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Praise be to God the Lord of the Worlds and peace and blessings be upon the master of humankind Mohammad and his pure Progency and his relatives and may God curse their enemies until the Day of Judgment.

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Ahmed 2006

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List of Abbreviations

LC	Liquid Chromatography
GC	Gas Chromatography
LLC	Liquid-Liquid Chromatography
PC	Paper Chromatography
TLC	Thin Layer Chromatography
HPLC	High Performance Liquid Chromatography
MPa	Mega Pascal
N	Number of theoretical plates
t_R	Retention time
W_b or W	Width of peak at baseline
HETP or H	Height Equivalent to Theoretical Plates
L	Length of column
R_S	Resolution between two peaks
α	Column selectivity or Separation factor
k'	Capacity factor
LSC	Liquid-Solid Chromatography
RBC	Reversed Bonded phase Chromatography
IEC	Ion Exchange Chromatography
SAX	Strong Anion Exchanger
SCX	Strong Cation Exchanger
ppm	Part Per million
M	Molarity

IPD	Indirect Photometric Detection
IPC	Indirect Photometric Chromatography
S	Observed signal
C_S	Concentration of sample ions
C_E	Concentration of eluent ions
€$_S$	Molar absorptivity of sample ions
€$_E$	Molar absorptivity of eluent ions
S/N	Signal to Noise ratio
(i.d)	Internal diameter
RSD	Relative Standard Deviation
UV	Ultra Violate
R^2	Correlation coefficient
R	Regression
IC	Ion Chromatography

Supervisor certification

I certify that this thesis was prepared under my supervision at the Department of Chemistry, College of Science, Al-Nahrain University as a partial requirements for the **Degree of Master of Science in Chemistry.**

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Date:

In view of the available recommendation, I forward this thesis for debate by the Examining Committee.

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Examining Committee's Certification

We, the Examining Committee, certify that we read this thesis and have examined the student *(Ahmed zuhair Mohammed Hussain)*, in its contents and that, in our opinion; it is adequate as a thesis for the Degree of Master of Science, in Chemistry.

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CHAPTER ONE

INTRODUCTION

CHROMATOGRAPHY

1.1 DEFINITION OF CHROMATOGRAPHY:

Chromatography is a method of chemical analysis and processing that is rapidly replacing some of the more traditional techniques of sample identification and purification [(1)]. Chromatography is the general name given to the methods by which two or more compounds in a mixture are physically separated themselves by distributing between two phases: 1) a stationary phase, which can be a solid or a liquid, supported on a solid; and 2) a mobile phase, either a liquid in which case the technique is called liquid chromatography (or LC) or a gas called gas chromatography, (or GC) which flows continuously through the stationary phase. The separation of individual components results primarily from differences in their affinity for the stationary phase [(2)]. A sample mixture is introduced into the mobile phase of a chromatograph that carries it to the head of a column, which is the heart of the separation system. Because all compounds have different molecular properties (e.g., polarity, molecular size, boiling point), separation of the individual components in the mixture occurs and they elute from the end of the column at different intervals. By monitoring the eluting compounds with a suitable detector, qualitative and quantitative analysis can be achieved or purified compounds are recovered [(1)]. Separation is; therefore, based on differences in migration rates among the sample components [(3)].

1.2 HISTORY OF CHROMATOGRAPHY:

Chromatography was discovered and named in 1906 by Michael Tswett, a Russian botanist, when he was attempting to separate colored leaf pigments (chlorophyll) by passing a solution containing them through column packed with adsorbent chalk particles. The individual chlorophyll passed down the column at different rates and was separated from each other.

The separated pigments were easily distinguished as colored bands hence the term comes from, chrom (color) + graphy (writing).

The next major development was that of liquid-liquid (partition) chromatography (LLC) by Martin and Synge [(2)] in 1941. Instead of only a solid adsorbent they used a stationary liquid phase spread over the surface of the adsorbent and immiscible with the mobile phase. The sample components partitioned themselves between the two liquid phases according to their solubilities. For this work, Martin and Synge received Nobel Prize in chemistry in 1952.

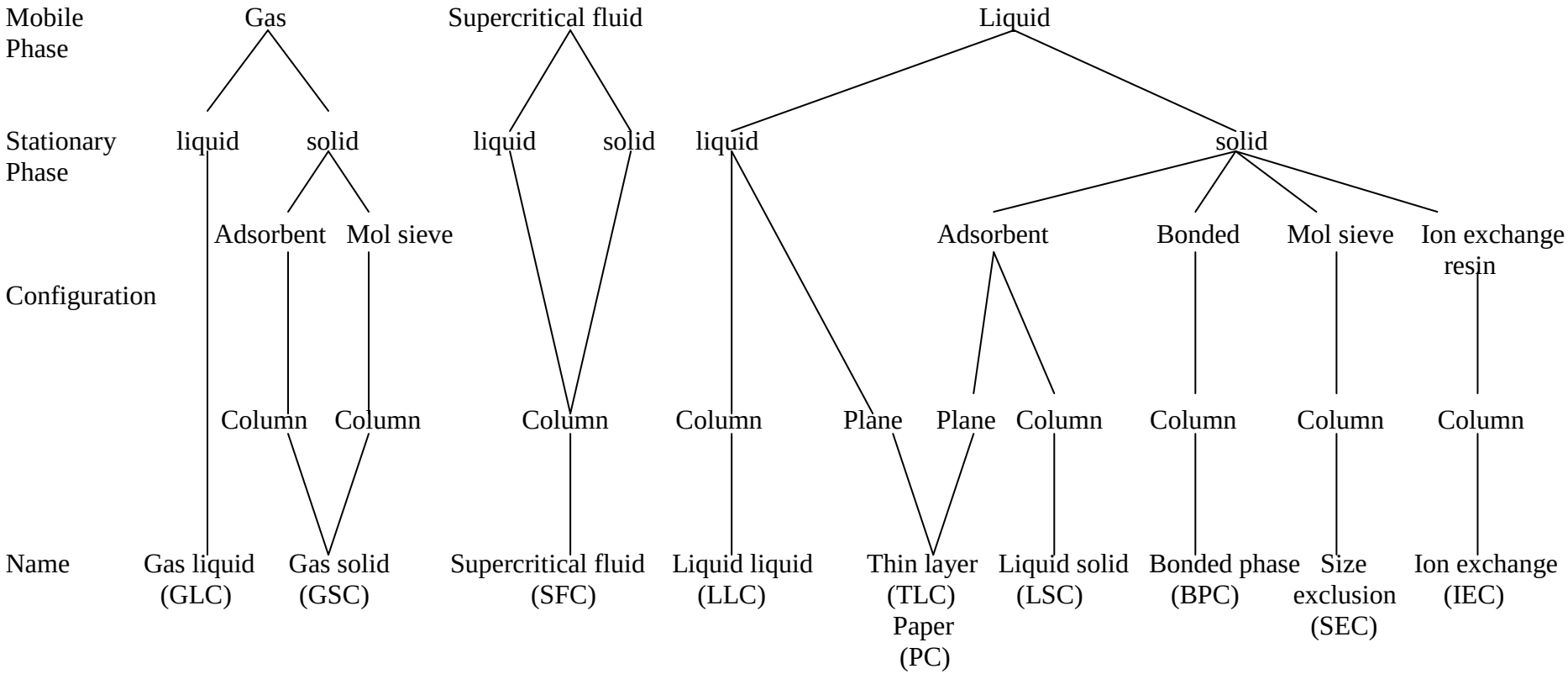
In the early days of column chromatography, reliable identification of small quantities of separated substances was difficult, so paper chromatography (PC) was developed. In this "planar" technique, separations are achieved on sheets of filter paper, mainly through partition. Appreciation of the full advantages of planar chromatography then led to thin-layer chromatography (TLC), in which separations are carried out on thin layer of adsorbent supported on plates of glass or some other rigid material. TLC gained popularity after the classic work by Stahl[(2)] in 1958 standardizing the techniques and materials used. To aid or enhance the separation of ionic compounds by PC or TLC, an electric field can be applied across the paper or plate. The resulting techniques are referred to as paper or thin-layer electrophoresis, respectively.

Martin and James[(2)] first described GC, in 1952 and has become the most sophisticated and widely used of all chromatographic methods, particularly for mixtures of gases or for volatile liquids and solids. Separation time in a matter of minutes have become commonplace even very complex mixtures. The combination of high resolution, speed of analysis, and sensitive detection have made GC a routine technique used in almost every chemical laboratory.

High-performance liquid chromatography (HPLC) is rapidly becoming as widely used as gas chromatography and is often the preferred technique for the rapid separation of non-volatile or thermally unstable samples [(2)].

Table (1-1) shows a complete classification scheme. The classification scheme lists not only the states of the two phases but also the configuration of the chromatographic bed. There are two popular configurations for the bed, a column and a planar surface [(4)].

Table (1-1) Classification of Chromatography Techniques [4].



1.3 BASIS OF THE SEPARATION PROCESS:

Chromatographic separation process based on the difference in the surface interactions of the analyte and eluent molecules. Let us consider a separation of a two-component mixture dissolved in the eluent. Assume that component **A** has the same interaction with the adsorbent surface as an eluent, and component **B** has strong excessive interaction. Being injected into the column, these components will be forced through by eluent flow. Molecules of the component **A** will interact with the adsorbent surface and retard on it by the same way as an eluent molecules. Thus, as an average result, component **A** will move through the column with the same speed as an eluent. Molecules of the component **B** being adsorbed on the surface (due to their strong excessive interactions) will sit on it much longer. Thus, it will move through the column slower than the eluent flow [(5)].

1.4. GAS CHROMATOGRAPHY:

In (GC), the sample is injected and vaporized (if not a gas) into the head of the chromatographic column. Elution is brought about by the flow of an inert gaseous mobile phase (carrier gas) [(6)]. The separation mechanism in GC involved two kinds of interactions, one between the solute molecules themselves and the other between the solute and the stationary phase [(7)]. The most important parameters for separation of sample components are the nature of the stationary phase and column temperature [(8)]. In general, GC can be used to analyze volatile and thermally stable samples, and this is not a limitation imposed on liquid chromatography [(9)].

1.5 LIQUID CHROMATOGRAPHY:

The separation of sample components as they pass down the column is due to differential distribution of the sample components between liquid mobile phase and stationary phase [9]. There are two types of LC, classical and high performance liquid chromatography (HPLC).

Classical LC use a large column (~ 50x 2cm) packed with stationary phase of large particles size (50-250µm in diameter) sample volumes in the millilitres range are often common. The mobile phase is generally gravity-fed at low flow rate, because the deep pores of the packing limits mass transfer, the analysis times are usually in order of hours [9]. Liquid chromatography techniques can be used for the determination of ionic species as well as other compounds [10]. However, HPLC uses high pressure to force solvent through closed columns containing very fine stationary phase particles that give high resolution separations .The HPLC systems consist of a solvent delivery system, a sample injection valve, a column, a detector, and data processing device or a computer to control the system and display results. Some systems include an oven for temperature control of the column.

The HPLC uses stainless steel or plastic columns that are 5-30 cm in length, with an inner diameter of 1-5 mm. The typical particle size packed in an HPLC columns are in the rang of 3-10µm to increase resolution afforded by decreasing the particle size. HPLC requires pressure of ~ 7-40 MPa (1000-6000 pounds/inch^2) to attain flow rates of ~0.5-5ml/min [11].These components and other factors that give the quality of a high performance chromatographic separation. The chromatographic efficiency is usually expressed by the number of theoretical plate (**N**) which is related to the retention of the solute ($\mathbf{t_R}$) compared with the width of its peak at base line ($\mathbf{W_b}$).

$$\mathbf{N=16\ (t_{Ri}/w_b)^2} \text{ ---------- } \mathbf{(1\text{-}1)}$$

The efficiency parameter **N** is useful when comparing chromatographic separations under different conditions and is related to the height equivalent to theoretical plates, [HETP], **H** by:

$$H = L/N \text{ ---------- (1-2)}$$

Where **L** is the length of the column [12]. The efficiency of separation in HPLC is higher due to the large number of mass transfer equilibria obtained with small values of **H**. This resulted from using small particle size of the stationary phase as describe by Van-Deemter and others [6]. Resolution (R_S) is the efficiency of a chromatographic system; it defines the degree of separation between two adjacent peaks [12].

$$R_S = 2 (t_{R2}-t_{R1}/W_2+W_1) \text{ ----------(1-3)}$$

Where t_{R2} and t_{R1} are the retention times and W_1 and W_2 are the widths of the peaks at the base line, of peaks 1 and 2, respectively. The larger resolution is better the separation. Resolution may also be described by three terms, efficiency, selectivity, and capacity factors as shown in the equation below [12]:

$$R_S = 1/4 (N)^{1/2} [(\alpha-1)] [(k'_2)/1+ k'_2] \text{ --------- (1-4)}$$

Where (α) selectivity, and (k_2') capacity factor.

The small particle sizes represent a good compromise between efficiency, pressure drop, analyses time, and reproducibility of packing. HPLC is not limited in application by component volatility or thermal stability as in GC, this makes it the method of choice for the analyses of most known samples including polymers, polar, ionic, and thermally unstable material. Choice of stationary phase and proper control of the composition of mobile phase can lead to better separation and high column efficiency. Other advantages of liquid chromatography methods in that many detectors that are used in HPLC are non-destructive, thus facilitating sample recovery and providing the opportunity for subsequent spectro-analytical and other studies [13]. HPLC has been used for analysis of wide variety of pharmaceutical products, body fluid, and environmental samples [14].

1.6 MODES OF LC:

Liquid chromatography can be classified into many classes based on the type of stationary phase interaction with sample components as well as with the mobile phase. Among these modes are adsorption, partition, ion exchange and ion pair chromatography in addition to other special modes of separation [15]. Separation modes can be chosen for a particular application depends on the properties of analyte to be separated, and can be optimized by choosing different combination of mobile phase and stationary phase materials [16].

1.6. I NORMAL PHASE (ADSORPTION) CHROMATOGRAPHY:

This mode of separation was the first discovered form of liquid chromatography or liquid-solid chromatography (LSC). This type of separations is usually called normal phase mode. The basis for normal phase separation is the selective adsorption of the polar sample components to the active adsorbent sites on the surface of the stationary phase. Adsorption chromatography has been performed on hydrophilic adsorbents such as silica and alumina with nonpolar to moderately polar solvents [9].

It involves no partition of the sample solute in the stationary phase [17]. Therefore, careful adjustment of the polarity of the mobile phase for stable activity of the polar sites is needed for reproducible separation [9]. The solute is adsorbed when the attraction forces between the solvent and the solute, and the solvent and the stationary phase. Attraction forces are hydrogen bonding, van der Waals, acid-base and complexation interactions [18]. The effectiveness of separation depends upon adsorbent, which should have large surface area of uniform size [19].

Normal phase is used in the conventional sense to mean system in which the stationary phase is more polar than the mobile phase. Bonded phase appear to be slowly replacing traditional solid silica and alumina as adsorbents in normal-phase

LC, although silica and alumina still find wide spread use. Functional studies of liquid-solid adsorption chromatography LSC continued to appear [6].

1.6. II REVERSED PHASE (PARTITION) CHROMATOGRAPHY:

The interaction and separation in partition chromatography (reversed phase mode) are based on non-polar stationary phase. The retention of an analyte depends on the degree to which it is partitioned into liquid organic stationary phase and determined by the hydrophobic interactions of analyte with a relatively polar mobile phase [20].

Partition chromatography is usually carried out with liquid stationary phase. The liquid stationary phase is bonded chemically to an inert solid surface. The main advantage of this bonded stationary phase is its quite stability which can not be easily removed or lost during use [8].

Reversed bonded phase chromatography (RBC) involved a relatively non polar stationary phase that is used in conjugation with polar mobile phase to separate a wide verity of less polar solutes [20]. Two fundamental types of stationary phase are used; the most common being non-polar groups bonded on silica. The most often used are the organic groups $-CH_3$, C_8H_{17}, and $-C_{18}H_{37}$. Of these the 18-Carbon chain (the octadecyl group) is the most common, the abbreviation ODS and C_{18} are used for this type of stationary phase. The second type of stationary phase solid support used for reversed phase chromatography is composed of organic polymer beads. A typical polymer is a resin composed of polystyrene cross-linked with divinyl benzene.

Reversed phase is quite popular since the peaks in reversed phase separation tend to be narrow and symmetrical and the adsorption / readsorption equilibrium reactions tend to be fast [12]. The functional groups that are usually used as liquid stationary phase may be aliphatic or aromatic hydrocarbon, amino, cyano, ion exchange groups such as sulfonic acid and quaternary ammonium derivatives. These attached functional groups to the support play an important role in separation of sample components [20].

1.6. III ION EXCHANGE CHROMATOGRAPHY:

Ion exchange chromatography (IEC) is an electostatic method for separation of ionic species based on their differential migration on an ion exchange column [21]. The column contains the stationary phase, often suitably modified silica or resin particales. The most commonly used resin in ion exchange chromatography is the styrene/divinylbenzene copolymer. Functional groups are placed on the benzene rings to provide the ion exchange sites. Depending on the type of ions that are available for exchange on the resin (R), the process may be called cation or anion exchange chromatography [22]. The mechanism of interaction of the solutes with the stationary phase determines the classification of the mode of liquid chromatography. In ion chromatography the basic interaction is ionic. The stationary phase is charged due to fixed anions or cations, which are neutralized by counter ions of the corresponding opposite charge. The counter ions can be exchanged by other ions either from the mobile phase or from the sample, hence the name ion-exchange chromatography [23]. Ion exchange reactions are usually reversible; the ion exchange process can be described in terms of equilibria equations [22].

Figure (1-1) illustrates the principle of retention by exchange of anions in anion-exchange chromatography and equation (1-5) describes it as equilibrium.

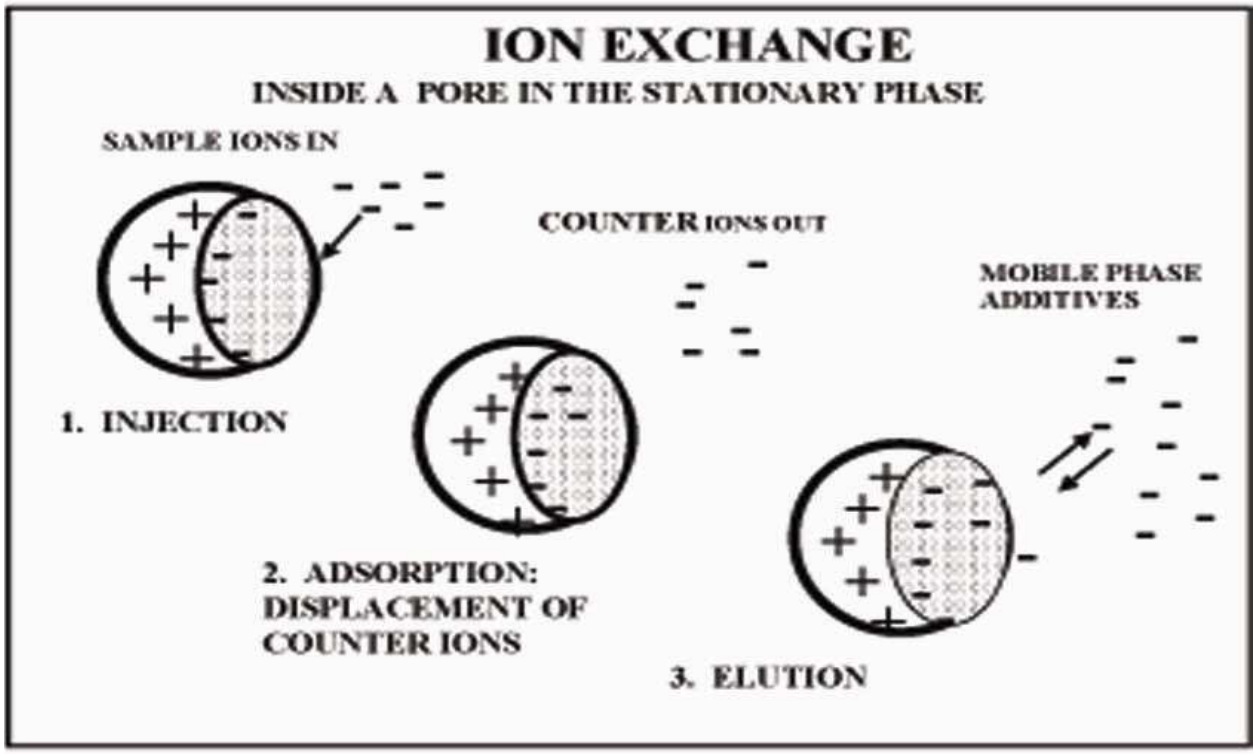


Figure (1-1) ***The principle of retention by exchange of anions in anion-exchange chromatography.***

$$M^+C^- + A^- \text{<--------> } M^+A^- + C^- \quad \text{---------- (1-5)}$$

The functional groups on the stationary phase surface are fixed positively charged species (M^+). At equilibrium these positively charged functional groups are neutralized by the counter ions from the running mobile phase (C^-). In the second and the third steps, the anionic sample components (A^-) enter the column and distribute between the stationary and the mobile phases by displacing the counter ions, and being displaced by the mobile phase ions back and forth.

The distribution equilibrium is determined by the competition between the sample components and the anions of the mobile phase on the charged sites of the stationary phase. The electroneutrality of the solution must be maintained during the ion-exchange process; therefore, the exchange is stoichiometric so that a single monovalent anion A^- displaces a single monovalent counter ion C^- . The process of cation retention is similar, however, the stationary phase is negatively charged and the counter ions are positively charged [23]. The terms stronge or weak ion exchanger refer to the acid/base strength of the functional groups. Sulfonic acid, R-$SO3^-$ H^+, and carboxylic acid, R-COO^- H^+, functional groups, are considered as strong and weak cation exchangers, respectively, while the quaternary ammonium group, R-N^+ (CH3)3 Cl^-, and the tertiary group, R-N(CH2CH3)2H^+ Cl^-, are considered as srong and weak anion exchangers, respectively [22]. Ion-exchangers are characterized both by the nature of the matrix used as a support and the nature of the ionic functional groups on the surface.

Some of the common types functional groups used in ion exchange chromatography are listed in table (1-2).

Table (1-2): Types of functional groups commonly used in ion Exchange chromatography.

CATION EXCHANGERS	ANION EXCHANGERS
Sulfonic acid $-SO_3^- H^+$	Quaternary amine $-N(CH3)_3^+ OH^-$
Carboxylic acid $-COO^- H^+$	Quaternary amine $-N(CH3)_2(EtOH)^+ OH^-$
Phosphonic acid $PO_3^- H^+$	Tertiary amine $-NH(CH_3)_2^+ OH^-$
Phosphinic acid $HPO_2^- H^+$	Secondary amine $-NH_2(CH_3)_2^+ OH^-$
Phenolic $-O^- H^+$	
Arsonic $-HAsO_3^- H^+$	
Selenonic $-SeO_3^- H^+$	

Cation-exchangers functional groups can function as such only when they are ionized, therefore they are classified into strong acid and weak acid types accordingly. The strong acidic functional groups are ionized over a wide pH range, in contrast to the weak acidic functional groups, which are ionized over a limited pH range. Sulfonic acid exchangers are strong acid types, whilst the remaining cation-exchangers' functional groups in Table (1-2) are weak. The weak acidic functional group requires the use of pH higher than its pKa. Similarly, anion-exchangers are classified as strong and weak base exchangers. Quaternary amine functional groups form strong anion-exchangers, whilst less substituted amines form weak base exchangers. The strong base will be positively charged over a wide pH range, therefore will be able to function as an anion-exchanger, in contrast to the weak anion-exchangers.

A weak anion-exchangers Resin-NH2 for example, requires pH sufficiently low enough to protonate the amine group into Resin-NH3+. Most of the ion-chromatography separations, using silica or polymeric ion exchangers perform on strong anion-exchanger (SAX) or strong cation exchangers (SCX). The types of matrixes used as support for stationary phases in ion chromatography can be divided to three: silica-based, synthetic organic polymers and hydrous oxides [(23)]. In the stationary phase, the ions are immobilized and it travels through column in the mobile phase. The separation of analyte ions depends upon the differential affinities of a functional group for different analyte ions. The relative affinities of analyte ions for the stationary phase are known as the selectivity. Selectivity is optimized by many parameters including type of functional group of the stationary phase, and concentration and characteristics of eluent ions [(24)]. The first two parameters determined by the design of the ion exchange column and usually optimized for anions, or cations. The other parameters can be adjusted by the analysis.

The variable number of functional group sites in the stationary phase is known as the capacity and it is usually expressed as the number of equivalents per gram of resin. A higher capacity results in longer retention of the analyte ion. It is independent of selectivity; “capacity can be increased or decreased without altering selectivity” [(25)] and is determined by taking a weighted amount of the cation exchanger in the H^+ form, replacing the H^+ with standard alkali metal, and titrating the librated H^+ with standard base. Anion exchangers in OH^- or Cl^- form are treated similarly using an appropriate counter ion to liberate the OH^- or Cl^-, which then titrated with standard acid or Ag^+, respectively [(26)]. Weak acid and base ion exchangers have capacities that are pH dependent.

The high selectivity for H^+ on a weak acid exchanger and OH^- on a weak-base ion exchanger, which is due to association, is responsible for the pH dependence [9].
Ion exchange chromatography has been applied for the separation of variety of charged organic and biochemical systems including drugs, amino acids, vitamins as well as inorganic ions.

The classical application of IEC concentrated heavily on the separation of inorganic ions, particularly closely related elements in the lanthanide and actinide series, as well as other radioisotopes. Modern LC techniques can be adapted to these separations, with important advantages in terms of automation, increase analyses speed, and important assay precision [20].

1.7 DETECTION SYSTEM:

The detectors in HPLC are employed to continuously monitor the column eluent. The detector signal is generally amplified and processed to a potentiometric recorder to obtain a permanent signal record with time of the analysis in the form of a chromatogram [27].

A wide variety of HPLC detectors have been developed with high sensitivity and universal detection requirements. The HPLC detectors can be generally classified as either responsive to a change in the property of the mobile phase, when a solute (sample component) is present or to a property of the actual solute itself [28]. These include UV-Visible spectrometer, Refractive index, Conductivity, Fluorescence and Electrochemical detectors [29]. However, the detectors used for the detection of ionic species are Conductivity, UV-Visible detectors and other.

1.7.I CONDUCTIVITY DETECTOR:

Electrical conductivity is a universal property of all ionic solutions. The conductivity is proportional to the concentration of the analyte after a chromatographic system equilibrates with the eluent; the magnitude of the response is proportional to the difference in conductance of the analyte. In order to detect a small analyte signal it is necessary to employ an eluent which gives a relatively low conductance [30, 31]. This system consists of suppressed and unsuppressed conductivity detection.

The suppressed conductivity detection is based on the use of a two columns arrangement followed by a conductivity detector .The first column serves to separate the ions of interest, while the second, suppressor column, is another ion exchange column of different functional groups, which acts to neutralize the charge of the eluent ions. Although there are different designs for such columns, these columns are packed with small polymer beads, but these beads carry acidic protons (H^+) on their surface of polymer [32]. The suppressor column is, therefore, used to lower the high eluent conductance, in order to suppress the background signal. The suppressor column becomes exhausted in the course of normal usage and must be periodically generated or replaced.

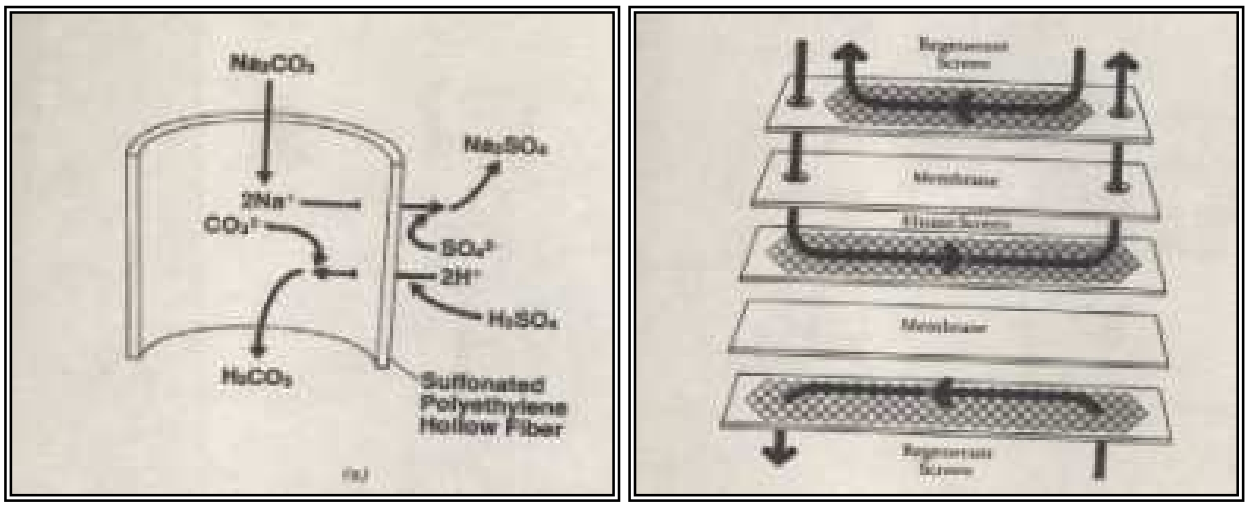
The use of a suppressor column is not without problems-eventually the resin becomes exhausted and needs to be regenerated, which is inconvenient. Also, the slightly ionized carbonic acid produces a small continuous base line conductance signal, so that the vacancy peak from the sample injection is detected and produces a negative peak that can interfere with other analyte eluting at that time .The use of a second column also results in some zone broadening, of which decreases the over all efficiency of the analysis.

The suppressor column may be in two designs:

A. a hollow fiber.

B. a sandwich.

The two designs are shown in Fig. (1-2)



(A) ***(B)***

<u>Figure (1-2):</u> The design of suppressor column for ion chromatography: (A) hollow fiber and (B) membrane sandwich.

The first problem has been involved using continuous, flowing suppressor streams that contact the chromatographic stream through a porous membrane.

The hollow-fiber ion exchange tubing packed with plastic beads to decrease the internal volume and zone spreading has been used [20, 33].

In anion analysis the aqueous sodium hydroxide used to elute anions, such as chloride, and nitrate, from a strong anion ion-exchange column .The resulting eluent, containing sodium, chloride, nitrate and hydroxide ions, which passed through a cation ion- exchange column (suppressor column) in the acid (H^+) form.

The sodium ions were replaced by protons, converting the hydroxide ions to virtually un-ionized water but leaving the chloride and nitrate ions as the strongly ionized mineral acids [8].

(eluent) $Na^+OH^- + H^+R^- \longrightarrow HOH + R^-Na^+$

$Na^+Cl^- + H^+R^- \longrightarrow H^+ + Cl^- + R^-Na^+$

$Na^+NO_3^- + H^+R^- \longrightarrow H^+ + NO_3^- + R^-Na^+$

The solutions of sodium carbonate or bicarbonate that were used as the eluent, they would be converted into the weakly ionized carbonic acid and the conductivity also effectively suppressed. The carbonate and bicarbonate ions ,both of which are basic ,combine chemically with the protons on the polymer surface forming carbonic acid which ,being unstable in aqueous. Solution decomposes to carbon dioxide gas and water in this way the carbonate and bicarbonate ions are removed from the solution [32].

$HCO_3^- + H^+ \longrightarrow [H_2CO_3] \longrightarrow H_2O + CO_2$

$CO_3^{-2} + 2\,H^+ \longrightarrow [H_2CO_3] \longrightarrow H_2O + CO_2$

In unsuppressed IC, the second column is not used [8] .Detection is not as good because the eluting ions maintain a large background at the detector at all times.

Very good performance is still possible when special eluting (with low conductance) or low concentrations are used. This represents an indirect detection mode because the eluting ion is displaced (charge exchange) by the analyte ions as they elute. Conductivity is normally not considered to be an indirect detector even though it does function in that mode in single-column IC. Sensitivity transfer in the indirect mode has been demonstrated with good results [34, 35].

The signal in this simple method is proportional to the difference of the equivalent conductivities of the sample and the eluting ions.

The sensitivity depends on the background conductivity which is the total conductivity of the eluent. Many investigations have been undertaken to achieve high sensitivity detection by the selection of appropriate eluent materials for unsuppresser ion chromatography. Typical examples are the used of [36, 37] salicylate [36, 38] as eluent in anion chromatography. Salicylate has a strong eluting ability, which leads to a low level of background conductivity.

Gjerde *et al*. [39] is the first who used direct conductivity detection in (1979). A dilute solution of a carboxylic acid salt such as benzoate, phthalate in (10^{-5}-10^{-4}) M range were used as the eluent along with a low capacity ion exchange column for direct conductivity detection of common inorganic anions at low ppm levels. In this system a pH adjustment of the mobile phase was found necessary to ensure reproducible chromatographic performances.

Gjerde and Fritz [35, 40] found that the sensitivity of conductivity detection was increased when weak acid eluent, such as benzoic acid, when used in anion chromatography. This effect was attributed to the shift of the acid dissociation equilibrium in the separation column. However, the baseline is likely to drift because of the high background conductivity.

In this system, both positive and negative analyte peaks were produced depending on the equivalent conductance of the analyte components and the pH of the mobile phase, which determined the ionic form of the analyte [41, 42]. One characteristic of the unsuppressed IC with conductivity detection is the appearance of the injection peak; this is due to the displacement of the eluent ions by the injected ions [43, 44].

1.7.II UV-VISIBLE DETECTOR:

This detector is functioning as a solute-specific detector and may be used for component that exhibit absorption in the UV-Visible region. Detection of some ions is not generally applicable except at very low wave length such at 215nm [45]. Many inorganic cations and anions do not have significant absorption in UV-VIS range of the spectrum; therefore direct detection can not be used. However there are cases where the ions can be detected directly by their UV detection in the (185-220) nm range [46].

Cochrane and Hillman [47] have reported the separation of nitrate and nitrite as examples of ions that can be detected as 205 nm following their separation. Other ions that can be determined at 205 nm are acetate, formate, bromide, iodate, iodide, bromate, and thiocyanate .Direct UV detection is difficult when separation encountered one of the species is UV transparent.

The UV/VIS detector may also be used in an indirect mode. Small and Miller [48] reported this approach, as a detection technique for ionic species. Indirect photometric detection (IPD), in which transparent sample ions were eluted with a light-absorbing ion. The elution of sample ions was observed as troughs in a high absorbance background.

The indirect photometric detection (IPD) technique also known as indirect photometric chromatography (IPC) makes use of conventional HPLC equipment with UV detectors for the analysis of transparent ionic species. The Mobile phases in IEC-IPD should have the ability of displacing the analyte ions from the stationary phase and selectively separating them [49]. The eluent in (IPD) should possess several characteristic such as ion exchange capability, ion-exchange selectivity and a large molar absorptivity [48]. The most common (IPD) mobile phases used for anion separation are benzoate, phthalate, sulfobenzoate and salicylate [50, 51].

These eluents have showed good separation profiles for many analyte anions with reasonably low detection limits [52, 53]. However, the elution of analyte ions will be determined by the effective charge of the eluent, which in turn is dependent upon the pH of the mobile phase. Precise control of the mobile phase pH was found to be important to ensure reproducible elution and retention times [49, 54]. In this technique, the ion exchange column is first equilibrated with the light absorbing eluent preferably at a very low concentration. The resultant high background absorbance signal is offsetted electronically. A decrease in the background signal is observed as the non-UV absorbing analyte ion is eluted from the column [50, 51]. Negative analyte peaks are obtained instead of the conventional positive peaks, as shown Fig. (1-3).

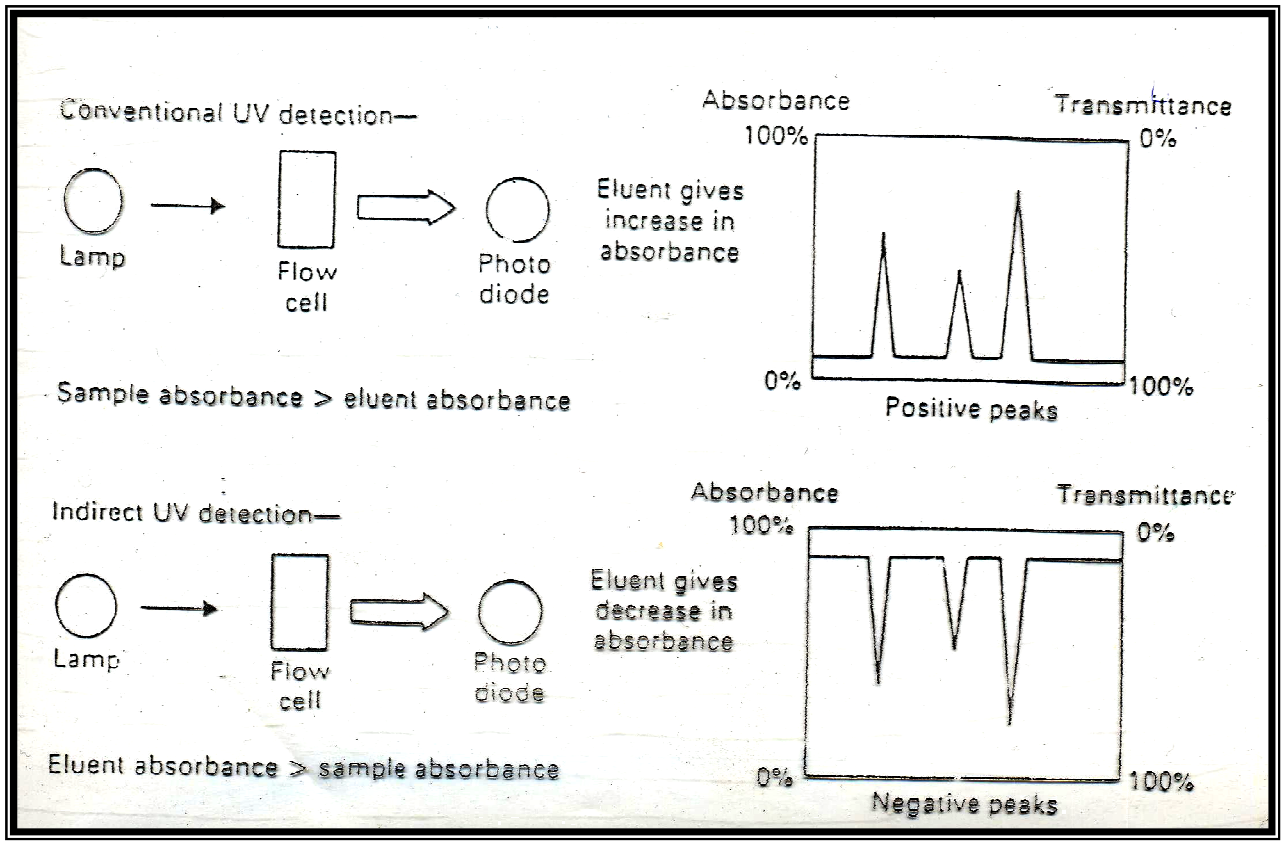


Fig.(1-3): ***Comparison between Conventional and Indirect Photometric Chromatography.***

The measured signal in IPD is the difference between the base-line signal and the signal when the analyte elutes. The observed signal, **S**, may expressed as:

$$S=C_S€_S+ (C_E-C_S) €_E-C_E€_E=Cs (€s - €_E) \text{ ---------- (1-6)}$$

Where Cs and C_E concentrations of the sample and eluent ions, respectively. $€_s$ and $€_E$ are the molar absorptivities for the sample and eluent ions, respectively. Since the molar absorptivity of the sample ions assumed zero, eq. (1-6) becomes:

$$S \alpha Cs€_E \text{ ---------- (1-7)}$$

This relationship reveals that the response will be high with a large eluent molar absorptivity. However, the response alone is not a sufficient measure of performance; noise should be consider as well. The base-line noise related to the background signal, which controlled by the eluent concentration:

$$\text{Noise (N)} \alpha C_E \text{ ---------- (1-8)}$$

It follows from equations (1-7) and (1-8) that the signal-to-noise ratio (S/N) is equal to:

$$S/N \alpha C_SC_E/ C_E \text{ ---------- (1-9)}$$

This relationship shows that a small signal can seen using a very dilute eluent with large molar absorptivity [50' 51].

The signal -to noise ratio (S/N) in (IPD) is proportional to the analyte ion concentration and inversely proportional to the eluent concentration and detector noise. In practice, very low eluent concentrations would result in extremely large retention volumes for the anions, and at the limiting conditions analyte ions would not be displaced by eluent ions from the ion-exchanger [49'55].

Maki and Danielson [56, 57] have used sodium naphthalenetrisulfonate as mobile phase in anion exchanger chromatography with IPC detection.

This mobile phase showed particular promise for the separation and detection of NO_2~, Br", NO_3~, SCV^2, SCN-, and I- in less than 18 min. with detection limit 0.4-l ng for all anions.

Changing the mobile phase to naphtholdisulfonate has allowed the separation of the same anions with detection limit of 0.2-10ng and separation time of 8 minutes. Comparison between indirect UV and direct conductivity detection for anion exchange chromatography using naphthalene mono-, di-and tri-sulfonate as mobile phase for the separation of the several anions using anion exchange column has been reported recently [58].These three eluents required no pH adjustment with detection limit for chloride 0.04 ng and 0.l ng with conductivity and indirect photometry respectively [46].

Michio Z. [59] has used the eluent 0.7 M sulfosalicylic acid containing 5×10^{-5} M chlorophosfonazo III as a color-forming reagent to separate Magnesium, Calcium, Strontium and Barium. The separating ions after passing through a cation-exchange column were detected directly by a spectophtometric detector.

This mobile phase showed particular promise for the separation and detection of Mg^{+2}, Ca^{+2}, Sr^{+2} and Ba^{+2} in less than 15 min. with detection limit 2-50 ng for all cations. The separation and determination of alkaline earth metals have been of great interest in analytical chemistry, because these metals exist widely in nature and occur together in significant amounts. They seem to play an important role in the field of biology.

Small and Stevens, [60] have used the eluent 1.25×10^{-3} M copper sulfate, which absorbs UV at 216 nm for displacing ions to separation of Sodium, Potassium, Calcium and Magnesium. This separation was achieved using a split column technique where in two columns of equal length but containing resins of different specific capacities connected in series and appropriately switched with IPC detection. This mobile phase showed particular promise for the separation and detection of Na^{+}, K^{+}, Mg^{+2} and Ca^{+2} in less than 10 min.

Kazutoku, O.; [61] has used of a pure silica gel (Pia Seed 5S-60-SIL), synthesized by the hydrolysis of pure teratraetoxysilane [$Si(OCH_2CHG_3)_4$], as a cation-exchange stationary phase in ion chromatography for common mono- and divalent cations (Li^+, Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}). Using aromatic monoamines at pH 5.0 as eluents, the Pia Seed 5S-60-SIL, silica gel acted as an advanced cation-exchange stationary phase for these mono- and divalent cations. Excellent simultaneous separation and highly sensitive indirect-photometric detection at 275 nm for these cations were achieved on a Pia Seed 5S-60-SIL column (150 × 4.6mm i.d.) in 20 min with 0.75 mM tyramine [4-(2-aminoethyl) phenol]-0.25 mM oxalic acid at pH 5.0 containing 1.5 mM 18-crown-6 (1, 4, 7, 10, 13, 16-hexaoxacycIooctadecane) as the eluent. Using dilute oxalic acid (0.2 mM oxalic acid) as the eluent, the Pia Seed 5S-60-SIL silica gel also behaved as an advanced cation-exchange stationary phase for these mono- and divalent cations. Excellent simultaneous separation and highly sensitive indirect-conductimetric detection for these cations were also achieved on the column in 20 min with 0.2 mM oxalic acid at pH 3.6 containing 1.5 mM 18-crown-6 as the eluent.

1.8 THE AIM OF THIS WORK:

Aim of this work is to analyses a variety of cations ranging from small singly charged ones to large and doubly charged type (Li^{+}, Na^{+}, K^{+}, Mg^{+2} and Ca^{+2}) by indirect photometric detection (IPD) and compared with unsuppressed conductivity detection using diphenylammonium chloride as eluent. This eluent have a good ion exchange capability as well as a large molar absorptivity to allow the use of very dilute mobile phase concentration.

CHAPTER TWO

EXPERIMENTAL PART

2.1 INSTRUMENTS AND EQUIPMENTS:

Two high performance liquid chromatograph were used in this work one of them was in the department of chemistry at AL-Nahrain University, Shimadzu (Kyoto, Japan) which consisted of a system controller model SCL-10 AVP, Conductivity detector model CDD-10AVP, a liquid delivery pump model LC-10AVP, a degasser model DGU-RA and Rheodyne manual injector model 3298 (USA), equipped with 10µl sample loop. The HPLC system has been interfaced with computer via a Shimadzu class-VP5 chromatography data system program supplied by the manufacture; Epson LQ-300 printer model P852A (Japan). The other one was in the Chemistry Unit at AL-Nahrain University for Medicine, Shimadzu (Kyoto, Japan) which consisted of a system Fraction controller model FRC-10A, Diode array detector model SPD-M 10AVP, a liquid delivery pump model LC-8AVP, a degasser model DGU-12AVP, Auto injector SIL-10AVP, equipped with 10µl sample loop. The HPLC system has been interfaced with computer via a Shimadzu class-VP5 chromatography data system program supplied by the manufacture; Canon LBP 810 printer model (Japan).

Orion expandable ion analyzer model EA 940 equipped with printer and glass combination electrode were used to measure the pH of the solution (USA). Shimadzu UV-Visible spectro photometer model UV-1650 PC (Kyoto, Japan). The UV-Visible system has been interfaced with computer via a Shimadzu UV-probe data system program. Cation exchange column from Dionex ion PAC HPLC-CS3 column (4 (i.d) mm × 250 mm, S/N 3583, P/N 037024).

2.2 CHEMICALS:

The chemicals were used in this work; include diphenylamine, lithium chloride, sodium chloride, potassium chloride, magnesium dichloride, calcium dichloride, methanol (analar), hydrochloric acid, sodium hydroxide. The above chemicals and reagent were obtained either from Fluka or BDH companies.

2.3 PREPARATION OF MOBILE PHASE:

All solutions and dilutions were prepared using deionized water (resistivity ~ 18 MΩ).

A 1×10^{-3} M of diphenylamine stock solution was prepared by adding 0.0423g of diphenylamine to 250 ml volumetric flask containing about 5 ml of 0.1 M hydrochloric acid and diluted to mark with deionized water and mix thoroughly in sonicator for 2 hour . A 1×10^{-7} M was prepared by diluting 0.1 ml of 1×10^{-3} M diphenylamine stock solution and adding 60 ml of pure methanol the pH of solution was adjusted by adding 0.1 M sodium hydroxide to reach pH 4.90, then the volume was completed to 1 L with deionized water. Eluent was degassed under vacuum for 15 min.

2.4 SAMPLE PREPARATION:

Stock solutions of 1000 ppm of each cations (Li^+, Na^+, K^+, Mg^{+2} and Ca^{+2}) were prepared, by dissolving the appropriate amounts of the each analyte in the same mobile phase in 100 ml volumetric flask according to the amounts listed in table (2-1) and dilute to the mark with deionized water. Store each volumetric flask in a refrigerator. Other standard solutions were prepared by subsequent dilution of stock solutions. The solvent used to prepare these solutions was usually the same as the mobile phase employed for their separation. Mixtures of four or more of the above analyte were also prepared by mixing the appropriate volumes of the stock solutions.

Table (2-1): Weight of compound required to prepare 100 ml of a 1000 mg/L standard solution.

Cation	Compound	Weight (g)
Li^{+}	Lithium (LiCl)	0.6108
Na^{+}	Sodium (NaCl)	0.2542
K^{+}	Potassium (KCl)	0.1907
Mg^{+2}	Magnesium ($MgCl_2.6H_2O$)	0.8365
Ca^{+2}	Calcium ($CaCl_2.2H_2O$)	0.3668

2.5 ANALYSIS OF SAMPLE:

All prepared standard solutions and their mixtures have been chromatographically analyzed on the cation exchange column with diphenylammonium chloride at pH 4.90 as Eluent at optimum flow rate 1 ml/min with UV-Visible and conductivity detectors.