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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 theioretical plates
t_R	Retention time
W_b or W	Width of peak at baseline
HETP or H	Hight Equivalent toTheoretical Plates
L	Length of column
Rs	Resolution between to peaks
α	Separation factor
Ḱ	Capacity factor
LSC	Liguid-Solid Chromatography
RPC	Reversed Bonded phase Chromatography
IEC	Ion Exchange Chromatography
DMSO	Dimethyl sulfoxide
IPD	Indirect Photometric Detection
IPC	Indirect Photometric Chromatography
S	Observed signal
Cs	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 Violet
R	Correlation cofficient
R^2	Linear regression Ion Chromatography
IC	Ion Chromatography
PVC	Poly vinyle chloride

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عنوان الاطروحه: *الفصل الكروموتوغرافي لبعض الايونات الموجبة و تقديرها بواسطة كاشف التوصيلية و التحليل الضوئي غير المباشر.*

CHROMATOGRAPHY

1.1 CHROMATOGRAPHIC SEPARATION TECHNIQUE:

Chromatography is primarily a separation technique. It 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 techniques are divided up according to the physical states of the two participating phases [(2)]. When applied to the collection of pure materials, it is called preparative chromatography. The aim is to extract as much material in as pure as state as possible, two chromatographic variants are used:

a) Batch or conventional liquid chromatography (Fig.1.1a).

b) Continuous liquid chromatography (Fig.1.1b).

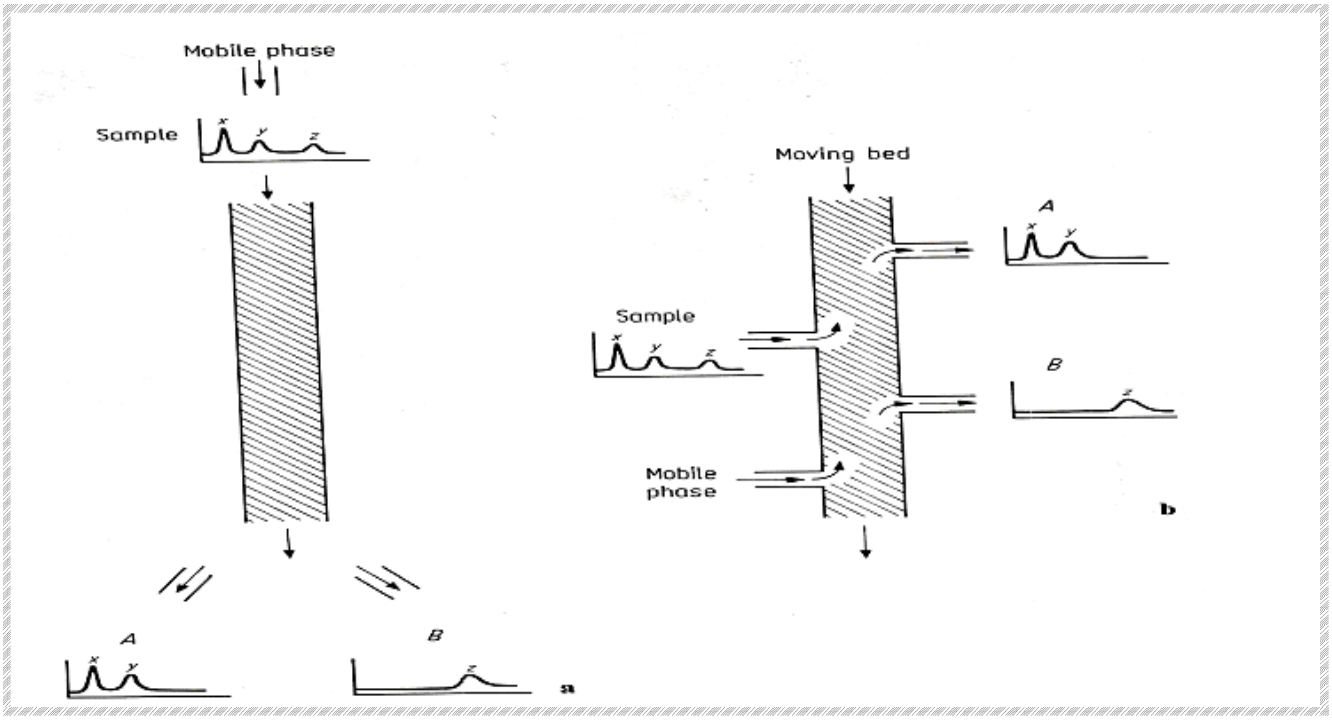


Fig. (1.1) Batch (a) and continuous (b) liquid chromatography

In batch chromatography one phase moves (the mobile phase) and the other remains stationary. In continuous chromatography the conventional stationary phase is moved counter currently in a "moving bed" against the mobile phase. If the two immiscible phases in a partitioning system are not held statically but move relative to one another through a tube or column, the analytes will be carried with the moving phase through the column [3]. However, they will be partly retained depending on their interaction with the second static phase, and even small differences in the distribution cofficients will cause the components to be separated (Fig 1.2) [4].

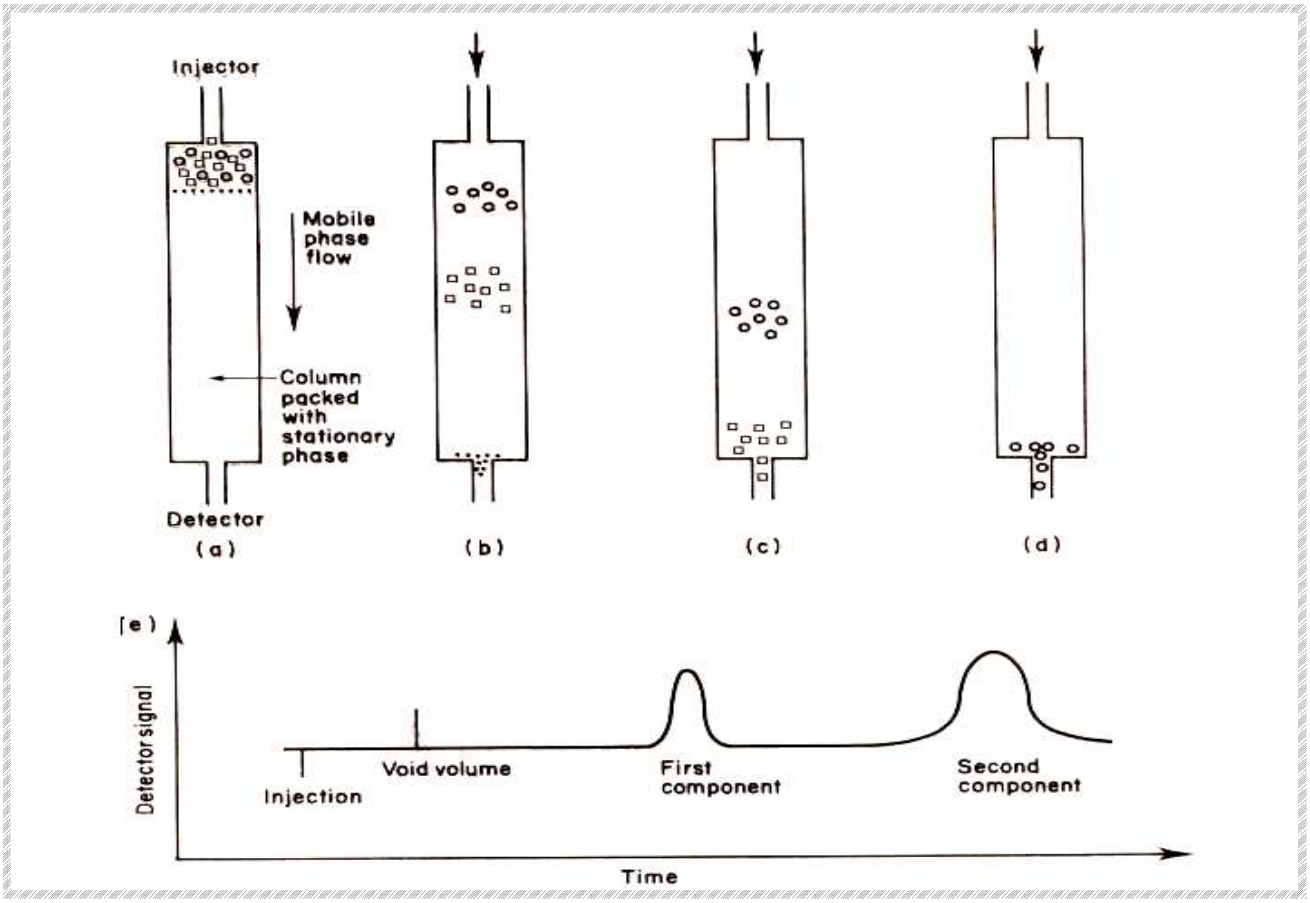


Fig. (1.2) Chromatographic separation on a column. (a) Introduction of the sample. (b) Elution of unretained components at column void volume. (c) Elution of more weakly retained compound. (d) Elution of more strongly retained component. (e) Chromatogram recorded by detector at end of the column.

By adjusting the flow-rate of the bed and the mobile phase, one can enrich and separate a component or even a group of component in one direction or the other. This type of chromatography, where the appartus used is normally highly specialized, has been reviewed [5,6]. Therefore, and because continuous liquid chromatography is the province of the chemical engineer, continuous chromatography is excluded here.

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 [7] 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 [7] 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 [7] 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 times 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 [7].

1.3 MODES OF CHROMATOGRAPHY:

The term "chromatography" has also come to include a number of related separation techniques using similar equipment but based on different physical concepts. These include size exclusion chromatography (SEC) based on the molecular size differences of the analytes and ion-exchange chromatography (IEC) in which the separation depends on the interactions between ionized groups on the analytes and the stationary phase; these techniques will be briefly included as appropriate [8].

The stationary phase can be either a solid, or a liquid spread as a thin film on the walls of the column or over an inert support.

The mobile phase can be either a gas, a liquid, or a supercritical fluid and is often termed the "eluent" if the analytes are carried(or eluted)out of the column system before detection [3].

This basic concept has developed into a wide range of analytical chromatographic methods broadly based into two main groups depending on whether the mobile phase is a gas or a liquid (Table 1-1).

Table (1-1): Methods of chromatography.

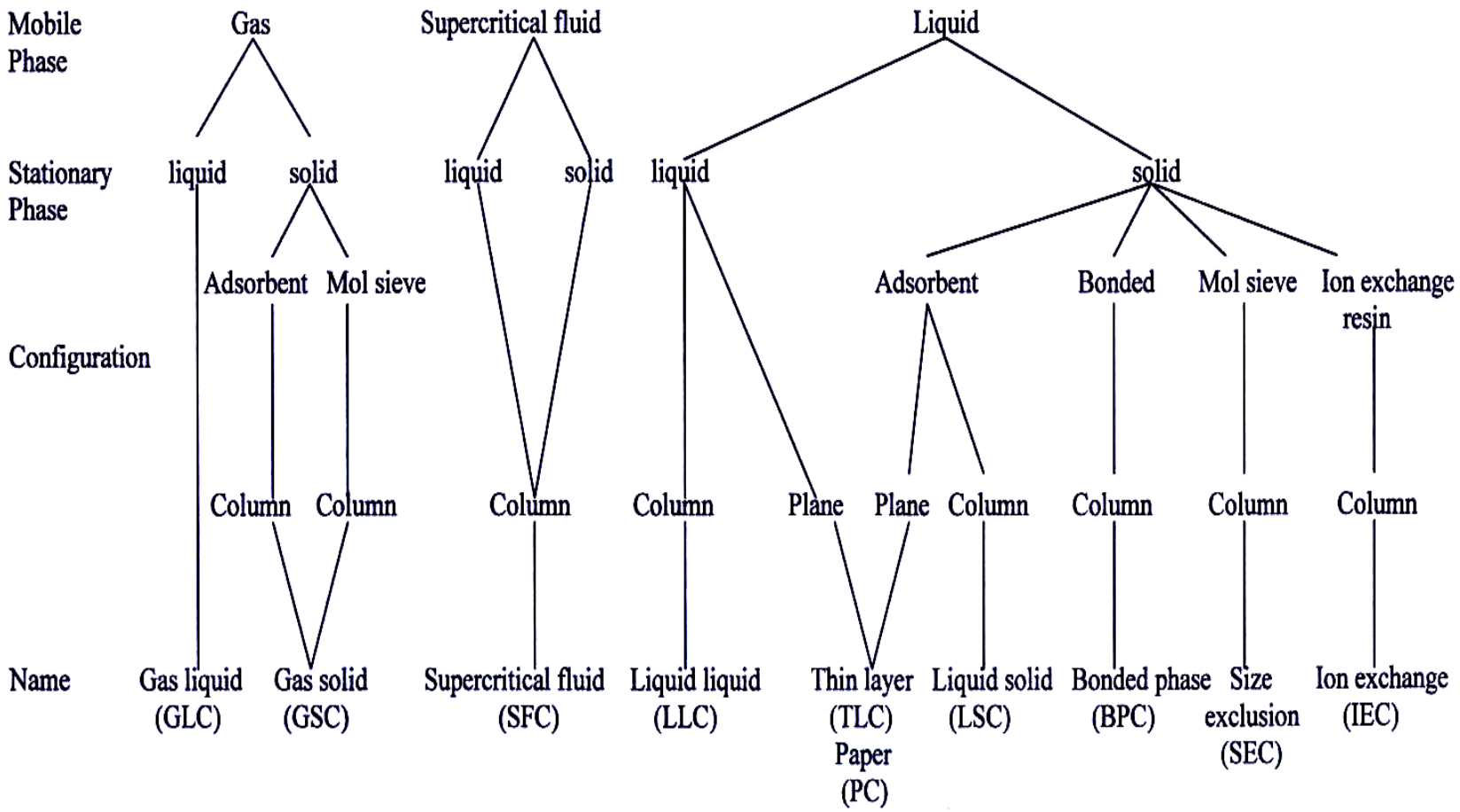
Mobile phase	Stationary phase	Chromatographic Method	Abbreviation
Gas	Liquid	Gas-Liquid chromatography	GLC
	Bonded Liquid	Gas-Liquid chromatography	GLC
	Solid	Gas-Solid chromatography	GSC
Liquid	Liquid	Liquid-Liquid chromatography	LLC
		Droplet counter-current chromatography	DCC
	Bonded Liquid	High-performance liquid chromatography (reversed-phase)	HPLC
	Solid	High-performance liquid chromatography	HPLC
		Thin-layer chromatography	TLC
Supercritical fluid	Bonded liquid	Supercritical fluid chromatography	SFC

The distribution and retention of an analyte in a chromatographic separation may result from adsorption onto a solid stationary phase and/or a partition process between gas and/or liquid phase [(9)].

Figure (1.3) shows a complete classification scheme of the different chromatographic techniques. It includes the most popular techniques; gas and liquid chromatography (GC and LC). The classification shows not only the physical states of the two phases but also the configuration of chromatographic bed, there are two popular configurations for the bed, a column and a planar surface [(10)].

In column chromatography; the stationary phase is held in a narrow tube through which the mobile phase is forced under gravity or external pressure (pump). The stationary phase in planar chromatography is supported on flat plate or in the interstices of paper. Here the mobile phase moves through the stationary phase by capillary action or under the influence of gravity [(11)].

Figure (1.3) Classification of Chromatography Techniques[10].



1.4 BASIS OF THE SEPARATION PROCESS:

Chromatographic separation process is based on the difference in the surface interaction of the analyte and eluent molecules. Let as 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 in to 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 [(12)].

1.5 GAS-LIQUID CHROMATOGRAPHY:

Gas-liquid chromatography (GLC) has found widespread application in many areas of chemical analysis and a wide range of compounds can be examined [(13)]. Gas-liquid chromatographic equipment was commercially available [(14)] and the rapid development of modern-day gas-liquid chromatography as a widely used routine analytical method was under way. The separation is carried out using a gaseous mobile phase, which transports the analyte through a column containing the liquid stationary phase [(15)]. Although this chromatographic technique had preceded GLC [(16)]**,** it has always been very limited in its application and even now it is not widely used. The retention of the analyte depends on the degree of its interaction with the liquid phase and its volatility.

The column can be heated, typically within the range 50-300°c, to increase the volatility of the sample and reduce retention times [17].

In general, GC can be used to analyzed volatile and thermally stable samples, and this is not alimitation imposed on LC [18]. As an analytical technique, GLC is very simple to operate and is capable of high resolution, selectivity and sensitivity [17]. This was followed by the introduction by Lovelock of the argon ionisation[19] and electron affinity detectors [20].

1.6 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 [18]. 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 milliliters 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 [18]. Liquid chromatography techniques can be used for the determination of ionic species as well as other compounds [21]. However, HPLC uses high pressure to force solvent through closed columns containing very fine stationary phase particles that give high resolution separations. The HPLC system 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 column that are 5-30 cm in length, with an inner diameter of 1-5 mm. The typical particale size packed in an HPLC column 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 ~0.5-5 ml/min [22]. 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 **(t_R)** compared with the width of its peak at base line **(W_b)**.

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

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

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

Where **L** is the length of the column [23]. 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 [11]. Resolution **(Rs)** is the efficiency of a chromatographic system; it defines the degree of separation between two adjacent peaks [23].

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

Where tr2 and tr1 are the retention times and w1 and W2 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 [ ] :

$$Rs = 1/4\ (N)^{1/2}\ [(\alpha\text{-}1)/\ \alpha]\ [\acute{K}_2/(1+\acute{K}_2) \text{----------(1-4)}$$

Where **(α)** selectivity, and **($\acute{K}_2$)** capacity factor.

The small particle size 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 [24]. HPLC has been used for analysis of wide variety of pharmaceutical products, body fluid, and environmental samples [25].

1.7 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY:

HPLC and its various techniques has been the domain of organic chemistry for along time. It has been recognized for twenty five years that HPLC can equally well be utilized to separate inorganic compounds [26]. It has even turned out that separations, which were definitely unfeasible before, can easily be effected now using HPLC techniques. The natural limitations of HPLC applicability will be valid also in the field of inorganic chemistry of course [27]. That means that only those compounds are amenable to HPLC investigations which are soluble in a solvent being compatible with HPLC columns. It is a " high performance" process that is the result of technological advances in synthesis of very small stationary phase particles which are as small as 2µm [28] ,Instrumentation, in addition to the manufacturing of reliable solvent delivery pumps, which can deliver 0.001-5mL/min with very high precision [(-2,+2)%] and at constant high pressure ranging from

1-39.2Mpa [29]. There are two distinctly different classes of soluble compounds in inorganic chemistry.

First, there are ions(-anions as well as cations-) which in general are easily dissolved in aqueous solutions.

Second, there are molecular compounds which are soluble in organic solvents [27].

Accordingly, two different branches of HPLC have come into use in inorganic chemistry. For the separation of ions in aqueous solution the technique of ion chromatography (IC) has been developed. The non dissociated molecular compounds can be separated in reversed-phase systems using the well-known bonded alkyl silica phased and mostly organic eluents like methanol or acetonitrile (RPLC) [27]. In a few cases also normal-phase LC on silica or alumina has been used.

1.8 APPLICATION OF HIGH – PERFORMANCE LIQUID CHROMATOGRAPHY:

Virtually all compounds which will dissolve in an aqueous or organic solvent can be separated and quantified by HPLC, with a sensitivity dependent on their ease of detection. As these criteria include almost all non-polymeric and non-gaseous compounds of interest in present-day chemistry, a detailed list of applications would be virtually limitless [25].
A bibliography of sources of application methods covering the main chemical types[30].

Generally, HPLC has been most widely applied in areas where GLC is unsuitable, in particular the separation of relatively polar or involatile compounds. The most important groups of analytes are pharmaceutical compound, including penicillins, tetracyclines, barbiturates, opium alkaloids and many other major drug groups [31].

HPLC has also found application in the study of agrochemicals, although for the more volatile organohalogen and organophosphorus insecticides and herbicides, the high sensitivity and selectivity of the electron capture, thermionic and flame photometric detectors has meant that GLC is often still preferred [32].

In addition, the need for the positive identification of pesticides and pollutants in complex matrices, will require the combination of high-resolution separation and a sophisticated detector provided by open-tubular GC-MS [33].

HPLC has proved especially useful for the separation of many biological samples, naturally occurring compounds, including carbohydrates, lipids and vitamins as well as pollutants, food additives, food colors and preservatives [34].

In recent years, there has been particular interest in the application of HPLC in the reversed-phase mode [35], with wide-pore column materials and hydrophobic chromatography for the separation and isolation of biopolymers, proteins and peptides, with the retention of biological activity [36].

HPLC can also be applied to the analysis of inorganic complexes or chelates, organometallic compounds and simple anions and cations[37].

By scaling up the size of columns and pumps, HLPC can be carried out preparatively on the milligram to kilogram scale.

Eluents used in normal-phase chromatography can usually be readily removed but the aqueous-organic eluents used in reversed-phase chromatography normally have to be separated from the analyte by freeze-drying [35].

1.9 MODES OF LIQUID CHROMATOGRAPHY AND OPERATION OF HIGH-PERFORMANSE LIQUID CHROMATOGRAPHY:

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 [38]. Instrumental liquid chromatography is characterized by the use of online detection system, controlled eluent flow rate and defined eluent composition [39].

The aim is to increase, compared to column or thin-layer chromatography, the efficiency of separation and the reproducibility and accuracy of retentions and peak areas in order to achieve quantitative and qualitative separation. Because a wide range of separation modes can be used, high-performance liquid chromatography (HPLC) is an extremely versatile technique and can be used to determine virtually any non-gaseous analyte, as long as it is soluble in an organic or inorganic solvent. It is this versatility that has made it one of the most important techniques available to the analytes and is the reason for its rapid growth since the late 1970s [40].

One of the greatest advantages of HPLC is that the separation can be carried out by making use of a wide range of different interactions between the analyte and the chromatographic system. The two principal modes are normal-phase and reversed-phase liquid chromatography but the same instrumentation [41], with different column packing and eluents, can also be used for separations based on size exclusion and ion exchange chromatography. Although the descriptions, normal-phase and reversed-phase are widely used and are useful guides to the operation of a particular HPLC separation, they are approximat ions and in reality the situation is more complex.

More than one mechanism may be in operation at the same time, A single column may be used for different primary modes by changing the solvent [42]. Most silica columns, even after being coated with an alkyl-bonded phase, will still possess some free acidic silanol groups on their surface and will interact with basic analytes by a mixture of ion-exchange and reversed-phase chromatography. The separation of ionisable analytes may thus be effected by parameters, such as pH and ionic strength, that have little effect on non-ionised analytes. Because the silica matrix of the stationary phase has a definite pore size, high-molecular-weight analytes may suffer exclusion from some pores and a from of size exclusion chromatography can be superimposed on the separation [43]. Simple theory of bonded-phase separations regards the column as a liquid-liquid partition system and this adequately describes much of the analyte behavior. However, the bonded phase is rigidly held and has a defined depth, and thus a more detailed treatment of the mechanism of retention must also include consideration of the layering of the stationary and mobile phase. On the other hand, silica columns are described as adsorption chromatography but some mobile-phase constituents can caot the surface and make it behave in a similar manner to a liquid phase. The selection of the appropriate method for a particular analysis is therefore complex and the experience of the operator can be an important factor [44].

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 [47].

1.9. I NORMAL PHASE (ADSORPTION) CHROMATOGRAPHY:

This mode of separation was the first discovered form of liquid chromatography or liquid-solid chromatography (LSC). 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 [18].
It involves no partition of the sample solute in the stationary phase [48]. Therefore, careful adjustment of the polarity of the mobile phase for stable activity of the polar sites is needed for reproducible separation [18]. 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 [49]. The effectiveness of separation depends upon adsorbent, which should have large surface area of uniform size [50].

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 appears 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 [11].

1.9. 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 [51].

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 [52].

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 [51]. 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 $-CH_3$, $C8H_{17}$, 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 [23]. 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 [51].

1.9. III ION EXCHANGE CHROMATOGRAPHY:

Ion chromatography (IC) has been rapidly employed in a wide application range including environmental analysis since 1975 when Small et al. published it [51]. Since it has made a remarkable development in these years, it may be said that ion chromatography has established a solid base for a method to analyze inorganic ions. There are many different ways of determining ions qualitatively and quantitatively.

There are many important fields of application for ion chromatography such as:

1. the routine investigation of aqueous system such as drinking water, rivers, effluents and rain water.
2. the analysis of ions in chemical product, foods, cosmetics, pharmaceutical.
3. ultratrace analysis such as in the semi-conductor and power industry.

Ion chromatography can be used for the analysis of anions, cations, organic acids and amines plus analytes such as carbohydrates.

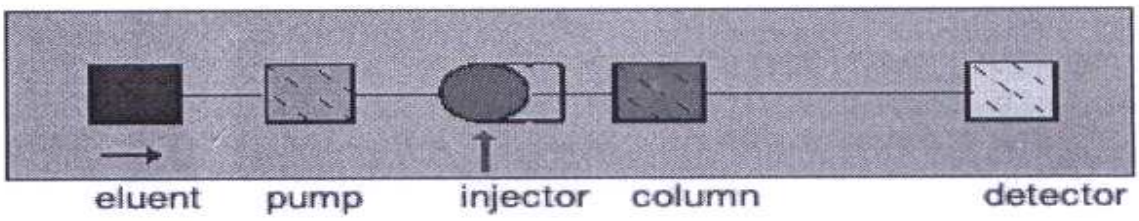


Fig. (1.4) Unsuppression in Ion Chromatography System

The above schematic in fig.(1.4) represents a non-suppressed ion chromatography system. The sample is introduced on-to the system via a sample loop on the injector.

When in the inject position the sample is pumped onto the column by the eluent and the sample ions are then attracted to the charged stationary phase of the column. The charged eluent elutes the retained ions which then go through the detector (which is most commonly conductivity) and are depicted as peaks on a chromatogram [(2)].

The different modes of chromatography (anion exchange, cation exchange and ion exclusion) simply relate to the different types of columns used to achieve the separation of the ions. The eluent used depends on the column type and also the mode of detection.

Modern ion exchange chromatography began with a report by Small, Steven and Bauman [(54)], where they described a way to combine an ion exchange chromatographic separation with simultaneous conductometric detection for the determination of anions including chloride, sulfate, nitrate, and phosphate, or cation including sodium, ammonium, potassium and calcium [(55)].

Inorganic cations and anions cannot be separated by reversed-phase chromatography using ion suppression or ion-pair chromatography but they can be separated using ion-exchange chromatography.

Ion exchange chromatography (IC) is based on a stichiometric chemical reaction between ions in a solution and the oppositly charges groups functional groups on the column resin. In the simplest case in cation chromatography these are sulfonic acid groups or carboxylic acid groups (such as maleic acid) and in anion chromatography quaternary ammonium groups [(2)]. 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 $-N(CH3)_2(EtOH)^+ OH^-$
Phosphoric acid $PO_3^- H^+$	Tertiary amine $–NH(CH_3)_2^+ OH^-$
Phosphonic acid $HPO_2^- H^+$	Secondary amine $–NH_2(CH_3)_2^+ OH^-$
Phenolic $-O^- H^+$	
Arsonic $-HAsO_3^- H^+$	
Selenonic $-SeO_3^- H^+$	

There is avariety of cation columns available, however the modern ones contain carboxylic acid functional groups. A large number of applications for silica-gel-based ion exchangers exist [(2)]. These columns allow simultaneous separation of alkali metals and alkaline earths plus the separation of transitional metal and heavy metal ions is also possible. Small amines can also be analysed using cation exchange columns [(2)].

The eluents used for non-suppressed cation exchange are weak acids with a complexing agent such as dipicolinic acid, the concentration of which can effect the elution of calcium and heavy metals such as iron, zinc and cobalt [(2)].

Anion exchange chromatography froms the largest group of IC methods mainly because there are few alternatives with such simplicity, sensitivity or selectivity. The two forms are anion exchanged with or without suppression and of these two methods are the most widely used. Eluents for suppressed chemistries tend to be either carbonate based or hydroxide [2].

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 [56]. 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 pH dependence [18].

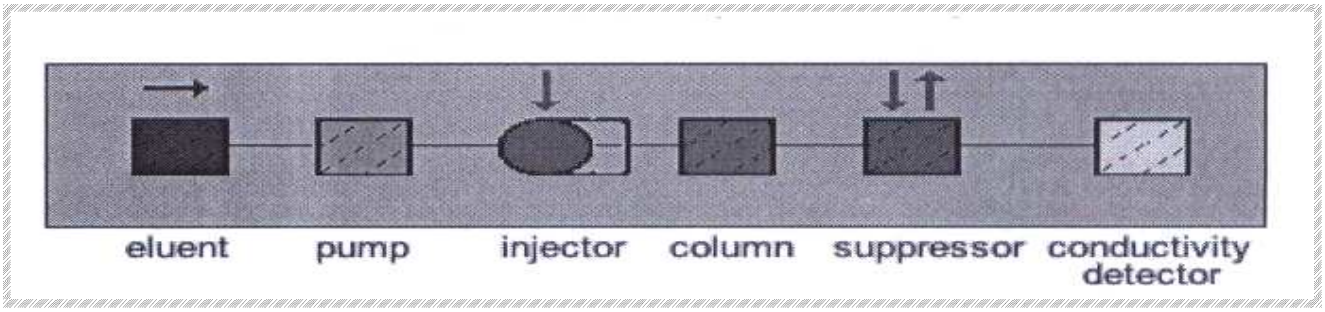


Fig. (1.5) Suppression in Ion Chromatography

Often, a device called a suppressor is used and is placed between the column and detector as shown above Fig. (1.5).

When suppression is used the detector is almost certainly conductivity. The greatest achievement of suppression is to increase the sensitivity of the anion, however at the same time the background conductivity of the eluent is greatly reduced. The same suppressor units can also be used to increase the sensitivity of organic acids. The suppressor used in anion chromatography is simply a cation exchanger and its job is to remove cation and replace them with an H^+ [2].

Ion exclusion chromatography (IEC) is mainly used for the separation of weak acid or bases [2]. The greatest importance of (IEC) is for the analysis of weak acids such as carboxylic acid, carbohydrates, phenols or amino acids.

Although this method was also popular for ionized organic compounds during the early days of HPLC, because the ion-exchange stationary phases then available were based on relatively soft polymers, the separations were inefficient and most of these analytes are now examined by ion-pair chromatography.

Subsequently, advances in polymerization methods have led to more rigid ion-exchange column materials but these have not yet proved popular in general use.

Most ion-exchange chromatography is a specialised area, primarily of interest to the transition-metal inorganic chemist. However, in recent years the analytical separation of many common anions and cations has been revolutionied by the introduction of "ion chromatography" based on the technology of HPLC equipment and offering similar resolution and speed of analysis.

There has also been a limited but useful application of silica gel as an ion-exchange column for the separation of basic drug compounds.

Ion exchange chromatography has been applied for the separation of variety of charged organic and biochemical systems, including drugs, serums, vitamins and pharmaceutical preparation as well as inorganic ions [51].

The key element was their development of a device, later known as suppressor column to lower the background conductometric signal resulting from the liquid ionic mobile phase while enhancing the conductometric signal from analyte ions [57], which is illustrates in Fig (1.6).

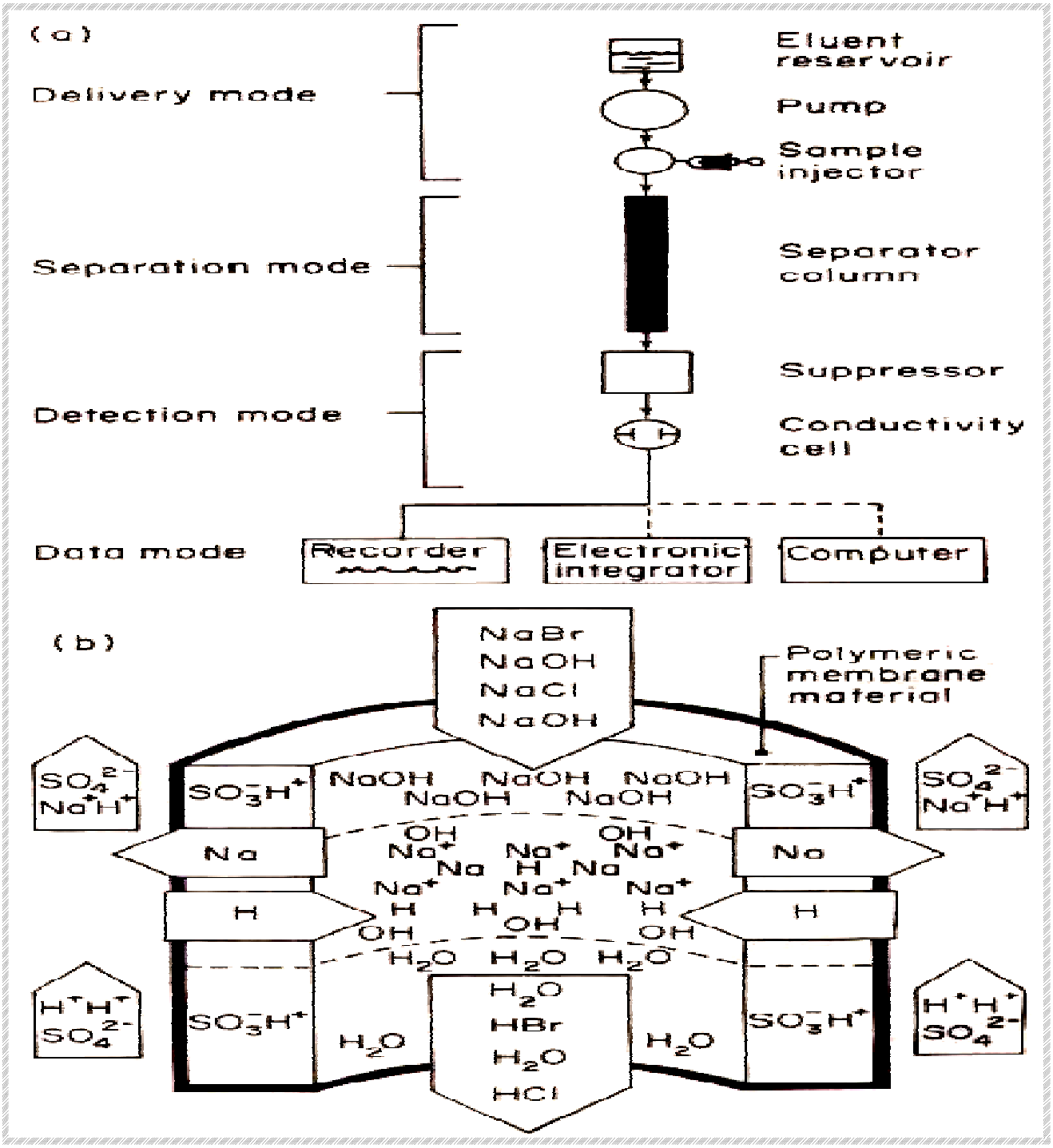


Fig.(1.6) (a) Configuration of ion chromatography with ion-exchange separation column and conductometric detector. (b) Ion chromatography suppressor fibre exchanging hydrogen ions for soduim ions during an anion separation. The halide ions remain as the mineral acids.

1.10 DETECTION SYSTEM:

The detectors in HPLC are employed to continusly 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 from of a chromatogram [58].

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 [59].

The detectors used in HPLC are reflective index (RI), UV visible Fluorescence, Conductivity and Electrochemical as well as other hyphenated detectors [60]. However the most common detectors used for the detection of ionic species are UV-VIS and conductivity detectors.

1.10. I ELECTRICAL CONDUCTIVITY DETECTOR:

Electrical conductivity is a universal property of all ionic solutions the conductivity is proportional to the concentration of the analyte.

The measurement of the resistance of an ionic solution, using an alternative voltage to eliminate spurious effects due to electrode polarization can be used as a detection principle, for ionic species. One of the potential advantages of such a detector is the very small dead volume[61]. When used with buffer solutions detection limit is in the low ppm range[62].The use of conductivity was first supported in 1979 by Gjered et .al . [63].

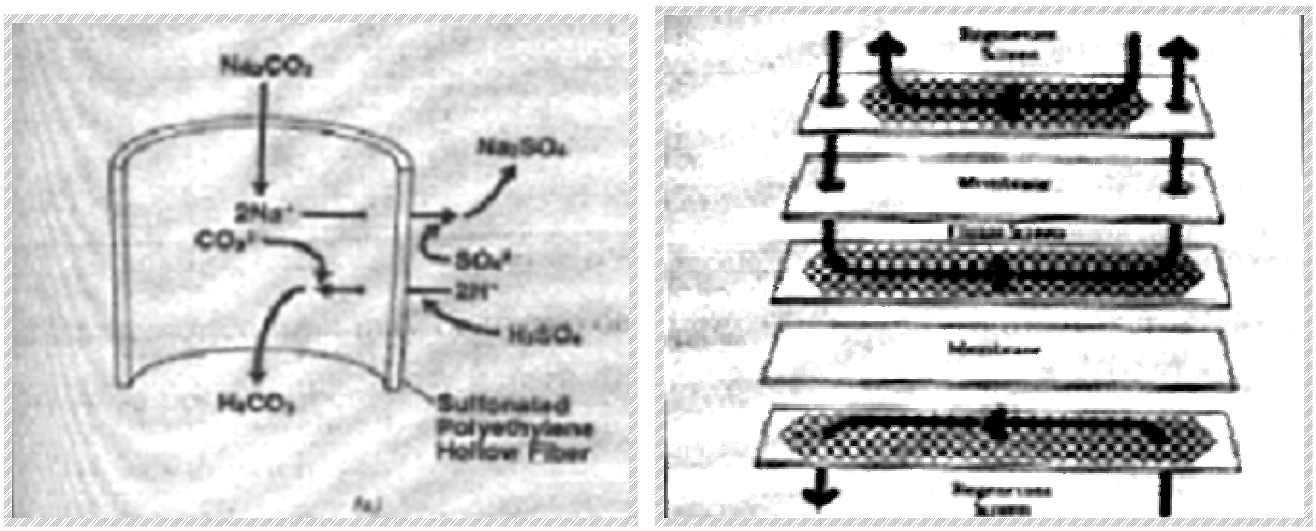
The combination of the conductivity detector with ion-exchange chromatographic columns has led to a technique called "ion chromatography ", which has known a rapid development since his first presentation by Small et. al.[54].

In order to suppress the overwhelming conductivity of the background eluent electrolyte, Small et.al.introduce [53], between the ion-exchange separation column and the conductivity cell, an ion-exchange suppressor column with opposite charge groups, i.e. an anion-exchange suppressor column for cation separation and vicversa.

In this system a pH adjustment of the mobile phase was found necessary to ensure reproducible chromatographic performance [63]. The eluent is thus neutralized and the conductivity of the eluate ions can be conveniently measured.

The suppressor column increases the analysis time and the bond broadening and may give some undesired effects (ion-exclusion, reaction with some ions).

The suppressor column may be in two designs shown in fig (1.7)



(A) (B)

Fig. (1.7): The design of suppressor column for ion chromatography (A) a hollow fiber. (B) a sandwich.

It has to be replaced or regenerated after a few analysis are performed but this operation can be done automatically in modern instruments. Alternatively, an ion-exchange hollow fiber may be used for eluent nutralisation[64]. The technique has been applied to organic as well as inorganic ions.

In order to detect a small analyte signal it is necessary to employ an eluent which gives a relatively low conductance [65,66].

The fast and recent development of ion analysis with a conductivity detector has led some manufactures, who, for patent reasons, cannot include a suppressor column in their instruments, to develop conductivity meters with electronic background suppression.

This technique, however, cannot be applied to strongly conducting eluents and gives higher detection limits i.e. poorer sensitivity than the previous one.

Conductance detection may give positive or negative peaks depending on eluent concentration and pH.

1.10. II UV- VIS DETECTOR:

UV-VIS detection may be employed in either the direct or indirect mode. This detector is functioning as a solute-specific detector and may be used for compounds that exhibit absorption in the UV-visible region. Detection of some ions is not generally applicable except at very low wavelength such at 215 nm [67].

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 [68].

Cochrane and Hillman [69] 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. Conductivity detection was 5 to 16 times more sensitive than indirect photometry for all analytes. Small and Miller [70] reported this approach, as a detection technique for ionic species. Indirect photometric detection (IPD), in which transparent sample ions were eluted with a hight-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 phase in IEC-IPD should have the ability of displacing the analyte ions from the stationary phase and selectively separating them [71].

The eluent in (IPD) should possess several characteristic such as ion exchange capability, ion-exchange selectivity and a large molar absorptivity [70]. The most common (IPD) mobile phases used for anion separation are benzoate, phthalate, sulfobenzoate and salicylate [72, 73]. The eluents have showed good separation profiles for many analyte anions with reasonably low detection limits [74, 75]. 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 [71, 75]. 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 [72, 73]. Negative analyte peaks are obtained instead of the conventional positive peaks, as shown in Fig. (1.8).

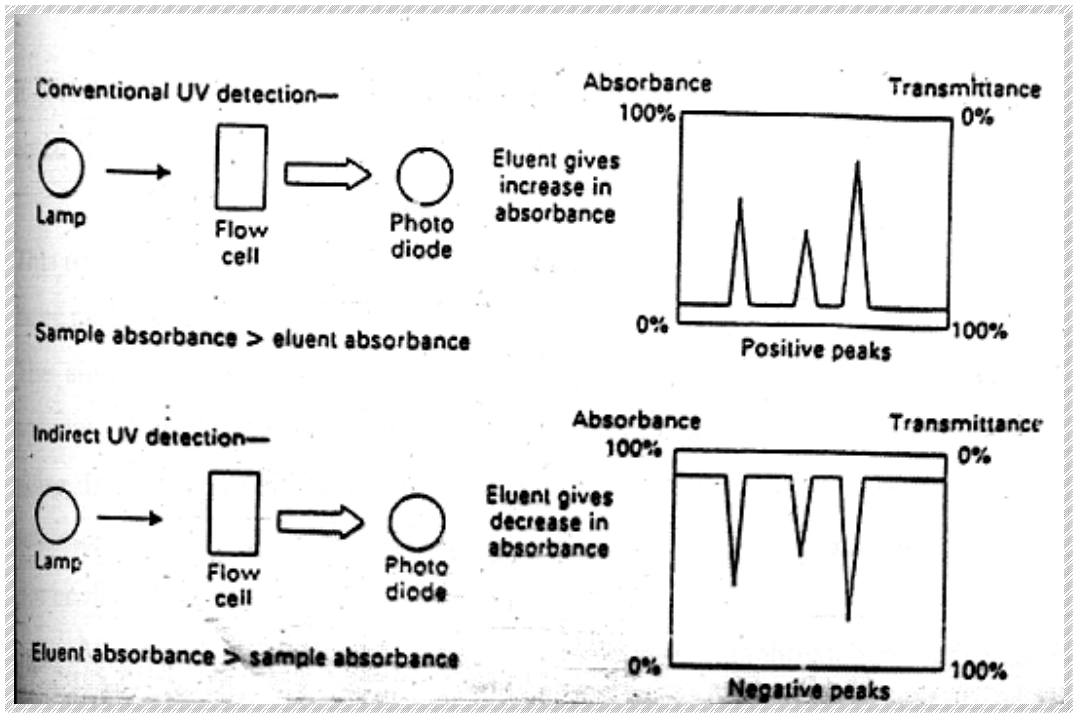


Fig. (1.8): Comparison between Conventional and Indirect Photometric Chromatography.

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:

$$S = C_s \epsilon_s + (C_E - C_s)\epsilon_E - C_E\epsilon_E = C_s(\epsilon_s - \epsilon_E) \quad \text{----------(1-6)}$$

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:

$$S \alpha C_s\epsilon_E \quad \text{----------- (1-7)}$$

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:

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

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

$$S/N \ \alpha \ C_s\epsilon_E/C_E \quad \text{---------- (1-9)}$$

This relationship shows that a small signal can seen using a very dilute eluent with large molar absorptivity [72, 73].

The signal –to noise ratio (S/N) in (IPD) is proportional to the analyte ion 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 [71, 77].

Maki and Danielson [78, 79] 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^-, SCN^-, and I^- in less than 18 min. With detection limit 0.4-1 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 colum has been reported recently [80]. These three eluents required no pH adjustment with detection limit for chloride 0.04 ng and 0.1 ng with conductivity and indirect photometry respectively [68].

Michio Z. [81] 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 separation ions after passing through a cation-exchange column were detected directly by a spectrophotometric detector.

This mobile phase showed particular promise for the separation and detection of Mg, Ca, Sr and Ba 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, [82] 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.; [83] has used of a pure silica gel (pia Seed 5S- 60-SIL), synthesized by the hydrolysis of pure tetratraetoxysilane [$Si(OCH_2CH_3)_4$], as a cation-exchange stationary phase in ion chromatography for common- 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

1.12 THE AIM OF THIS WORK:

Aim of this work is to analyse a variety of cations ranging from small singly charged ones to large, doubly charged and tripply charged type (Li^{+}, Ba^{+2}, Fe^{+2} and Fe^{+3}) by unsuppressed conductivity detection and compared with indirect photometric detection (IPD) using 4-aminodiphenylammonium 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.

2.1 INSTRUMENTS AND EQUIPMENTS:

Shimadzu (Kyoto, Japan) used in this work as the high performance liquid chromatograph was in the department of chemistry at AL-Nahrain University, which consisted of a system controller model SCL-10 Avp, Conductivity Detector model CDD-10 Avp, a liquid delivery pump model LC-10 AVp, 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 Shimadzu Class-VP5 chromatography data system program supplide by the manufacturer; 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 Shimadzu Class-VP5 chromatography data system program supplide by the manufacturer; Canon LBP 810 printer model (Japan).

Orion expandable ion analyzer model EA 940 (USA) equipped with printer and glass combination electrode were used to measure the pH of the solution.

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 following materials and chemicals were used in this work:

Chemicals	*Formula*	*Companies*
4-aminodiphenylamine	**(C6H7)2N3**	**Fluka**
Lithium bromide	**LiBr**	**Flaka**
Barium chloride	**BaCl2**	**Fluka**
Anhydrous ferrous sulfate	**FeSo4**	**BDH**
Ferric sulfate pentahydrate	**[Fe2(SO4)3.5H2O]**	**BDH**
Dimethelysulfoxide	**C2H5SO2**	**Fluka**
Hydrochloric acid	**HCl**	**BDH**
Sodium hydroxide	**NaOH**	**BDH**

2.3 PREPARATION OF MOBILE PHASE:

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

-1×10^{-3} M of 4-aminodiphenylamine stok solution was prepared by adding 0.029g of 4-aminodiphenylamine to 250 ml volumetric flask and diluted to mark with deionized water and mix thoroughly in sonicator for 1 hour.1×10^{-7} M was prepared by diluting 0.1 ml of 1×10^{-3} M 4-aminodiphenylamine stock solution and adding 50 ml of pure DMSO the pH of solution was adjusted by adding 0.1 M hydrochloric acid to reach pH 5.80, then

the volume was completed to 1L with deionized water. Eluent was degassed under vacuum for 20 min.

2.4 SAMPLE PREPARATION:

Stock solutions of 100 ppm of each cations (Li^{+}, Ba^{+2}, Fe^{+2} and Fe^{+3}) 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 three or more of the above analyte were also prepared by mixing the appropirate volumes of the stock solutions.

Table (2-1): Weight of compound required to prepare 100 ppm in 100 ml mobile phase.

Cation	Compound	Weight (g)
Li^{+}	Lithium (LiBr)	0.1251
Ba^{+2}	Barium ($BaCl_2$)	0.0152
Fe^{+2}	Ferrous ($FeSO_4$)	0.0272
Fe^{+3}	Ferric $[Fe_2(SO_4)_3].5H_2O$	0.0433

2.5 ANALYSIS OF SAMPLE:

All prepared standard solutions and their mixtures have been chromatographically analyzed on the cation exchange column with 4-aminodiphenylammonium chloride at pH 5.80 as Eluent at optimum flow rate 1 ml/min with UV-Visible and conductivity detectors.

1.3 MOBILE PHASE OPTIMIZATION:

The eluent in unsuppressed conductivity detections methods as mentioned before, should possess several characteristics such as ion exchange capability, chromatographic selectivity, and large molar absorptivity.

Since most of the aromatic weak acid (or base) salts have very low conductivity; therefore, they can be used to elute inorganic cations (or anions) and detected directly because of having large conductivity than the eluent.

4- aminodiphenylamine, $[(C_6H_7)_2N_3]$, was chosen as eluent in this study. 4-aminodiphenylamine has a basicity constants K_b value; 6×10^{-14} mol/L. From this value indicate about the basicity of compound is a weak base; therefore, arylamines are much weaker bases than ammonia and alkyl amines because of increasing the delocalization of arylamines which is responsible for the weak basicity.

The UV-Visible spectrum from 200-700 nm for 1×10^{-7}M 4-aminodiphenylammonium chloride at pH 5.80 is shown in Fig.(3-1). The spectrum showed maximum absorbance at 342.5 nm with molar absorptivities of 7.44×10^{7}L/mol.cm. Therefore the absorbance at 342.5 nm has been used as detection wavelength in this work.

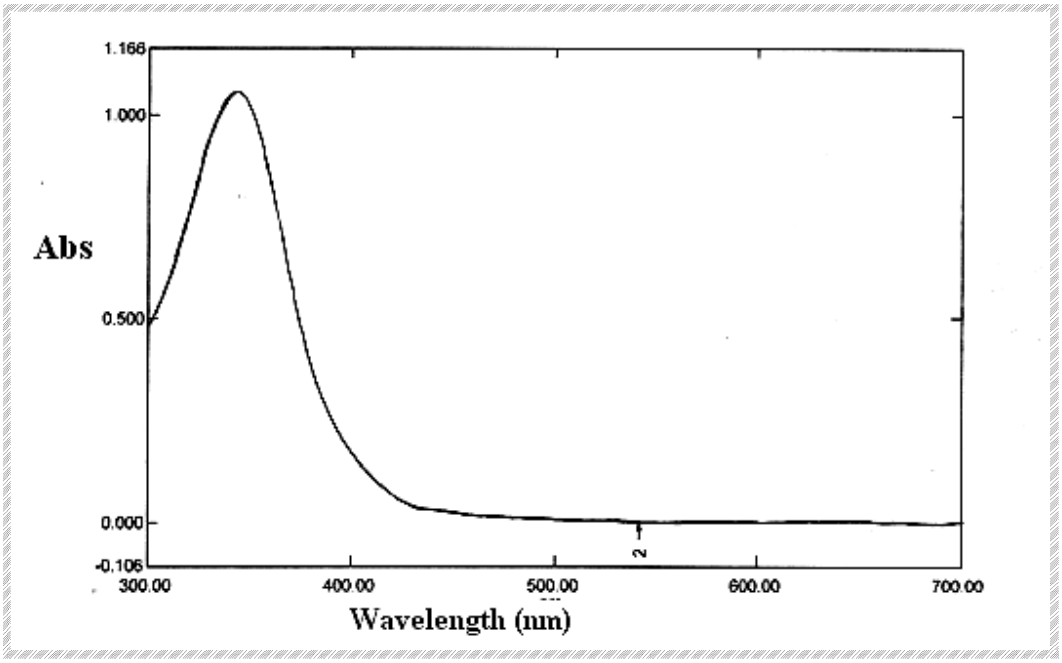


Fig.(3-1): The UV Spectrum for 1×10^{-7}M 4-aminodiphenylammonium chloride solution at pH 5.80

The pH of 1×10^{-7}M 4-aminodiphenylammonium chloride was changed from 2 to 11. The absorbance of each solution was measured at 342.5 nm. A plot of the absorbance versus the pH of 4-aminodiphenylammonium chloride is shown in Fig.(3-2).

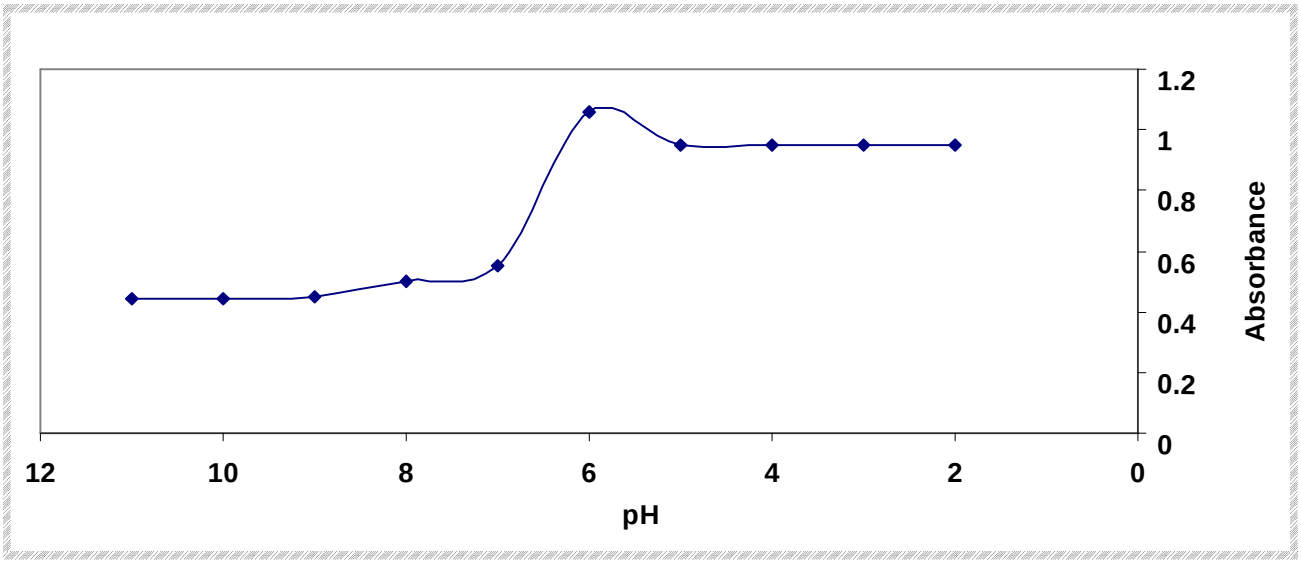


Fig.(3-2): The absorbance of 4-aminodiphenylammonium chloride at 342.5 nm versus pH values

The solubility of 4-aminodiphenylammonium chloride was very low at high pH values, in addition, the absorbance which was lower than that at low pH due to the formula of the association and dissociation of the compound , which involved a combination of 4-aminodiphenylamine and hydrochloric acid.

Optimum flow rate was measured by analyzing one of the ions which is (Fe^{+3}) at different flow rate ranging from (0.125-2.00) ml/min and calculating the **H** value for each run using the equation(1-2). A plot of **H** versus flow rate has given a minimum **H** at flow rate of near 1.0 ml/min as shown in Fig. (3-3).

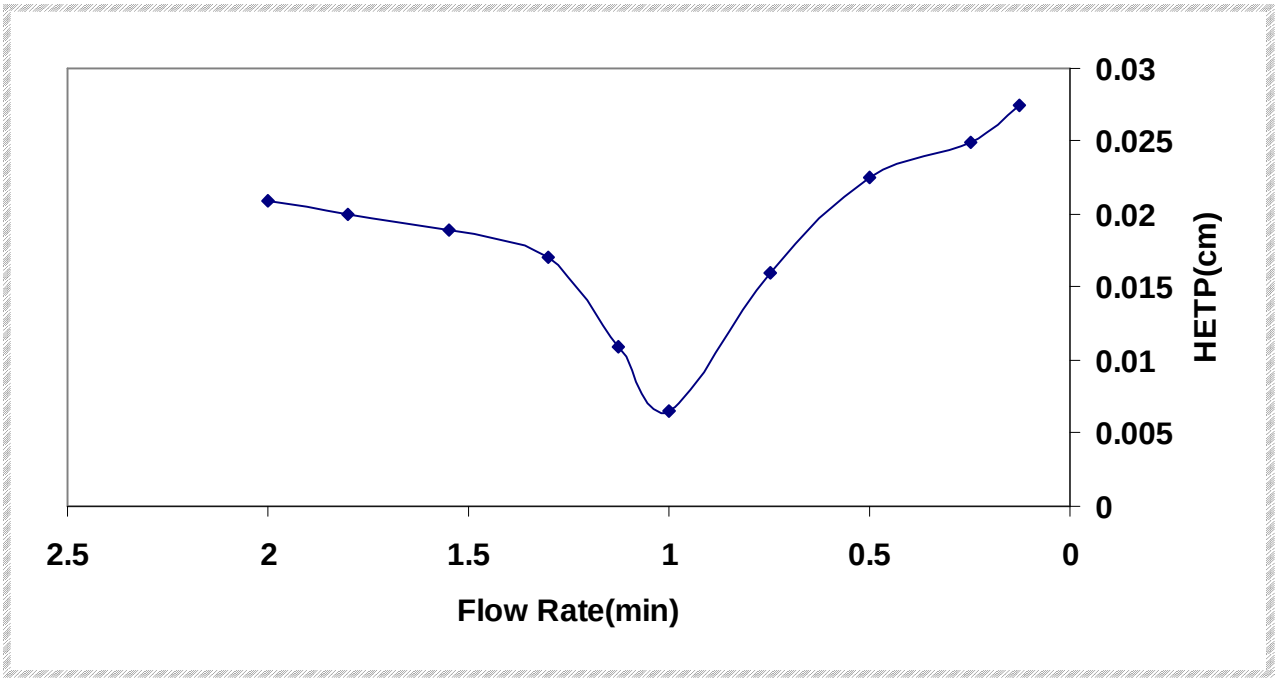


Fig.(3-3): Plot of Theoretical Plate Height (H) versus flow rate using Dioex Ion Pac CS3 column (4 (i.d) mm × 250 mm) with 1×10^{-7} M 4-aminodiphenylammonium chloride as eluent and 2.5 ppm Iron ion as analyte.

The effect of eluent concentration on the capacity factor was measured for several cations. The **K`** values were obtained using computer program supplied by the HPLC instrument. The result are listed in table (3-1). These values were also plotted versus eluent concentration as shown in Fig.(3-4). The capacity factor which is related to the retention time, regards as a function of eluent concentration as given by equation[54]:

$$\textbf{Log K` = constant- (Y/X) log \{E}^{\textbf{x}}\textbf{\}-----(3-1)}$$

Where $\mathbf{E^x}$ is the eluent concentration, **Y** and **X** are the change of eluent and analyte, respectively.

The constant represents[54]:

$$\textbf{Constant=(1/X) log } K^{S}_{E} \textbf{ + (Y/X) log C – log R}$$

Where K^{S}_{E} is the selectivity coefficient, **C** is the capacity of the resin and are **R** is the phase volume ratio at certain analysis conditions.

As shown in table (3-1) a high concentration of eluent, the capacity factor ranged from (0.86 -1.20) compared to that of lower concentration, which ranged from (1.87 -4.48).

However, the differences in **K`** were relatively small at larger concentration than at 1×10^{-7} M as indicated from the column selectivity, originally called the separation factor **α**-values of these analytes. The separation factor **(α)** is defined as the ratio of the capacity factors of two adjusted peaks and calculating by using this eq.(3-2):

$$\alpha = K`_2/K` \text{----------(3-2)}$$

In addition at large concentrations it were difficult to separate them due to overlapping peaks because of their short retention times.

Consequently, we choose 1×10^{-7} M as optimum eluent concentration, although it gave relatively large retention times with capacity factor ranged from (1.617 -2.55).

These range values of **K`** were considered as reasonable values.

While, at low concentration (1×10^{-8}) M the retention time of cations were longer and peak broadening started to be pronounced such as Fe^{+3}.

It is clear from the table (3-1) that the Fe^{+3} analyte can be detected at high concentration of eluent while the retention time decreased.

In general, when the concentration of eluent is increased, the retention time of analyte is decreased.

Table(3-1): Some chromatographic parameters of cations using Dionex Ion Pac CS3 column (4 (i.d) mm ×250 mm) with different eluent concentration

ION	Retention time (tR), Capacity factor (Ḱ) and Separation factor (α) at different eluent concentration (M)											
	1×10^{-6}			0.5×10^{-6}			1×10^{-7}			1×10^{-8}		
	t_R	k`	α	t_R	k`	α	t_R	k`	α	t_R	k`	α
Li^{+1}	1.22	0.86		1.31	1.12		1.62	1.70		1.82	1.81	
Ba^{+2}	1.38	0.92	1.09	1.40	1.22	1.13	1.88	1.82	1.22	2.40	2.38	1.56
Fe^{+2}	1.50	1.14	1.32	1.44	1.26	1.20	2.25	2.10	1.28	3.35	3.24	1.51
Fe^{+3}	1.52	1.20	1.38	1.48	1.31	1.29	2.85	2.44	1.31	3.92	4.48	1.56

It is obvious that the inclination of **K`** values versus the eluent concentration which gave nagative values for all cations because these valuse were very close to each other, so the separation of cations was very difficult as shown in Fig. (3-4).

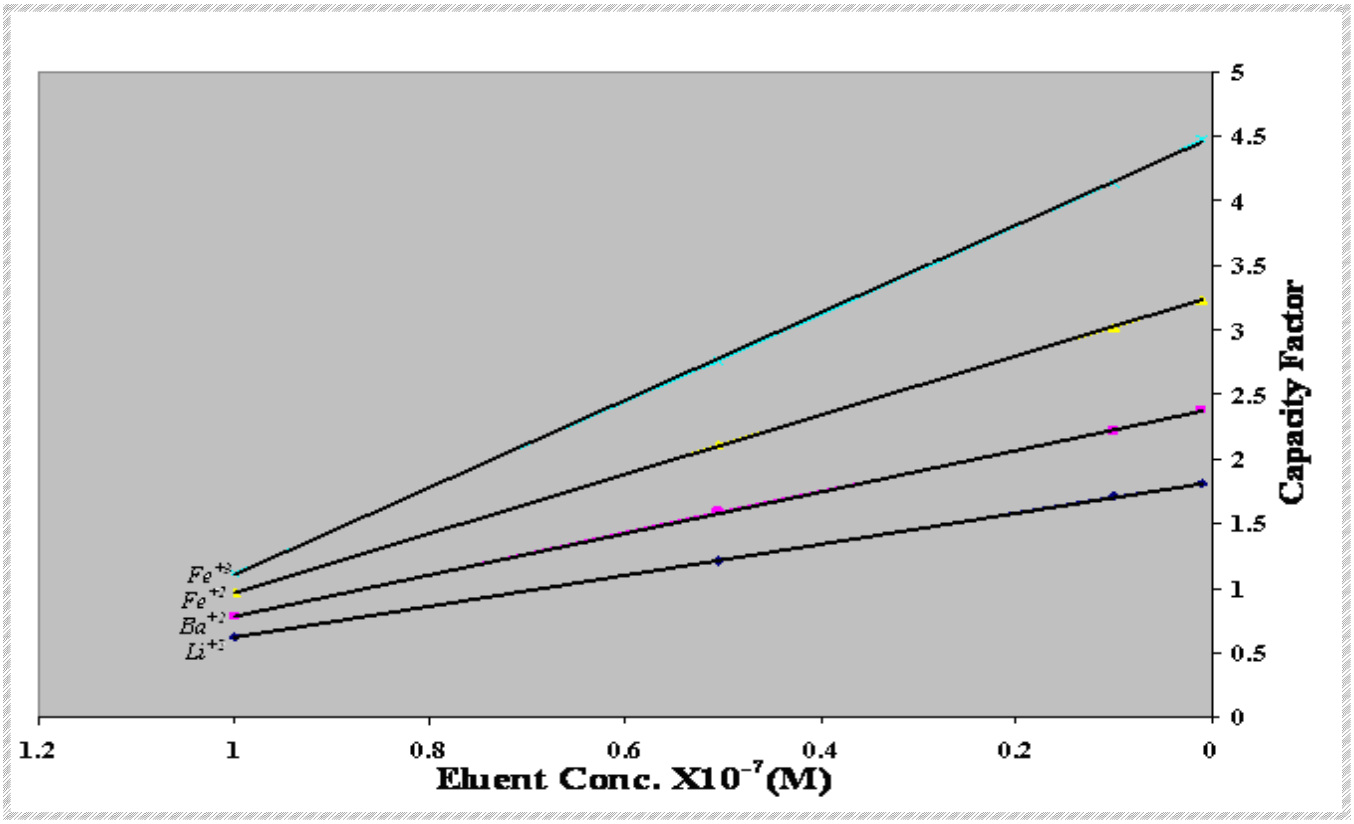


Fig. (3-4): Capacity Factors of some Cations using Conductivity Detector.

The chromatographic parameters for several cations were measured using the optimum condition obtained from above experiments, which were 1×10^{-7} M 4-aminodiphenylammonium chloride solution at pH 5.80 at eluent, flow rate 1ml/min and UV detection at 342.5 nm.

The peak shapes of the analyzed cations were some what distortion and the baseline was up-drifting using 1×10^{-7} M eluent concentration as shown in Fig.(3-5). This has been noticed with other eluent concentration (large or lower).

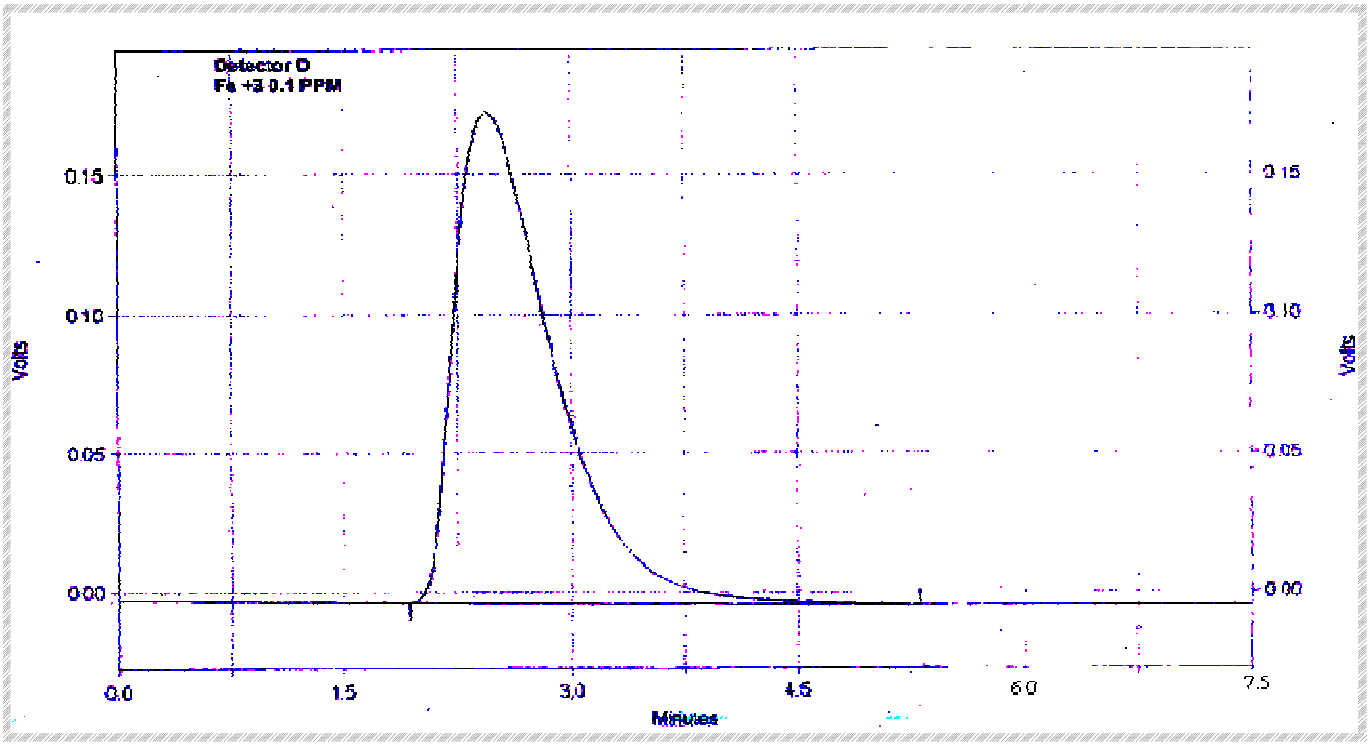


Fig.(3-5): Chromatogram of 0.1 ppm Fe^{+3} using Dionex Ion Pac CS3 column (4 (i.d)mm×250mm) with eluent concentration 1×10^{-7}M 4-aminodiphenylammonium chloride at pH 5.80, Flow Rate 1ml/min, Sample loop 10 µL using unsuppressed conductivity detector.

In order to improve the peak shape of the analyzed cation and to gate a stable baseline an organic modifier was examined, namely dimethyl sulfoxide (DMSO), and study its effects on the capacity factors and peak shapes. The effect of adding organic solvent to the eluent on the capacity factor **(K`)** of cations was studied using 1×10^{-7} M 4- aminodiphenylammonium chloride concentration at pH 5.80 .

The results are listed in table (3-2). These values were also plotted versus the (DMSO percentage) in mobile phase as shown in Fig. (3-6).

Table (3-2): Capacity factor (K`) of Cations using Dionex Ion Pac CS3 Column (4(i.d) mm × 250 mm), eluent 1×10^{-7} M 4- aminodiphenylammonium chloride at pH 5.80 with different percentage of Dimethyl sulfoxide in the mobile phase.

IONS	***Capacity Factor (k`) and separation Factor(α) at pH 5.8 in different presence of DMSO percentage***					
	1%		***3%***		***5%***	
	k`	***α***	***k`***	***α***	***k`***	***α***
Li^{+1}	1.42		1.65		1.87	
Ba^{+2}	1.56	1.20	1.83	1.23	2.09	1.26
Fe^{+2}	2.11	1.41	2.38	1.38	2.63	1.34
Fe^{+3}	2.70	1.56	3.14	1.54	3.26	1.51

The DMSO content has been found to affect the retention times of studied cation. In 1% of DMSO, the retentions of the separated ions were small so as the capacity factor (**K`**) (1.42 - 2.70) for all analyzed cations compared to (1.87 – 3.26) at 5% DMSO in 1×10^{-7}M 4-aminodiphenylammonium chloride at pH 5.80 as shown in Fig. (3-6) and summarized in table (3-2). This can be explained by the relative decrease in ionic strength of the eluent as the percentage of DMSO increase and more decreasing in the polarity of the eluent. This increase in **K`** was good enough to obtain a baseline separation for most of the analyzed cations.

However, the peak shapes as well as the baseline of the analyzed peakes were well enhanced as shown in Fig. (3-7).

Consequently, we found that the addition of 5% DMSO to be added to mobile phase provides good separation of the analyzed ions, although this was on the expense of the ionger retention time ($\mathbf{t_R}$). No attempt was made to use higher percentage of DMSO due to column manufacture recommendation, which may case swelling of the column packing material. Based on the above experiments, we have chosen 1×10^{-7} M 4-aminodiphenylammonium chloride solution in 5% DMSO at pH 5.80, flow rate 1 ml/min for un-suppressed conductivity detection.

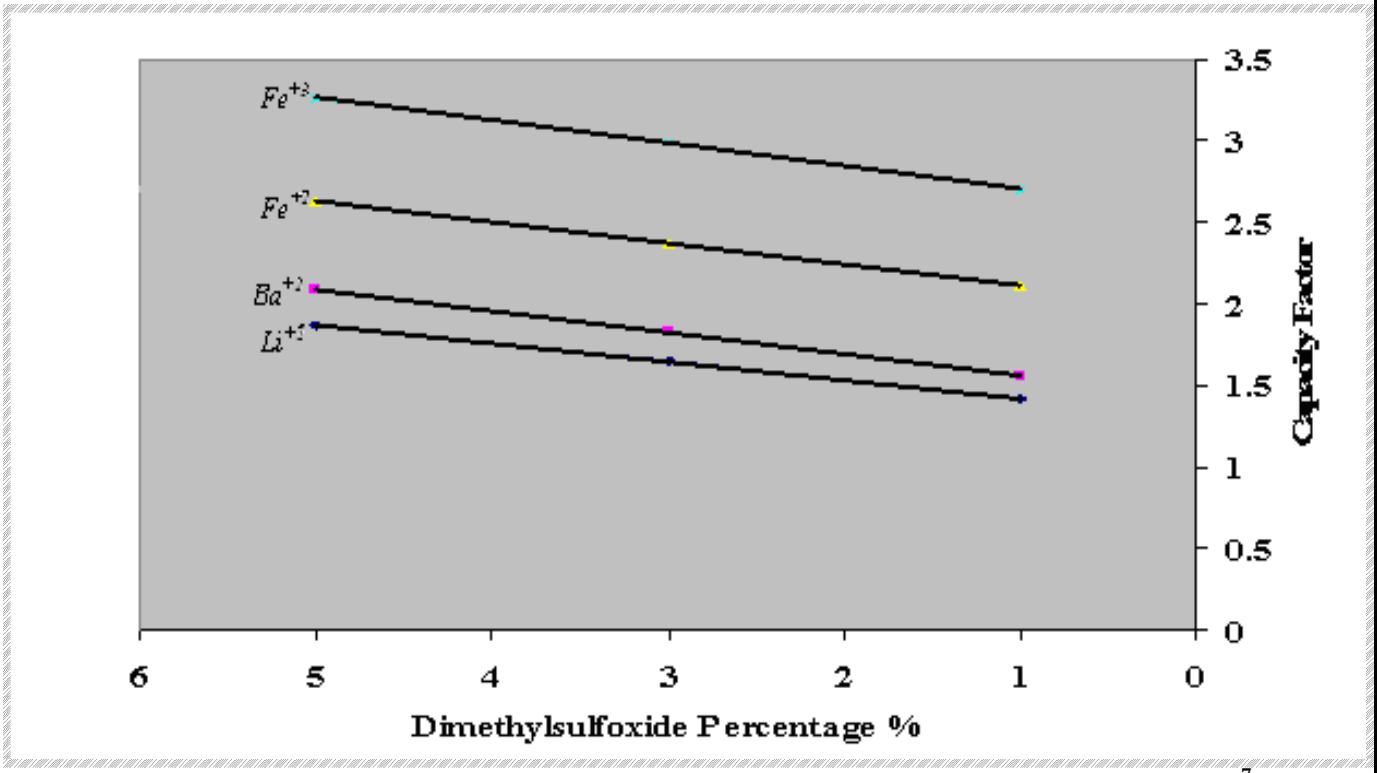


Fig.(3-6): Effect of organic Solvent on the Retentions of Cations with 1×10^{-7} M 4-aminodiphenylammonium chloride as eluent.

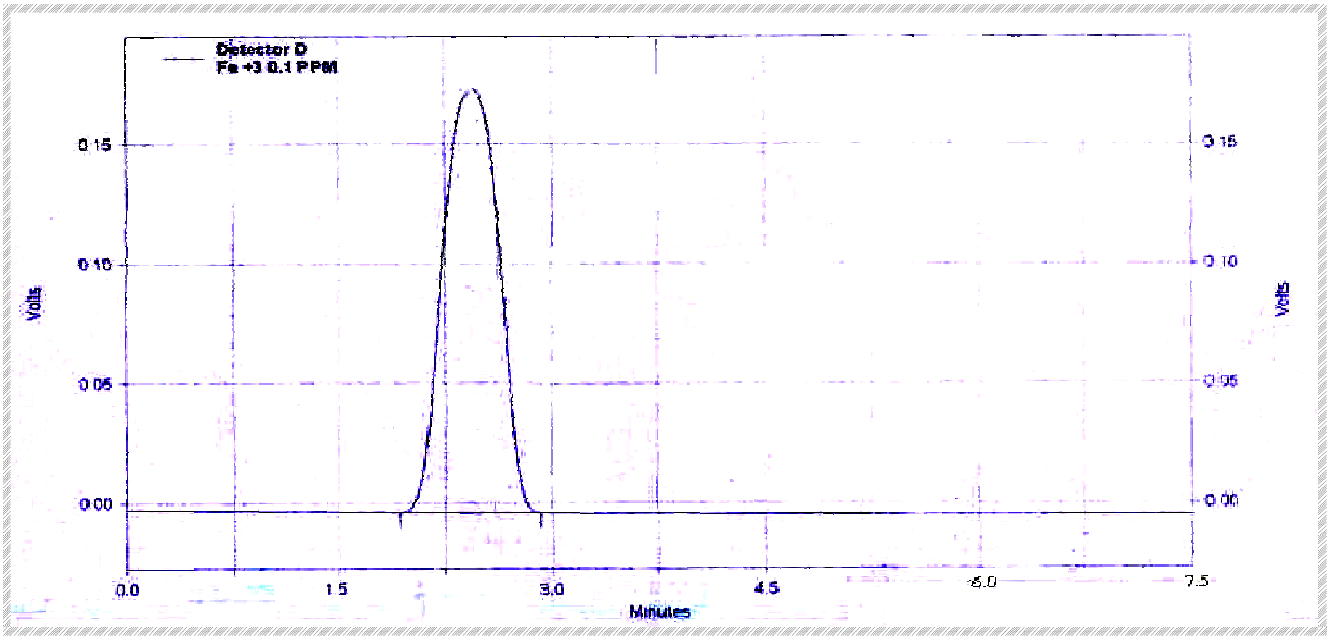


Fig. (3-7): Chromatogram of 0.1 ppm Fe^{+3}, conditions were the same as in Fig. (3-6), except using 5% DMSO.

3-2 CHROMATOGRAPHIC ANALYSIS:

Some singly and doubly charged cations at different concentrations were chromatographically analyzed at least three times with Dionex Ion Pac CS3 column, with optimized mobile phase eluent. The analyzed cations have given relatively sharp and symmetrical peaks as well as good detector response. The retention times for all analyzed cations were repproducible. The **RSD** in t_R were ranged from (0.099-0.825) % with an average of (0.435) %. The **K`**and **α** values gave the same results since it is closely related to the t_R of the analyzed ions. The large differences between the retention times of these cations have produced good resolution between the peaks as shown in table (3-3). Consequently; the possibility of separating these cations in mixture has been achieved.

Table (3-3): Retention times, resolutions and peak asymmetries at 10 % for some cations using Dionex Ion Pac CS3 column (4(i.d) mm × 250 mm), eluent 1×10^{-7} M 4-aminodiphenylammonium chloride at pH 5.80 in 5% DMSO, using unsuppressed conductivity detector.

Ion	*Retention time, t_R (min.)*	*Resolution , R_s*		*Peak Asymmetry at 10%*
Li^{+1}	1.62			1.11
Ba^{+2}	1.88	Ba^{+2}/Li^{+1}	0.98	1.20
Fe^{+2}	2.25	Fe^{+2}/Ba^{+2}	1.01	1.33
Fe^{+3}	2.85	Fe^{+3}/Fe^{+2}	1.24	1.41

The peaks asymmetries for the chromatographed cations were ranged from (1.11-1.41). The resolution **Rs** ranged from (0.98-1.24) with an average value of (1.310) as listed in table (3-3). Mixtures of some cations were chromatographed using the optimized conditions are shown in Fig. (3-8) and (3-9). In conductivity, positive position peaks were produced in this work because of the mobile phase has a very low conductance than the sample cations. We have some difficulties in separating the studied cations especially the first two were overlapping occured, although partial overlapping separation of Fe^{+3} from Fe^{+2} was obtained. This may be due to the large conductivities of these cations, which caused overlapping peaks as shown in Figs. (3-8) and (3-9).

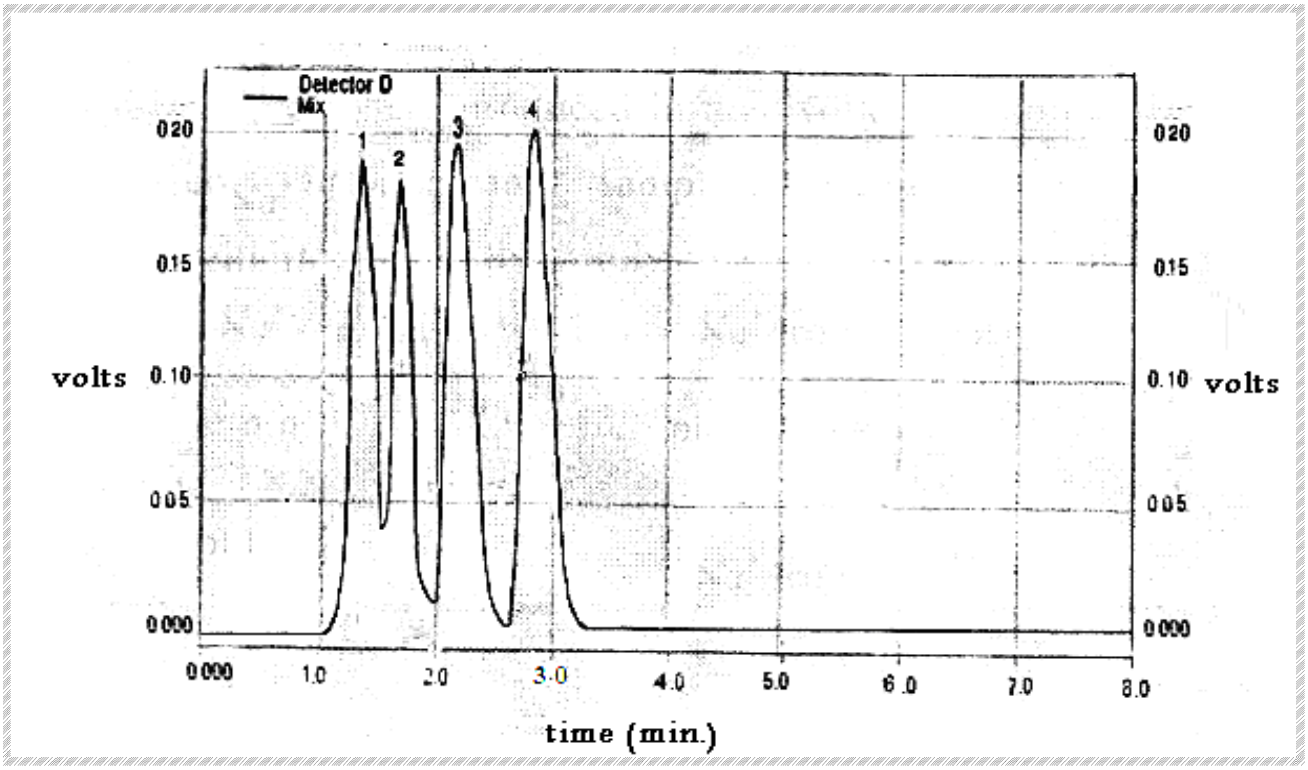


Fig.(3-8): Chromatogram of a mixture of fourcations {(1) 0.5ppm Li^{+1},(2) 1.5ppm Ba^{+2},(3) 2ppm Fe^{+2}, (4) 2.5 ppm Fe^{+3} (4) }, using Dionex Ion Pac CS3 column (4(i.d) mm × 250 mm) with eluent concentration 1×10^{-7} M 4-aminodiphenylammonium chloride at pH 5.80 in 5% DMSO , flow rate 1ml/min, Sample loop 10µL using unsuppressed conductivity detector.

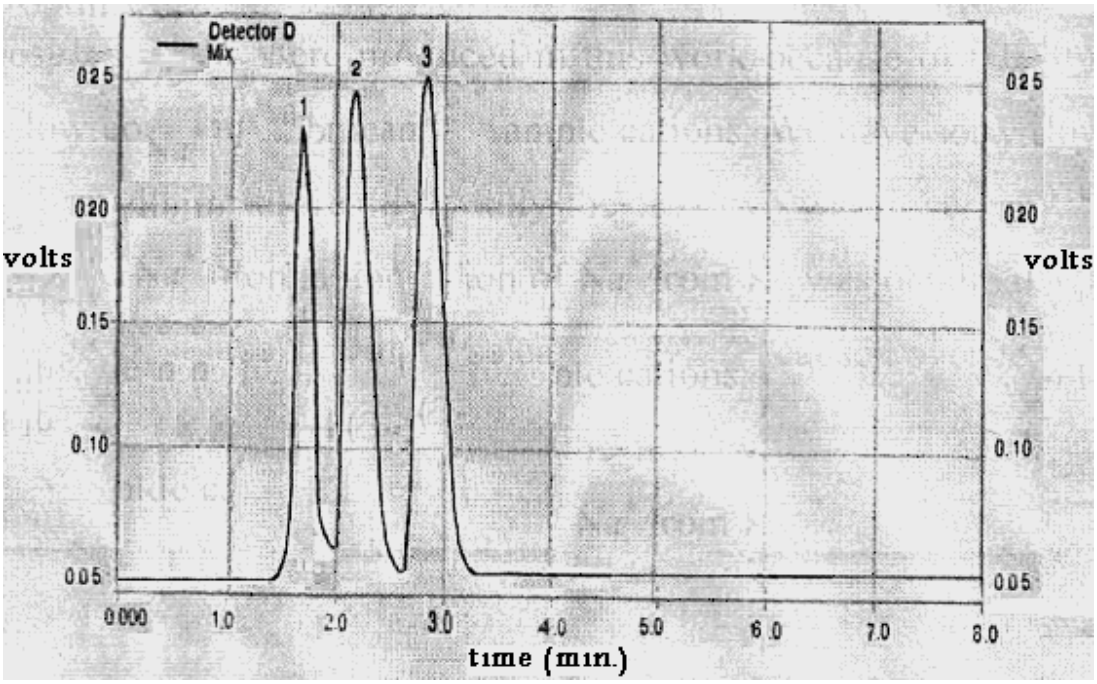


Fig.(3-9): Chromatogram of a Mixture of three cations {(1) 1.5ppm Ba^{+2},(2) 2ppm Fe^{+2},(3) 2.5ppm Fe^{+3} } using the same conditions as in Fig.(3-8).

However, the separation of other cations possible, such as the case in the separation of mixture containing Fe^{+3}, Fe^{+3} and Li^{+1} as shown in Fig.(3-10). Symmetrical peaks were obtained, although they were relatively broader which explained the diffuculty in separation of more sample components.

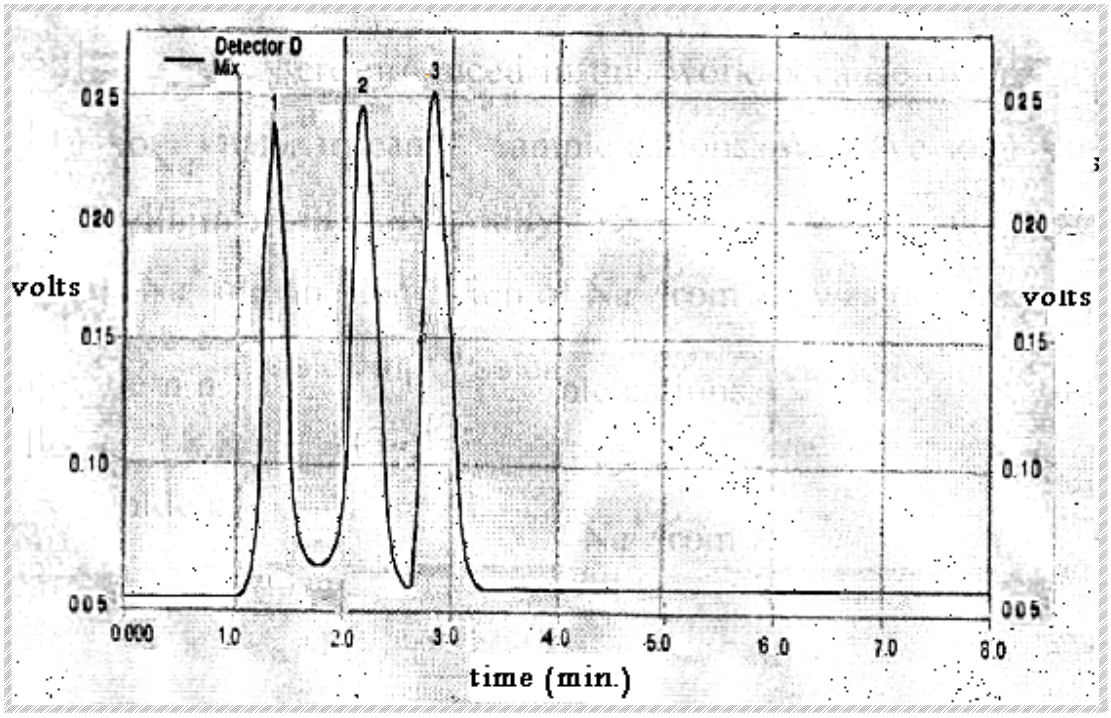


Fig.(3-10): Chromatogram of a mixture of three cations {(1) 0.5ppm Li^{+},(2) 2ppm Fe^{+2}, (3) 2.5ppm Fe^{+3}}, using the same conditions as in Fig.(3-8).

3.3 QUANTITATIVE ANALYSIS:

Different concentrations of each analyte cations chromatographed at least 3 times and the peak area was measured and averaged for each concentration. The average peak area of each concentration was plotted versus their concentration.

Linear calibration curves were obtained for all analyzed cations from at least 10 ppm down to the detection limit of each cation. Table (3-4) lists the linear least square equations for the analyte calibration lines.

The correlation coefficients were ranged from (0.9992-0.9998). The detection limits were ranged from [0.025-0.10] ppm. The slope values showed relatively high values which ranged from (0.0098-0.1342) with an average value for the four cations of (0.058525) as shown in table (3-4). Calibration curvs of cations studied in this work were plotted as shown in Fig. (3-11) using unsuppressed conductivity detection.

Table (3-4): Linear Equation, Regression (R) and Detection Limit for the analyzed cations using Dionex Ion Pac CS3 column (4(i.d) mm × 250 mm), eluent 1×10^{-7} M 4-aminodiphenylammonium chloride at pH 5.80 in 5% DMSO flow rate 1 ml/min, sample loop 10µL using unsuppressed conductivity detector.

Ions	Linear equation	Regression R	Detection limit (ppm)
Li^{+1}	Y=0.0098X + 0.155	0.9995	0.025
Ba^{+2}	Y=0.0218X +0.1339	0.9999	0.03
Fe^{+2}	Y=0.01342X + 0.1683	0.9996	0.05
Fe^{+3}	Y=0.0105X + 0.174	0.9994	0.1

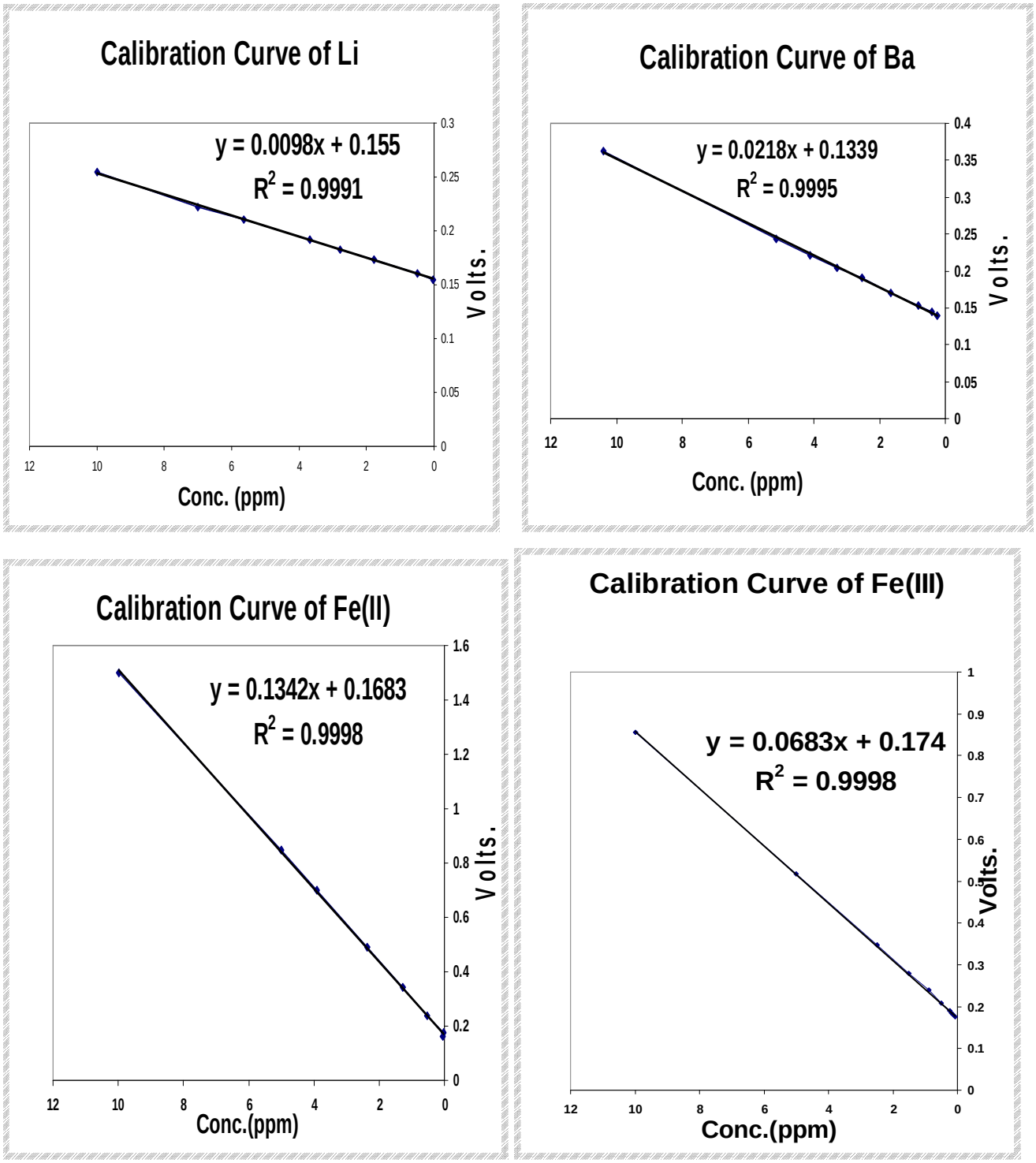


Fig (3.11): Calibration Curve for four cations, at the same conditions as in Fig. (3.8)

Prepared standard mixture solutions of the analyzed cations were injected for at least 3 times under the same condition and their concentration were calculated by measuring the peak area of each ion and using their respective linear equations.

The recovery were ranged from (88.40 – 96.20) % with an average of 93.1075 % as shown in table (3-5).

Table (3-5) : Recoveries for the analyzed cations using unsuppressed conductivity detector.

Ions	Concentration injected (ppm)	Concentration found (calculated)* (ppm)	% recovery
Li^{+1}	0.5	0.352	96.20
Ba^{+2}	1.5	0.626	95.33
Fe^{+2}	2.0	1.365	92.50
Fe^{+3}	2.5	2.210	88.4

* Using the linear equation for each ion.

3.4 INDIRECT PHOTOMETRIC DETECTION STUDY:

The same eluent with the optimum conditions and with IPD detection wavelength at 342.5 nm, was used to separate cations such as (Li^{+1}, Ba^{+2}, $Fe^{+2}$and Fe^{+3}) which did not absorb or give any signal at this wavelength., therefore, they can be used to eluent inorganic cations and detected indirectly because of these cations don't have absorbance at 342.5 nm, while 4-aminodiphenylammonium chloride gave higher absorbance at this wavelength.

Different concentrations of each analyte cations were chromatographed at least 3 times and each peak area was measured and averaged for each concentration. The average peak area of concentration was plotted versus their concentration.

Calibration curvs were obtained are shown in Fig. (3.12) using **IPD** at 342.5 nm.

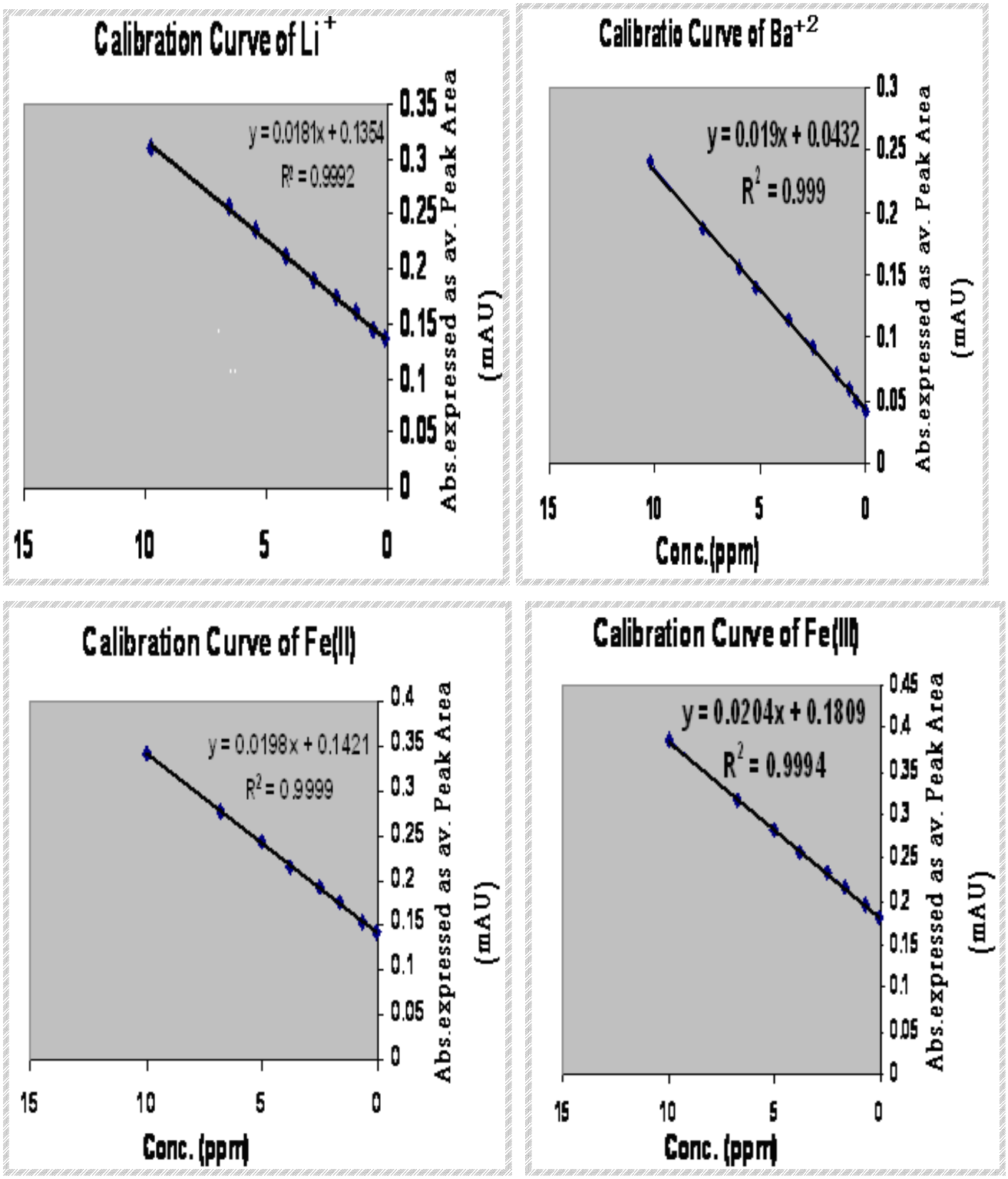


Fig. (3.12) Calibration Curve for four cations, using the same conditions as in Fig. (3.8). Using IPD at 342.5instead of unsuppressed Conductivity Detector.

Linear calibration curves were obtained for all analyzed cations from at least 10 ppm down to the detection limit of each cation.

Table (3-6) lists the linear least square equations for the analyte calibration lines. The correlation coefficients were ranged {0.9990-0.9999}. The detection limits were ranged from [0.02-0.05] ppm. The slop showed relatively high values (compared to un-suppressed conductivity detection method) which ranged from (0.0181-0.0204) with an average for the four cations of (0.019325) as listed in table (3-6).

Table (3-6) : Linear Equation, Regression (R) and Detection Limit for the analyzed cations using Dionex Ion Pac CS3 column (4(i.d) mm × 250 mm), eluent 1×10^{-7} M 4-aminodiphenylammonium chloride at pH 5.80 in 5% DMSO, flow rate 1 ml/min, sample loop 10µL using Indirect Detection Wavelength at 342.5 nm.

Ions	Linear equation	Regression R	Detection limit (ppm)
Li^{+1}	Y=0.0181X + 0.1354	0.9994	0.02
Ba^{+2}	Y=0.0190 + 0.0432	0.9992	0.03
Fe^{+2}	Y=0.0198X + 0.1421	0.9998	0.05
Fe^{+3}	Y=0.0204 + 0.1809	0.9996	0.05

Prepare standard mixture solutions of the analyzed cations by IPD were injected for at least 3 times with same condition and their concentration were calculated as described earlier using their IPD respective linear equations.

The recovery were ranged from (95.00-97.60) % with an average of 95.975 % as listed in table (3-7).

Table (3-7) : Recoveries for the analyzed cations using IPD method wavelength at 342.5 nm.

Ions	Concentration injected (ppm)	Concentration found (calculated)* (ppm)	% recovery
Li^{+1}	0.5	0.488	97.60
Ba^{+2}	1.5	1.440	96.00
Fe^{+2}	2.0	1.906	95.30
Fe^{+3}	2.5	2.375	95.00

* Using the linear equation for each ion.

A comparision between UV-Visible and unsuppressed conductivity detections showed that the slope values for the four cations with conductivity detection were less than that using IPC detection, which indicates a greater sensitivity for IPD.

The detection limit values were also higher than with conductivity. The recovery were lower with conductivity detection. This can be alttributed to the influence of changing in temperature during analysis and from day to day and to the boarder peaks obtained with conductivity compared to that of IPC method.

Mixture of cations were chromatographed using the optimized conditions are shown in figures {(3-13)-(3-15)}. As mentioned before, in IPC, negative position peaks would be produced with this technique and that were the case in this work. This indicate that these peaks were for cations that displaced the sites on the stationary phased that have been occupied by the flowing eluent giving a decrease in the base-line absorbance, which has been offset prior analysis.

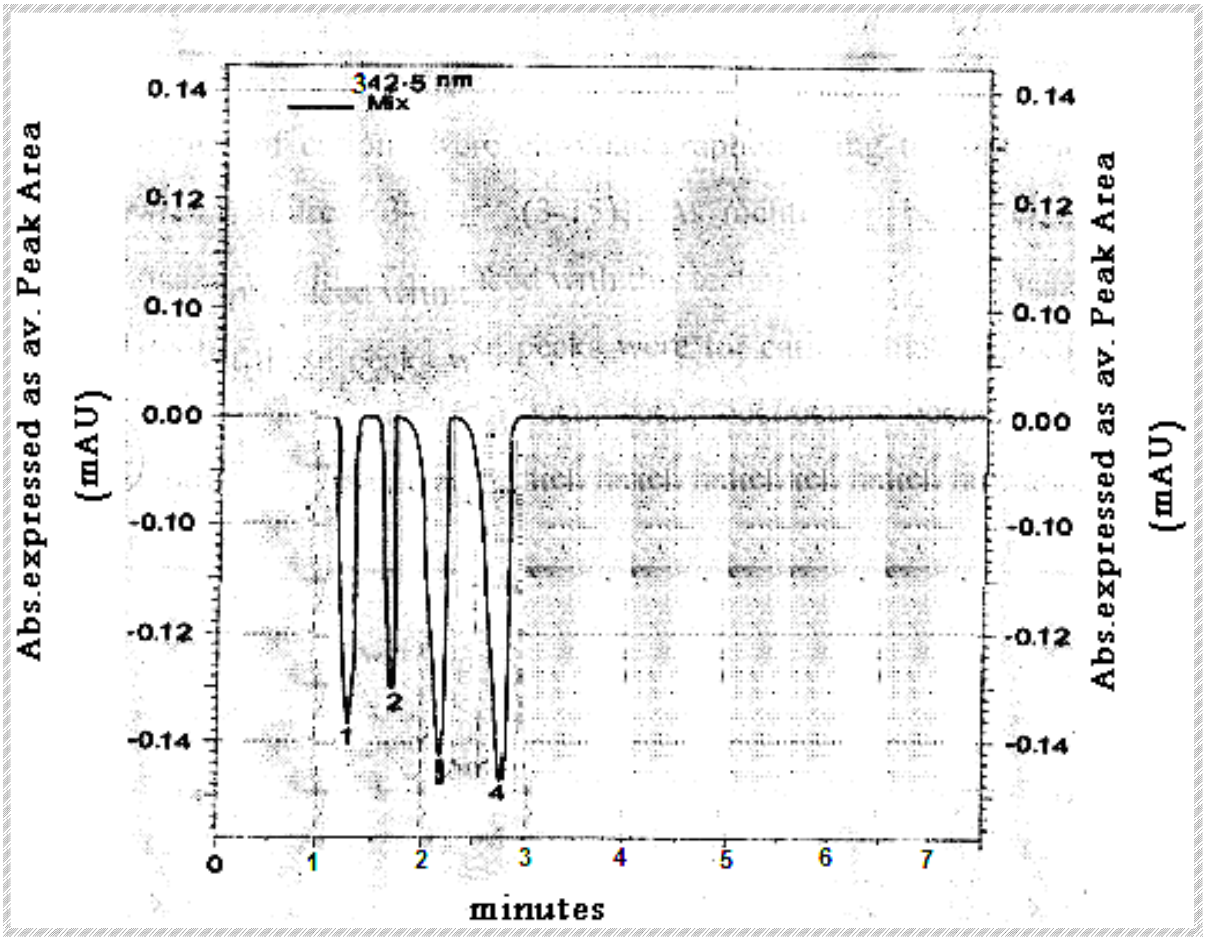


Fig. (3.13): Chromatogram of a mixture of four cations {(1) 0.5ppm Li^{+1}, (2) 1.5ppm Ba^{+2}, (3) 2ppm Fe^{+2}, (4) 2.5 ppm Fe^{+3} }, using Dionex Ion Pac CS3 column (4(i.d) mm × 250 mm) with eluent concentration 1×10^{-7} M 4-aminodiphenylammonium chloride at pH 5.80 in 5% DMSO , flow rate 1ml/min, Sample loop 10µL using Indirect photometric detection at 342.5 nm.

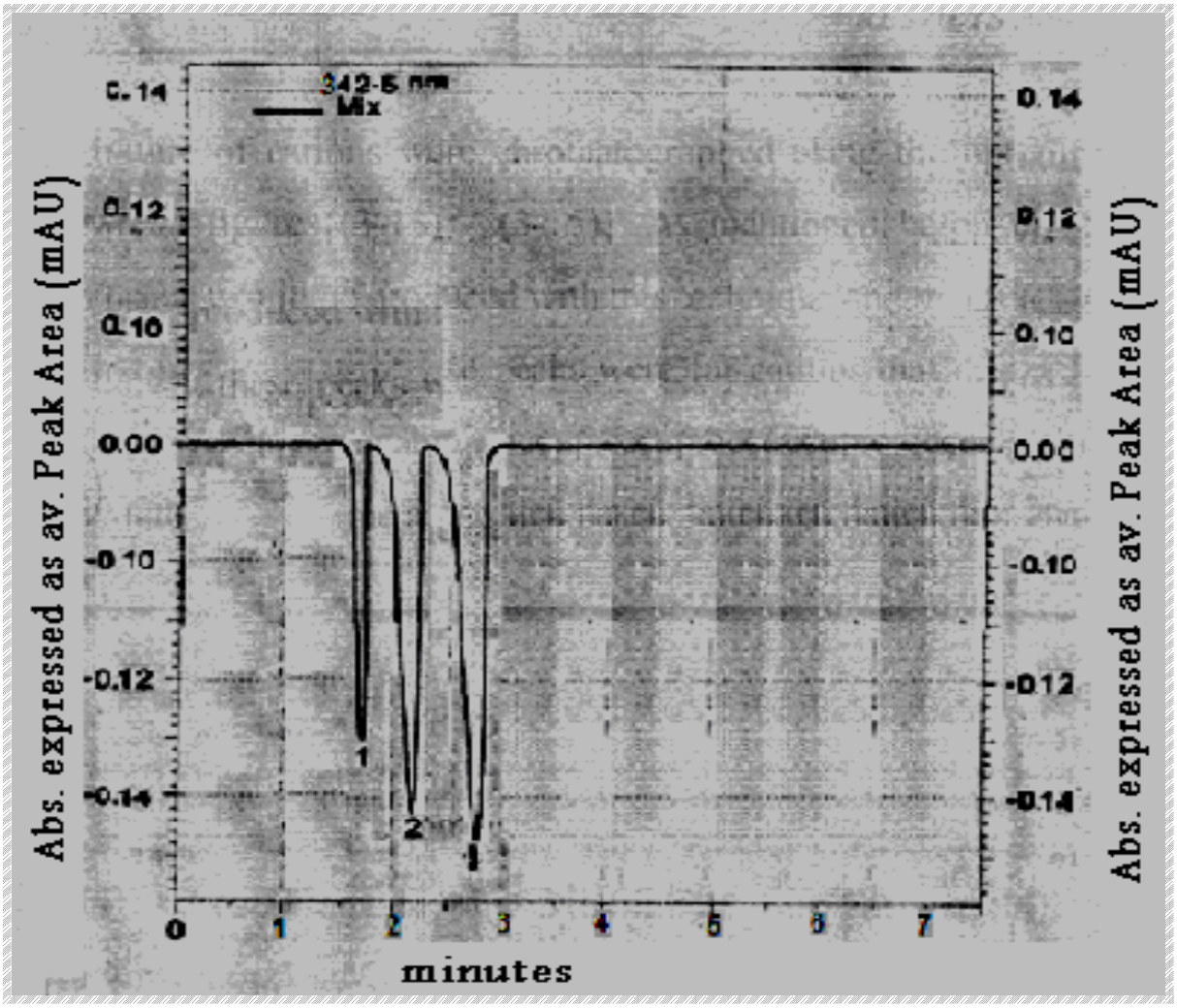


Fig.(3-14): Chromatogram of a mixture of three cations {(1) 1.5ppm Ba^{+2}, (2) 2ppm Fe^{+2},(3) 2.5ppm Fe^{+3} } using the same conditions as in Fig.(3-13).

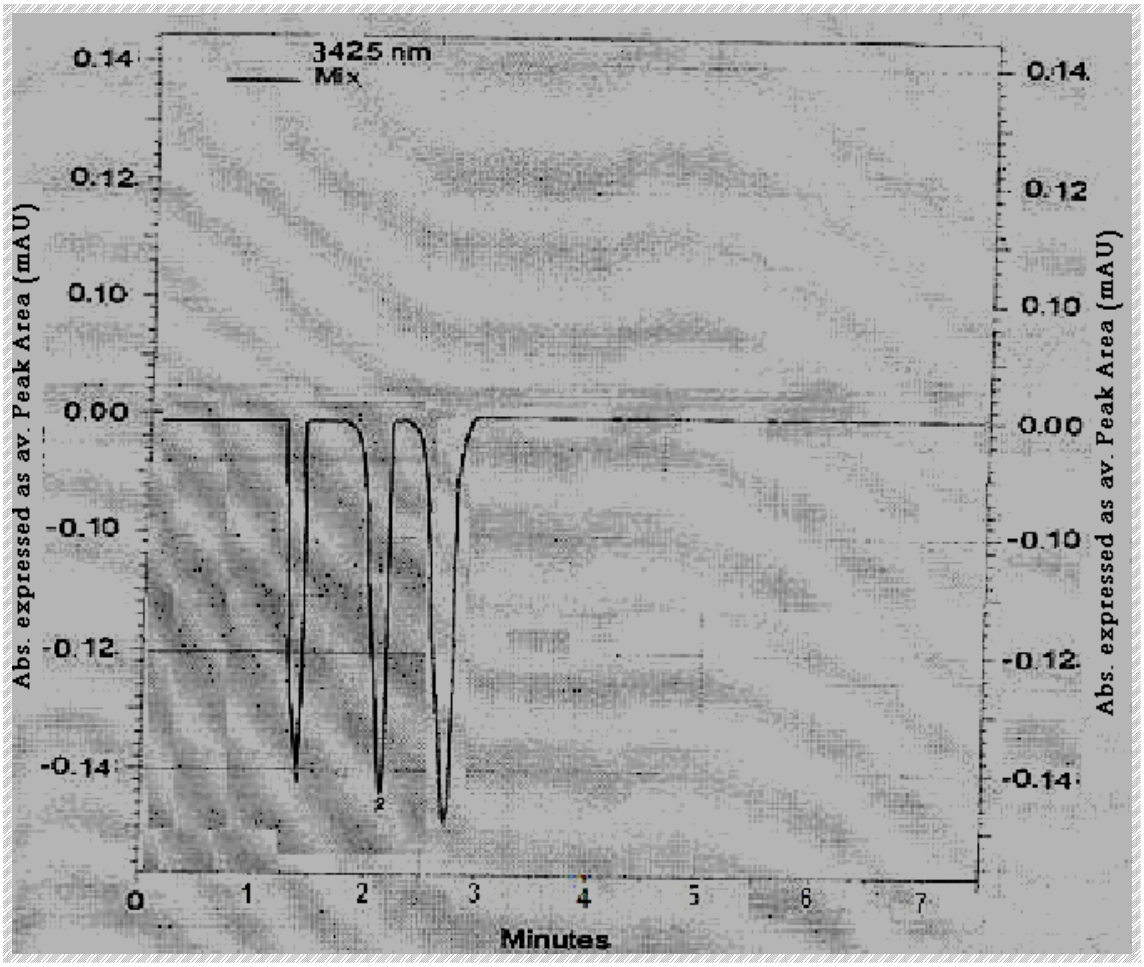


Fig.(3-15): Chromatogram of a mixture of three cations {(1) 0.5ppm Li^{+1}, (2) 2ppm Fe^{+2}, (3) 2.5ppm Fe^{+3} } using the same conditions as in Fig.(3-13).

The figures {(3.13), (3.14) and (3.15)} represent the separation of three cations which have given symmetrical peaks (except for Fe^{+3}) with good resolution in less than 4 minutes.

The peak asymmetryies for the chromatographic cation were ranged from (1.30-1.54). The RSD in t_R were ranged from (0.075-0.723)% with an average of (0.351) %.

The resolutin Rs ranged from (1.20-1.60) with an average value of (1.41).

Table(3-8) lists some of the chromatographic parameters for the analyzed cation.

Table (3-8): Retention times, resolutions and peak asymmetries at 10 % for some cations using Dionex Ion Pac CS3 column (4(i.d) mm × 250 mm), eluent 1×10^{-7} M 4-aminodiphenylammonium chloride at pH 5.80 in 5% DMSO, using UV-Visible detector.

Ion	***Retention time, t_R (min.)***	***Resolution , R_s***		***Peak Asymmetry at 10%***
Li^{+1}	1.42			1.30
Ba^{+2}	1.75	Ba^{+2}/Li^{+1}	1.20	1.40
Fe^{+2}	2.20	Fe^{+2}/Ba^{+2}	1.43	1.51
Fe^{+3}	2.70	Fe^{+3}/Fe^{+2}	1.60	1.54

3.5 CONCLUSION:

For both unsuppressed conductivity and indirect photometric detection, the ion exchange eluent play an important role in both the separation and detection of UV-transparent ionic species. It is found that in IPD the eluent have provided a simple chromatographic method for the analysis of divierse ionic species using conventional HPLC equipment with UV detector.

4-aminodiphenylammonium chloride eluent was used for the separation and detection of the small singly charged, multi charged, and triply charged of common and transition inorganic cations, such as (Li^{+1}, Ba^{+2}, Fe^{+2} and Fe^{+3}) with capacity factors (Ḱ) ranged from (1.70-2.44) using single cation exchange column. The peaks were symmetrically shaped and well resolved with an average value of Rs (1.076). The RSD in t_R was averaged (0.435) %. The average recovery was (93.11) % , with unsuppressed conductivity detection. But IPD has given peak symmetry ranged from (1.30-154) , and the average value of Rs (1.41). The RSD in t_R averaged avalue of 0.351 % . The average recovery was (96.00) % .

Quntitative chromatographic analysis of four cations was performed using conductivity and UV detection at 342.5 nm. Both detectors have given a linear calibration curves from their detection limits to at least 10 ppm. A mixture of four cations (Li^{+1}, Ba^{+2}, Fe^{+2} and Fe^{+3}) have been base-line separated using IPD technique, in less than 4 minutes.

3.6 SUGGESTION FOR FUTURE WORK:

We suggest the following for future work:

1.Using other cation exchanger column from different suppliers for comparison.

2.Use gradient elution which is possible with IPD, but not with conductivity.

3.Separate large and multi charged inorganic as well as organic cations in amixture

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SUMMARY

Cation exchange chromatography with both unsuppressed conductivity detection and indirect photometric detection (IPD) modes were used for the separation and detection of inorganic cations. Salt of weak base diphenylamine has been used as an eluent for several cations both with unsuppressed conductivity detection and IPD. 4-aminodiphenylammonium chloride possess ion exchange capability, chromatographic selectivity and large molar absorptivity.

4-aminodiphenylammonium chloride showed a good chromatographic performance toward the analysis of the cations (Lithium, Barium, Iron II and Iron III) using conventional HPLC equipment with either conductivity or indirect UV detectors. The analysis of these cations using (1×10^{-7} M) 4-aminodiphenylammonium chloride at pH 5.80 in 5% DMSO, with Dionex Ion Pac CS3 Column, and 1ml/min flow rate was achieved with unsuppressed column conductivity and IPD at 342.5 nm detection. Chromatogram of a separation mixtures containing four cations (Li^{+}, Ba^{+2},Fe^{+2} and Fe^{+3}) and other mixture have given well separated peaks using IPD technique. The capacity factor K` for the analyzed cations were ranged from (1.70-2.44), peak symmetries was ranged from (1.11-1.41) and resolution with an average value of (1.310) was optained. The average RSD for t_R was 0.435%. The average recovery was 93.1075 % and the average relative error percentage was 6.77 % with unsuppressed conductivity detection . However, using IPD, the peak symmetry was ranged from(1.30-1.54), and an average value of resolutin of 1.41 which indicate a good chromatographic performance. The RSD in t_R averaged 0.351 %. The average value of recovery was 95.975 %.

Calibration curve for all analyzed cations were linear from their detection limit to at least 10 ppm. The correlation coefficients for the linear calibration curve were ranged from (0.9991, 0.9998) with both detection techniques. The detection limit was ranged from (0.025, 0.1) ppm for unsuppressed conductivity detection compared to (0.02, 0.05) ppm for IPD.