funnel in batch extraction. As the liquid travels down the column the equilibrium is attained in the second plate and then in subsequent plates and this process continues until liquid comes out of the column.

The overall efficiency of the separation largely depends upon two factors:
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a) Height Equivalent of Theoretical Plate \((HETP)\) and

b) Number of theoretical plates \((N)\).

If \(L\) is the height of column and \(N\) is the number of plates, then \(HETP\) is given by the expression,

\[
HETP = \frac{L}{N} \tag{1.7}
\]

The efficiency of chromatographic column is determined by the total number of theoretical plates \((N)\) which is given by the expression

\[
N = 16 \times \left(\frac{V_R}{W}\right)^2 \tag{1.8}
\]

Where \(V_R\) is retention volume, while \(W\) is the base width of elution peak.

In chromatography, migration of solute is generally referred relative to that of wash liquid or mobile phase. A sample band which interacts with the stationary phase migrates down the chromatographic bed at some fraction of the velocity of the mobile phase. This fraction is called \(R_t\) the retardation factor,

\[
R_t = \frac{\text{Rate of movement of solute band}}{\text{Rate of movement of solvent front}} \tag{1.9}
\]

The degree of retardation of a particular substance in a mixture is often expressed quantitatively in terms of retention time \(t_R\). Retention time is defined as the time that elapses from the moment the sample is introduced, to the point of maximum concentration of the eluted peak. More approximately, retention volumes rather than times are used. The retention volume \(V_R\) of a given sample component that is equal to the total volume of the mobile phase needed to elute the centre of the chromatographic band, can be calculated from the retention time \(t_R\) and volumetric flow rate \(F\).

The retention volume is directly related to the fundamental chromatographic parameter \(K\) i.e. the distribution coefficient as follows:

\[
V_R = t_R \times F \tag{1.10}
\]

\[
V_s = V_m + K \times V_S \tag{1.11}
\]
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Where ‘V_m’ is the volume of mobile phase and ‘V_s’ is the volume of stationary phase in the column. Equation 1.11 is a fundamental equation in chromatography. It relates the relative volume of the component to the column dead volume and the product of the distribution coefficient and the volume of stationary phase present within the column. This equation is correct for the partition chromatography but for the adsorption chromatographic column ‘V_s’ would be replaced by ‘A_s’ (the surface area of the adsorbent present in the column) or possibly by ‘W_a’ (the weight of the adsorbent present in the column). In any given chromatographic analysis the adjusted retention volume ‘V_r’ is used.

\[ V_r = V_m - V_s = K \times V_s \quad \text{or} \quad K \times A_s \]  

(1.12)

The capacity factor or the retention factor \( K \) is given by the following expression:

\[ K' = \frac{V_k - V_o}{V_o} \]  

(1.13)

where ‘V_o’ is the void volume or dead volume. The capacity factor \( K' \) is related to the distribution coefficient \( K' \) through the following relationship:

\[ K' = \frac{\text{Solute mass in stationary phase}}{\text{Solute mass in mobile phase}} \]  

(1.14)

\[ K' = \frac{\text{Solute concentration in stationary phase} \times V_s}{\text{Solute concentration in mobile phase} \times V_m} \]  

(1.15)

\[ K' = K \times \frac{V_s}{V_m} \]  

(1.16)

The values of capacity factor \( K' \) are characteristics of individual solutes and the selection of chromatographic system that will selectively retard component mixture, is of primary interest. By choosing a proper combination of the mobile and stationary phases, the \( K' \) values will be different, for each component in a given mixture.
1.4.2 The rate theory of chromatography

A more realistic description of the processes at work inside a column takes account of the time taken for the solute to equilibrate between the stationary and mobile phase (unlike the plate model, which assumes that equilibration is infinitely fast). The resulting band shape of a chromatographic peak is therefore affected by the rate of elution. It is also affected by the different paths available to solute molecules as they travel between particles of stationary phase. If we consider the various mechanisms which contribute to band broadening, we arrive at the Van Deemter equation for plate height;

\[
HETP = \frac{A + B}{u + Cu}
\]  

(1.17)

Where ‘u’ is the average velocity of the mobile phase. \(A\), \(B\), and \(C\) are factors which contribute to band broadening.

A - Eddy diffusion

The mobile phase moves through the column which is packed with stationary phase. Solute molecules will take different paths through the stationary phase at random. This will cause broadening of the solute band, because different paths are of different lengths.

B - Longitudinal diffusion

The concentration of analyte is less at the edges of band than at the centre. Analyte diffuses out from the centre to the edges. This causes band broadening. If the velocity of the mobile phase is high then the analyte spends less time on the column, which decreases the effects of longitudinal diffusion.

C - Resistance to mass transfer

The analyte takes a certain amount of time to equilibrate between the stationary and mobile phase. If the velocity of the mobile phase is high, and the analyte has a strong affinity for the stationary phase, then the analyte in the mobile phase will move ahead of the analyte in the stationary phase. The band of analyte is broadened. The higher the velocity of mobile phase, the worse the
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broadening becomes.

**Van Deemter plots**

A plot of plate height Vs average linear velocity of mobile phase

A typical Van Deemter plot

![Van Deemter Plot](image)

Such plots are of considerable use in determining the optimum mobile phase flow rate.

**Resolution**

Although the selectivity factor $\alpha$, describes the separation of band centres, it does not take into account peak widths. Another measure of how well species have been separated is provided by measurement of the resolution. The resolution of two species, A and B, is defined as

$$R = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$$

(1.18)

Baseline resolution is achieved when $R = 1.5$ It is useful to relate the resolution to the number of plates in the column, the selectivity factor and the retention factors of the two solutes are,

$$R = \frac{\sqrt{N}}{4} \left( \frac{\alpha - 1}{\alpha} \right) \left( 1 + \frac{k'_A}{k'_B} \right)$$

(1.19)

To obtain high resolution, three terms must be maximized. An increase in $N$, the number of theoretical plates and by lengthening the column leads to
an increase in retention time and increased band broadening—which may not be desirable. Instead, to increase the number of plates, the height equivalent to a theoretical plate can be reduced by reducing the size of the stationary phase particles. It is often found that by controlling the capacity factor, $k'$, separations can be greatly improved. This can be achieved by changing the temperature (in Gas Chromatography) or the composition of the mobile phase (in Liquid Chromatography). The selectivity factor, ($\alpha$) can also be manipulated to improve separations. When $\alpha$ is close to unity, optimizing $k'$ and increasing $N$ is not sufficient to give good separation in a reasonable time. In these cases, $k'$ is optimized first, and then $\alpha$ is increased by one of the following procedures

1. Changing mobile phase composition
2. Changing column temperature
3. Changing composition of stationary phase
4. Using special chemical effects (such as incorporating a species which complexes with one of the solutes into the stationary phase)

1.4.3 Band broadening and column efficiency

To get optimal separation, sharp, symmetrical chromatographic peaks must be obtained. This means that band broadening must be limited. It is also beneficial to measure the efficiency of the column.

1.5 General Aspects of Adsorption

Adsorption is one of the general classes of phase transformation or molecular distribution process which include the partitioning of the sample between adjacent liquid phases. Two limiting types of the adsorptions are commonly recognized, physical adsorption and chemisorption. Physical adsorption resembles phase transformations. The energy involved in physical adsorption is generally small. The adsorption and desorption are normally rapid. The intra molecular forces responsible for physical adsorption are the Van der waals forces. In chemisorption an actual covalent or ionic bond is formed between adsorbing molecule and the adsorbent surface. The energy of
chemisorption is generally large. The adsorption and desorption are frequently slow. The adsorbate-adsorbent interaction is of primary important in determining the relative adsorption of sample molecule in liquid-solid chromatography. The adsorbate-adsorbent interaction can occur via five possible mechanisms:

1. Dispersion (Van der Waals forces, London dispersion forces)
2. Dipole interaction (Inductive forces).
3. Hydrogen bonding.
4. Dielectric interaction.
5. Columbic (electrostatic) interactions.

1.6 Optimization of Chromatographic Process

Optimization of the process is the key to chromatography. The resolution of the two components depends on a series of factors which are described below.

1.6.1 Dimension of the column

In early column chromatography [51-54] the ratio between length and breadth of the column varied from 5:1 to 10:1. In high efficiency columns a ratio between 100:1 and 1000:1 was used [55-56]. The efficiency of the separation evidently increases with increase in this ratio that is increase of column length but this is offset by longer time of analysis and difficulties experienced in filling the column uniformly. Column with 4 mm diameter can be packed well [57]. Columns of 6.5 mm diameter give zone breadths about four times those obtained on 4 mm diameter column of the same length. In special conditions high efficiencies may be obtained with columns having a diameter up to 11 mm [58-59].


1.6.2 Size of particle and sample

The particle size used in column chromatography is usually 100-200 mesh for lower concentration of the sample put on the column. \( HETP \) is independent of the sample size [60] but increases rapidly above a certain concentration limit. For analytical separation, it is almost preferable to work within a sample size range where \( K' \) values are constant i.e. sample size should be less than linear capacity of the column. High resolution is obtained for small quantity of sample [52-61].

1.6.3 The flow rate and viscosity of eluent

The resolution depends on the flow rate of the eluent. From practical point of view the optimal rate is 0.3 - 3.0 column volumes per hour for a column of 100 cm length [52]. The viscosity of the eluent does not affect the separation selectivity or the capacity factor in the resolution equation but these terms depend very much on the type of solvent used. High efficiency separations are achieved using solvents with lowest possible viscosity [62].

1.6.4 Separation time

In most cases the time of separation should be as small as possible, both for convenience and to allow a maximum number of separations per day.

1.6.5 Temperature

The temperature is an important parameter in liquid solid chromatography. In 1948, Le-Rosen and Rivet [63] studied the change in rate of zone transfer in liquid-solid chromatography. The retention of the solute could be made to increase, decrease or stay approximately constant by variation of temperature in the column. Maggs has published [64] new experimental results on the role of temperature and of moderator (a polar solvent added in small quantities to a non-polar eluent). In liquid-solid column chromatography the asymmetry of chromatography peak increases with increase in column temperature and decreases with the concentration of moderator. An important role is played by the polarity of the mobile phase in the dependence of the retention volume on the temperature.
1.6.6 Pressure

Modern liquid chromatography uses pumps creating the pressure of 34-340 atmospheres to transfer eluent through the column [65]. This allows the use of short column packed with finely granulated materials. By using such columns the separations can be carried out 100-1000 times more rapid than in conventional liquid chromatography.

1.7 Crown Ethers

Crown ether is a generic name given to macrocyclic polyether containing ethylene bridges separating by oxygen atoms. They typically containing central hydrophilic cavity, whose diameter varies from 1.2-2.6 Å. The central hydrophilic cavity is ringed with electronegative oxygen atoms, which in turn are surrounded by a collar of -CH₂ groups forming a framework which is flexible and exhibit hydrophobic behavior. The hydrophobic exteriors allow them to solubilize ionic substances into non-aqueous solutions and in membrane media. When the inorganic cation fits into the cavity of crown ether, it becomes a lipophilic species. This property of converting inorganic cation into lipophilic species has been utilized extractive separation analysis, ion transport through membrane media, phase transfer catalysis etc.

Macrocyclic compounds are uncharged and contain a cavity in which a cation can be encapsulated. The complexes thus formed are of great analytical interest. The crown ether was discovered by pure chance. C.J. Pedersen who was working as an industrial chemist for Du Pont [66]. Since the large number of crown ethers were synthesized and used as physical, organic, inorganic, biochemist and analytical chemists. The synthesis, properties and various applications of crown ethers have appeared in several monographs [67-121].

Dunitz crystal structure of 18-crown-6 and those of its K⁺ SCN⁻ complex indicate that host and its complex have different conformational organization [122]. The potential crown ethers of the pure host is filled with two inward-turned -CH₂ groups and electron pair of two oxygens face outward and away from the centre of the rectangular structure. Thus the free host dose not have a
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'crown shape' not a cavity. Only when oxygens become engaged with guest such as K⁺ only the presence of guest in the complex induces the electron pair to converge on the centre of a crown-shaped object. In other words, the guest conformationally reorganizes the host upon complexation. Many solvents are probably able to play the same role as guest. The complexed and uncomplexed structure of 18-crown-6 is shown in figure 1.2.

![Uncomplexed crown ether + KSCN → Complexed Crownether](image)

Figure: 1.2: Structure of uncomplexed and complexed 18-crown-6.

1.7.1 Preparation of Crown polymers

Numerous crown polymer and crown ether modified silica's which are able to bind definite inorganic salts are obtained by condensation, substitution or copolymerization reaction with cyclic polyether of different structures and ring size. The mode of synthesis of crown polymers is shown in Table 1.1. Dibenzo-crown compounds are condensed with formaldehyde in formic acid and thereby cross-linked by methylene groups. With monobenzo crown compounds, additional cross linking agents such as toluene, xylene, phenol or resorcinol are used. It is also possible to bind monobenzo- and dibenzo-crown compounds at a polystyrene surface via Methylene bridge by condensation reaction with formaldehyde. Substitution reaction can be performed with chloromethylated polystyrene as well as with silica gel. The various crown polymers with their structures are shown in figure 1.3.
C. J. Pedersen [1967] synthesized crown ethers and awarded the Nobel Prize in Chemistry in 1987 without having a Ph. D.
1.7.2 Poly[dibenzo-18-crown-6] in extraction chromatography

Aromatic crown polyethers were prepared by Pedersen by condensation reaction of catechol with dichloroethylether in the presence of butanol and sodiumhydroxide. It gives bis-phenol and a new product as [dibenzo-18-crown-6] (123-125). Poly[dibenzo-18-crown-6] is prepared by the condensation and polymerization of [dibenzo-18-crown-6] with formaldehyde (Figure 1.4). The review of use of poly[dibenzo-18-crown-6] for the chromatographic separation studies of various cation is given in Table 1.2

![Synthesis of dibenzo and Poly[dibenzo-18-crown-6]](image)

Figure 1.4: Synthesis of dibenzo and Poly[dibenzo-18-crown-6]

1.7.3 Properties of crown polymers

The most important properties of crown polymers are

1. They show high resistance to chemical, temperature and radiolysis.
2. They contain neutral ligands as anchoring group.
3. Simultaneous uptake of cation and anion to maintain electroneutrality.
4. Stripping of the solvent shell of cation and anion.
5. Stability of polyether complexes depends on cation, anion and solvent, size of polyether ring and on the number, type and
position of heteroatom.

6. Salt uptake with polymers containing only oxygen as heteroatom is independent of pH, where as with oxygen and nitrogen as heteroatom it is dependent at pH <3.

7. Elution by pure solvents.

8. Capacities are high.

1.7.4 Crown ether in extraction chromatography

In extraction chromatographic separation of particular cation, a simple rule helps in finding the suitable crown provided the following relationship is best fulfilled

\[
\frac{\text{Diameter of the cation}}{\text{Diameter of the crown ether ring}} = 0.8 \quad (1.20)
\]

Since the discovery, crown ethers are being used for the extraction chromatographic separation of number of metal ions. High performance liquid chromatographic separation studies of mercury were carried out using dibenzo18-crown-6 [126], in which mercury was eluted with 0.01 M methanol containing phosphate and borate buffers. Using various crown ethers lithium isotopes were separated by column chromatographic method. For such a studies aqueous lithium perchlorate and trichloroacetate salts were used [127]. Extraction chromatographic separation technique was also extended for the separation of calcium isotope using dicyclohexano-18-crown-6 [128-129] and isotope with 15-crown-5 and dicyclohexano-18-crown-6 [130]. Separation of uranium(IV) from uranium(VI) was carried out from hydrochloric acid medium using dibenzo18-crown-6 carrier powder [131]. Enhancement of extraction of uranium was observed when small quantity of potassium was added.

Crown ether was reacted with molybdophosphoric acid and tungstophosphoric acid to get number heteropoly adduct of crown ether and were used for the separation studies of s-block, p-block and some of the heavy metal ions [132-139]. In extraction chromatography the nature of inert support
is very important. Number of hydrophobic inert supports was used for the separation studies of various elements. These supports include silanised silica gel [140-143], TVEX [144], Chromosorb-P [145], Kieselghur [146], Silochrome and polymeric beads [147-148] and polyurathane foam [149]. Column chromatographic separation studies of the elements were also carried out by using crown ethers, ion exchangers [150-153], carbon [15] and zeolites [155].

1.7.5 Various polymeric crown ethers in Extraction chromatography

Numbers of polymeric crown ethers are prepared by condensation, substitution or copolymerization reactions with cyclic polyether. Poly-benzo-15-crown-5 was used for the separation of zinc and cadmium [156]. Poly-vinyl-benzo-18-crown-6 was used for the study of binding constants of potassium and cesium [157]. Calcium isotopoes were separated by using poly-18-crown-6 [158] and poly-thia-divinyl-18-crown-6 [159]. Complexation studies of copper, silver and gold were carried out using thio-crown-polymers [160-161]. Poly crowns were used [162] for the enrichment studies of silver, nickel and lead. Crown carboxylic resins were used for the separation studies of alkali metals [163-164]. Sorption studies of gold and other cations were carried out using crown polymers [165-166]. Sorption studies of alkali metals were carried out using crown polymers [167-169]. Crown ether carboxylic acid polymers were used for the separation of yttrium and strontium [170]. Substituted 18-crown-6 sorbed on an inert support was used for the separation of barium and radium and determination of radium in volcanic rocks [171]. Fluoremetric determination of nickel in human urine and serum was done using liquid chromatography in which 18-crown-6 was used as a mobile phase [172]. Scintillating extraction chromatographic resin prepared by coating patented crown ether on glass particles was used [173] for the detection of efficiencies of \textsuperscript{90}Sr and \textsuperscript{238}Pu. Tetrathia crown ethers were used [174] for the ion pair liquid chromatographic studies of silver(II) and palladium(II). Extraction chromatographic studies of strontium(II) were carried out [175] using
chromosorb-102 coated with dicyclohexano-18-crown-6, polymer supported dicyclohexano-18-crown-6 [176], di-tert-butyl cyclohexanecrown-6 [177] and PVC-based dicyclohexanecrown-8 for Cd(II) [178]. The inclusion membranes with calix [4]-crown-6 derivatives were used for the facilitated transport of Zn(II), Cd(II) and Pb(II) ion by Ulewicz et al.[179]. Freidzon et al. [180], has been reported complexation of alkali metal ions in the cavity of arylazacrown ethers which is helpful in the extraction of alkali metal ions. Extraction of Am and rare-earth elements from acidic solutions by alkali derivatives of dibenzo-18-crown-6 and dicyclohexanecrown-6 was carried out by Yakshin et al. [181]. Bartos reported the effect of 15-crown-5 and 18-crown-6 on uptake of Sr, Ba, and Ra by Tunnel-Structure ion Exchangers [182].
### Table 1.1: Synthesis mode of crown polymers

<table>
<thead>
<tr>
<th>Procedure of Synthesis</th>
<th>Matrix</th>
<th>Starting material for polymers</th>
<th>Anchor group</th>
<th>Structural framework</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Condensation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylene bridges</td>
<td>Dibenzo crown ether</td>
<td>Formaldehyde</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monobenzo crown ether</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toluene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polystyrene</td>
<td>Dibenzo crown ether</td>
<td>Toluene</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymerization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polystyrene</td>
<td>Mono vinyl monobenzo crown ether</td>
<td>Divenyldibenzo crown ether Divenylbenzen</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substitution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polystyrene, Amino bridges</td>
<td>Aminobenzo crownether, monocyclic cryptands</td>
<td>Chloromethylated polystyrene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polystyrene ether bridges</td>
<td>Hydroxy methyl monobenzo crown ether</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silica gel, Methoxy bridges</td>
<td>Hydroxy methyl monobenzo crown ether</td>
<td>Silica gel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silica gel, Alkane bridges</td>
<td>w-bromoalkane monobenzo crown ether</td>
<td>Silica gel</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1.2 Review of crown ether and adducts in Extraction Chromatography

<table>
<thead>
<tr>
<th>Element</th>
<th>Crown Ether Type</th>
<th>Condition</th>
<th>Remark</th>
<th>Refer. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li</td>
<td>B15C5;15C5;DC18C6;DB18C6</td>
<td>Chlorate, acetate</td>
<td>Li isotope sepn.</td>
<td>183</td>
</tr>
<tr>
<td>Alkali</td>
<td>Adduct DB18C6,(P. Molyb.)</td>
<td>HNO₃</td>
<td>Kd for K⁺ largest</td>
<td>184</td>
</tr>
<tr>
<td>Alkali</td>
<td>Adduct DB18C6, (P. Molyb.)</td>
<td>Acidic media</td>
<td>Sorption Studies</td>
<td>185</td>
</tr>
<tr>
<td>Alkali</td>
<td>Adduct crown ether (P.Tung.)</td>
<td>-</td>
<td>Kd alkail metals</td>
<td>186</td>
</tr>
<tr>
<td>Mg</td>
<td>15C5,DC18C6</td>
<td>Trichloro-acetate</td>
<td>Mg isotope</td>
<td>187</td>
</tr>
<tr>
<td>Alkaline</td>
<td>DB18C6 on silanised silica</td>
<td>NaClO₄ soln.</td>
<td>Ca,Sr,Ba sepn.</td>
<td>188</td>
</tr>
<tr>
<td>Pb</td>
<td>AdductDB18C6, B15C5 (P. Molyb.)</td>
<td>-</td>
<td>Pb environment sample</td>
<td>189</td>
</tr>
<tr>
<td>U</td>
<td>DB18C6 carrier powder</td>
<td>HCl</td>
<td>U(IV) and U(VI) sepn.</td>
<td>190</td>
</tr>
<tr>
<td>Ca</td>
<td>DC18C6</td>
<td>SCN⁻</td>
<td>Ca isotope sepn.</td>
<td>191</td>
</tr>
<tr>
<td>Ca</td>
<td>DC18C6</td>
<td>SCN⁻, Picrate</td>
<td>Ca isotope sepn.</td>
<td>192</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>Adduct12C4, DB18C6 (P.Molyb., Tung)</td>
<td>-</td>
<td>Effect of pH on sorption</td>
<td>193</td>
</tr>
<tr>
<td>Co</td>
<td>DB18C6 on silanised silica</td>
<td>Thiocynate</td>
<td>-</td>
<td>194</td>
</tr>
<tr>
<td>Ba</td>
<td>DB18C6 on silanised silica</td>
<td>Picrate</td>
<td>Ba sepn. From associated elements</td>
<td>195</td>
</tr>
<tr>
<td>Ba</td>
<td>DB18C6 on Kieselguhr</td>
<td>Picric acid,HNO₃</td>
<td>Sepn. of Ba from Sr, Ce, Zr etc.</td>
<td>196</td>
</tr>
<tr>
<td>Sr</td>
<td>1C6 deriv. On polymeric support</td>
<td>HNO₃</td>
<td>Radiochemical sepn.</td>
<td>197</td>
</tr>
</tbody>
</table>
1.7.6 Various Poly Crown Modified Silicas and other Polymeric Crown Ethers in Extraction Chromatography

Table 1.3 summarizes the use of poly crown modified silicas in extraction chromatography. Poly crown modified crown silicas are prepared by copolymerization of vinyl-modified silica with crown ether vinyl monomers. The modified silicas possess a hard core, being well suited for high pressure liquid chromatography. Modified silicas have been successfully applied for the chromatographic separation studies of number of alkali metals and heavy metal ions [202-210]. Crown carboxylic acid resins were used for the separation of alkali metals [211].

Table 1.4 summarizes the use of chromatographic methods for various elements with poly[di-benzo-18-crown-6] and derivatives [212-236].
### Table 1.3 Review of Chromatographic methods for various elements with Poly-crown modified silicas and various polymeric crown ethers

<table>
<thead>
<tr>
<th>Elements</th>
<th>Crown Ether Type</th>
<th>Condition</th>
<th>Remark</th>
<th>Refer. No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkali</td>
<td>P-B15C5 modified silica</td>
<td>H$_2$O, MeOH</td>
<td>Metal halide sepn.</td>
<td>202</td>
</tr>
<tr>
<td>Alkali</td>
<td>P-B18C6 modified silica</td>
<td>H$_2$O, MeOH</td>
<td>-</td>
<td>203</td>
</tr>
<tr>
<td>Alkali</td>
<td>12C4 modified silica</td>
<td>H$_2$O, MeOH</td>
<td>Na-K Sepn.</td>
<td>204</td>
</tr>
<tr>
<td>K</td>
<td>P-B15C5 modified silica</td>
<td>-</td>
<td>K-Sepn.</td>
<td>205</td>
</tr>
<tr>
<td>S-block</td>
<td>B18C6 modified silica</td>
<td>H$_2$O</td>
<td>Sepn. Of alkali metal chloride</td>
<td>206</td>
</tr>
<tr>
<td>S-block</td>
<td>B15C5-B21C7 modified silica</td>
<td>H$_2$O</td>
<td>Retention time study</td>
<td>207</td>
</tr>
<tr>
<td>S-block</td>
<td>15C5,18C6 modified silica</td>
<td>H$_2$O</td>
<td>-</td>
<td>208</td>
</tr>
<tr>
<td>Hg</td>
<td>18C6 deriv. modified silica</td>
<td>H$_2$O</td>
<td>Hg-Ag sepn.</td>
<td>209</td>
</tr>
<tr>
<td>Alkali</td>
<td>18C6 deriv. modified silica</td>
<td>-</td>
<td>Na-K halides sepn.</td>
<td>210</td>
</tr>
<tr>
<td>Ag, Ni, Pb, Fe</td>
<td>Poly crown</td>
<td>-</td>
<td>Enrichment of Ag,Ni,Pb ect</td>
<td>211</td>
</tr>
</tbody>
</table>
Table 1.4  Review of Chromatographic Methods for various Elements with Poly[di-benzo-18-crown-6] and derivatives

<table>
<thead>
<tr>
<th>Element</th>
<th>Condition</th>
<th>Remark</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-block</td>
<td>----</td>
<td>Selectivity of cations</td>
<td>212</td>
</tr>
<tr>
<td>Alkali, Ag, Tl</td>
<td>Aq. EtOH</td>
<td>Sorption studies</td>
<td>213</td>
</tr>
<tr>
<td>Tl</td>
<td>HCl</td>
<td>Tl from rock samples</td>
<td>214</td>
</tr>
<tr>
<td>Pb, Cd</td>
<td>Chloride media</td>
<td>Sorption behavior</td>
<td>215</td>
</tr>
<tr>
<td>Ag, Tl</td>
<td>----</td>
<td>Influence of anions</td>
<td>216</td>
</tr>
<tr>
<td>Zn</td>
<td>Thiocyanate</td>
<td>Sorption increased with KSCN</td>
<td>217</td>
</tr>
<tr>
<td>Heavy Metals</td>
<td>HCl</td>
<td>Sepn. of Tl, Fe, Ga</td>
<td>218</td>
</tr>
<tr>
<td>U</td>
<td>Picrate-EtOH</td>
<td>Sepn of U and Ti</td>
<td>219</td>
</tr>
<tr>
<td>U</td>
<td>HCl, H₂SO₄</td>
<td>Fe⁺⁺ interferes</td>
<td>220</td>
</tr>
<tr>
<td>Mo</td>
<td>HCl</td>
<td>Sepn. from other cations</td>
<td>221</td>
</tr>
<tr>
<td>U</td>
<td>HCl</td>
<td>Sepn. from other cations</td>
<td>222</td>
</tr>
<tr>
<td>Pb</td>
<td>HCl</td>
<td>Sepn. from other cations</td>
<td>223</td>
</tr>
<tr>
<td>Pb</td>
<td>HBr</td>
<td>Sepn. from other cations</td>
<td>224</td>
</tr>
<tr>
<td>Ba</td>
<td>HCl</td>
<td>Sepn. from other cations</td>
<td>225</td>
</tr>
<tr>
<td>U</td>
<td>Ascorbic acid</td>
<td>Sepn. from other cations</td>
<td>226</td>
</tr>
<tr>
<td>Th</td>
<td>Ascorbic acid</td>
<td>Sepn. from other cations</td>
<td>227</td>
</tr>
<tr>
<td>Ce</td>
<td>L-valine</td>
<td>Sepn. from other cations</td>
<td>228</td>
</tr>
<tr>
<td>La</td>
<td>L-valine</td>
<td>Sepn. from other cations</td>
<td>229</td>
</tr>
<tr>
<td>U, Th, Ce</td>
<td>L-arginine</td>
<td>Sepn. from other cations</td>
<td>230</td>
</tr>
<tr>
<td>Be</td>
<td>L-arginine</td>
<td>Sepn. from other cations</td>
<td>231</td>
</tr>
<tr>
<td>Pb</td>
<td>Hippuric acid</td>
<td>Sepn. from other cations</td>
<td>232</td>
</tr>
<tr>
<td>Pb</td>
<td>L-arginine</td>
<td>Sepn. from other cations</td>
<td>233</td>
</tr>
<tr>
<td>La</td>
<td>L-arginine</td>
<td>Sepn. from other cations</td>
<td>234</td>
</tr>
<tr>
<td>U</td>
<td>L-valine</td>
<td>Sepn. from other cations</td>
<td>235</td>
</tr>
<tr>
<td>Th</td>
<td>L-valine</td>
<td>Sepn. from other cations</td>
<td>236</td>
</tr>
</tbody>
</table>
Chapter 1: Introduction

From Table-1.2 to Table 1.4 it is clear that there is wide scope for the column chromatographic separation studies of various cations using poly[dibenzo-18-crown-6]. Also the literature survey reveals that there are no reports on the use of amino acids as medium i.e. counter ion. Hence we decide to work on the sorption study of metal ions using poly[dibenzo-18-crown-6] and amino acid medium.

The sorption of lead(II) in hippuric acid, L-arginine, L-valine, L-ascorbic acid, L-leucine and L-histidine are reported in this thesis. The importance of our work is “Development of simple column chromatographic method without using sophisticated instruments”. The simple instruments used throughout the experiments and experimental setup are given below.
1.8 Experimental Setup and Instruments

- Digital Flame Photometer
- Polydibeno-18-crown-6]
  100-200 mesh size
- Pyrex glass chromatographic column
- Spectrophotometer
- Digital pH meter
- Digital Flame Photometer
Chapter 1: Introduction

The thesis is divided into eight chapters as given below,

Chapter 1: General: Introduction and Literature Survey

Introduction to various chromatographic methods, historical perspective of chromatography, various chromatographic methods and parameters affecting chromatographic analysis have been described in this chapter. This chapter also includes review of use of polymeric crown ethers for chromatographic separation studies along with its preparation and characteristics.

Chapter 2: History of Lead

Historical perspective of lead(II) is given in this chapter.

Chapter 3: Sorption Study of Lead(II) in Hippuric acid Medium Using Poly[dibenzo-18-crown-6] and Column Chromatography

The method for separation of lead(II) was developed by using poly[dibenzo-18-crown-6] as stationary phase in column chromatography and hippuric acid as a medium.


The separation method for lead(II) was developed by using poly[dibenzo-18-crown-6] and column chromatography. The separation of lead(II) was carried out by using L-arginine medium.

Chapter 5: Sorption Study of Lead(II) in L-valine Medium Using Poly[dibenzo-18-crown-6] and Column Chromatography

The column chromatographic separation method was developed for separation of lead(II) using poly[dibenzo-18-crown-6]. The separation of lead(II) was carried out by using L-valine medium.
Chapter 6: Sorption Study of Lead(II) in L-ascorbic acid Medium Using Poly[dibenzo-18-crown-6] and Column Chromatography

The column chromatographic separation method was developed for separation of lead(II) using poly[dibenzo-18-crown-6]. The separation of lead(II) was carried out by using L-ascorbic acid medium.

Chapter 7: Sorption Study of Lead(II) in L-leucine Medium Using Poly[dibenzo-18-crown-6] and Column Chromatography

The method for separation of lead(II) was developed by using poly[dibenzo-18-crown-6] as stationary phase in column chromatography and L-leucine as a medium.

Chapter 8: Sorption Study of Lead(II) in L-histidine Medium Using Poly[dibenzo-18-crown-6] and Column Chromatography

The method for separation of lead(II) was developed by using poly[dibenzo-18-crown-6] as stationary phase in column chromatography and L-histidine as a medium.
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