3-1 ***Mobile phase optimization:***

The eluent in IPD, as mentioned before, should possess several characteristics such as ion exchange capability, chromatographic selectivity, and large molar absorptivity. Some weak aromatic acids, such as phthalic, benzoic, sulfo-benzoic, salicylic acid, and others, which possessed such characteristics, are considered as potential IPD eluent at certain pH values.

Phthalic acid, $C_8H_6O_4$, (H_2P), was chosen as eluent in this study. Phthalic acid dissociates with two K_a values; ($1.12x10^{-3}$, and $3.91x10^{-6}$) mol/L. The singly and doubly charged phthalate can be obtained at different pH values. The first dissociated form, the singly charged, hydrogen phthalate, gave at pH 2.90, a HP^-/H_2P ratio of 37%. While the doubly charged, at pH 5.40, gave a P^{-2}/HP^- ratio of 98%. At higher pH values, the interferences of excess OH^- will predominate and consequently affect the eluent chromatographic performance.

The UV-Visible spectrum from 200-500 nm for 0.15 mM phthalate at pH 5.40 is shown in Fig. (3-1).The spectrum showed maximum absorbances at 228, and 276 nm, with molar absorptivites of 7560, and 1286 L /mol. cm, respectively. The high absorbance at 228nm is large enough to be used as detection wavelength in this work.

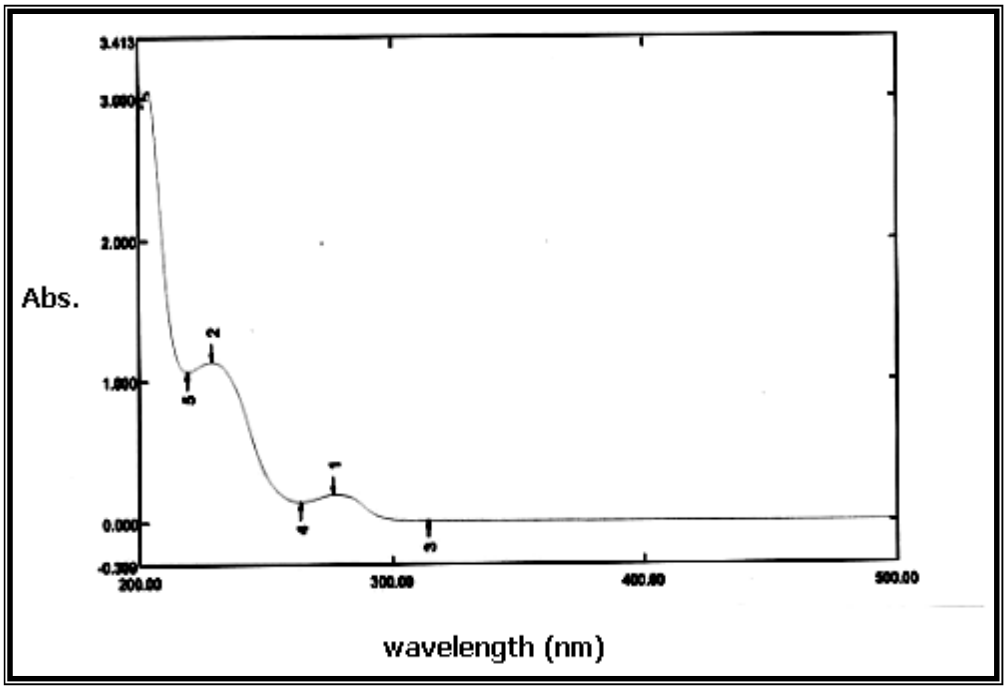


Fig. (3-1): The UV Spectrum for 0.15mM Phthalate Solution at pH 5.40.

The pH of 0.15mM Phthalate solution was changed from (2-8). The absorbance of each solution was measured at 228 nm. A plot of the absorbance versus the pH of phthalate is shown in Fig. (3-2).

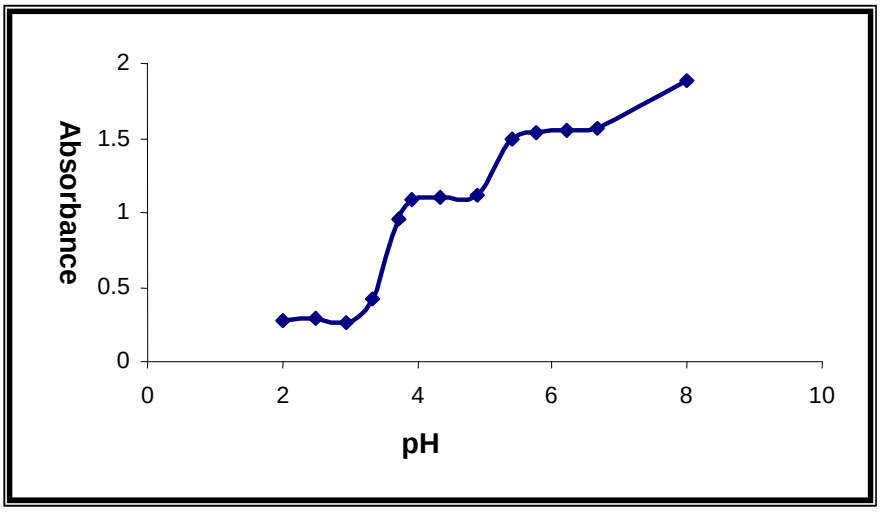


Fig. (3-2): The Absorbance of Phthalate at 228 nm versus pH Values

At low pH values, the solubility of phthalic acid is very low in addition; the absorbance was lower than that at higher pH due to the formula of the compound, which is a combination of phthalic acid and hydrogen phthalate. The auxochromic effect that affect the π-π^* transition causing shift to shorter wavelength. While at higher pH, the higher auxochromic affect of the n-π^* transition caused shifting to longer wavelengths.

The optimum flow rate was measured by analyzing one of the ion (Cl^-) at different flow rate ranging from (0.20-2.20)ml/min and calculating the H value for each run using eq. (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):

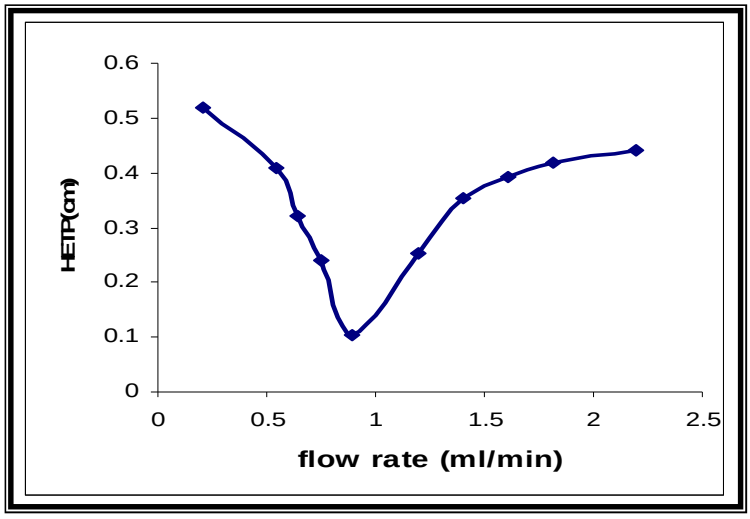


Fig. (3-3): Plot of Theoretical Plate Height H, versus flow rate, using Dionex Ion Pac AS4A Column [25×0.4 (i.d) cm], with Sodium Phthalate as Mobile Phase Eluent and 2ppm Chloride Ion as Analyte.

The effect of eluent concentration on the capacity factor was measured for several anions. The Ḱ were obtained using computer program furnished by the instrument. The results are listed in the Table (3-1). These values were also plotted as shown in Fig. (3-4).The capacity factor, which related to the retention time, is a function of eluent concentration as given by equation [21]:

$$\text{Log } \acute{K} = \text{constant} - (y/x) \log \{E^x\} \quad \text{--------- (3-1)}$$

Where E^x is the eluent concentration, y, and x are the charge of eluent and analyte, respectively. The constant represent:

$$\text{Constant} = (1/x) \log K^S_E + (Y/X) \log C - \log R$$

Where K^S_E is the selectivity coefficient, C is the capacity of the resin, and R is the phase volume ratio at certain analysis conditions.

In table (3-1) high concentration of eluent, the capacity factor ranged from (0.80-3.58) compared to that of lower concentration, which ranged from (1.39-2.27) using 0.05mM. However, the differences in Ḱ were relatively small at larger concentration than at 0.15mM as indicated from the separation α-values of these analytes. In addition, at large concentrations it were difficult to separate them due to overlapping peaks because their short retention times. Consequently, we choose 0.15mM as optimum eluent concentration, although it gave relatively large retention times with capacity factor ranged from (1.28-5.02).

Table (3-1): Some Chromatographic Parameters of Anions Using Dionex Ion Pac AS4A Column (25×0.4 (I.d) cm), with Different Eluent Concentration.

Ion	Capacity factor (k') and separation factor (α) and retention time (t_R) at different eluent concentration (mM)															
	1.00			0.50			0.25			0.15			0.05			
	t_R	k'	α	t_R	k'	α	t_R	k'	α	t_R	k'	α	t_R	k'	α	
SCN^-	1.14	0.80		1.36	1.08		1.53	1.16		2.31	1.28		2.63	1.39		
Cl^-	1.36	0.98	1.23	1.56	1.34	1.24	1.87	1.51	1.3	2.63	1.69	1.32	3.47	1.80	1.29	
Br^-	1.87	1.23	1.24	2.15	1.69	1.26	2.38	1.90	1.2	3.68	2.07	1.22	4.21	2.27	1.26	
NO_3^-	2.64	2.05	1.67	3.27	2.68	1.58	4.57	2.93	1.5	6.42	3.20	1.54	— *	— *	— *	
SO_4^{-2}	3.52	3.58	1.74	4.36	4.33	1.61	5.28	4.64	1.5	9.52	5.02	1.57	— *	— *	— *	

* Not measured because they were not detected clearly.

These were considered as reasonable values of Ḱ. While, at lower concentrations than that the retention time of anions were longer and peak broadening started to be pronounced and some analytes such as NO_3^- and SO_4^{-2} were not eluted clearly even after 30 minutes. It is clear that as the concentration of eluent increases the retention time of the analytes decrease. That is manifested in the slope of Ḱ values versus the eluent concentration, which gave negative values for all anions as shown in Fig. (3-4).The chromatographic parameters for several anions were measured using the optimum conditions obtained from above experiments, which were 0.15mM phthalate solution at pH 5.40 as eluent, flow rate of 1ml/min and UV detection at 228nm, to analyze anions such as (SCN^-, Cl^-, Br^-, NO_3^-, and SO_4^{-2}) which did not absorb or give any signal at this wavelength.

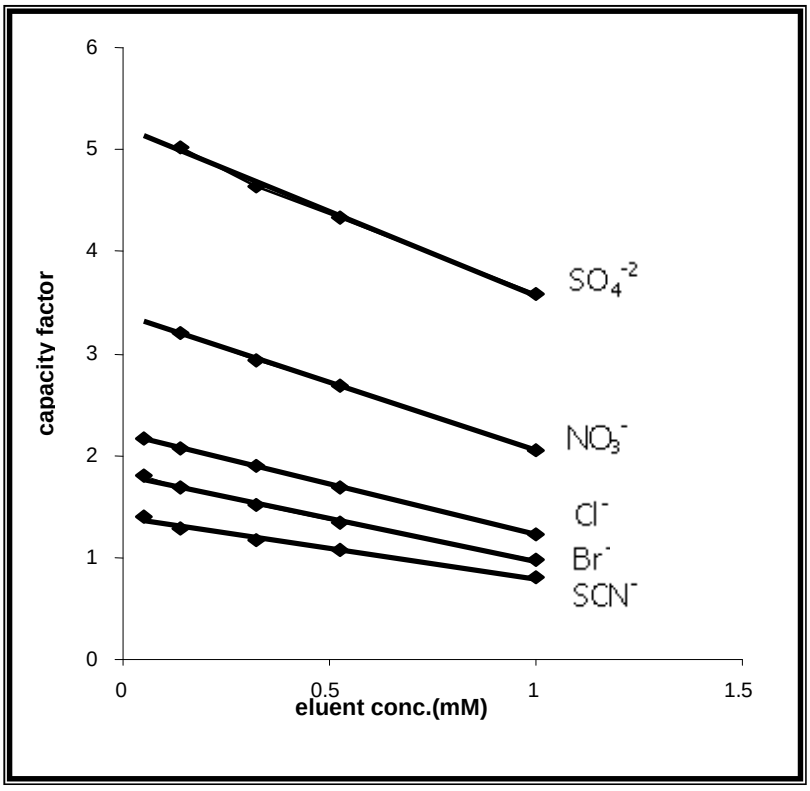


Fig. (3-4): Capacity Factor of Five Anions with Eluent Concentration at pH 5.40, Flow Rate 1ml/min, Sample Loop 10µL and Detection Wavelength at 228nm.

The peak shapes of the analyzed anions were somewhat broaden and the baseline was drifting using 0.15mM eluent as shown in Fig. (3-5).This has been noticed with other eluent concentration (larger or lower).

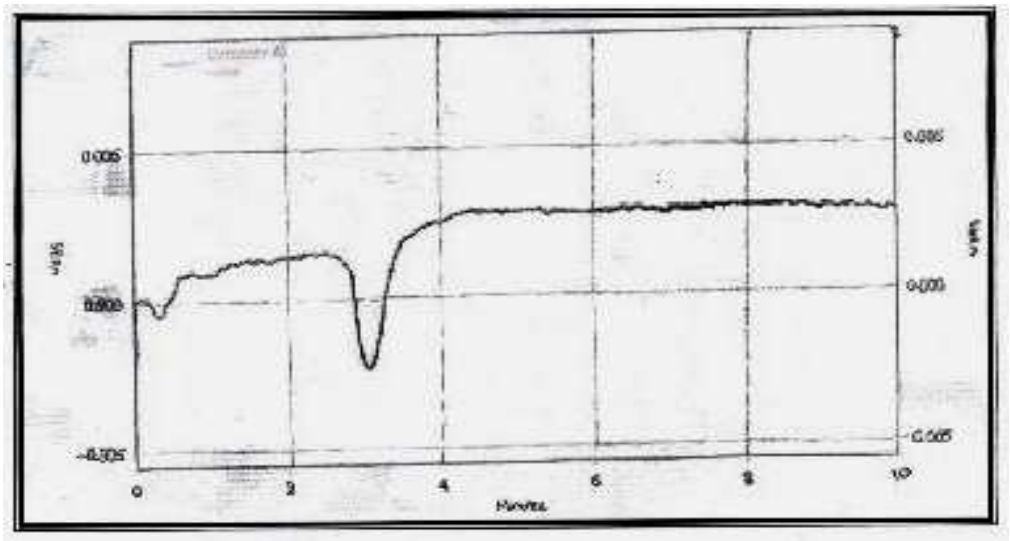


Fig. (3-5): Chromatogram of 1ppm Cl^- using Dionex Ion Pac AS4A Column [25×0.4(i.d) cm]. Eluent 1.5×10^{-4} M Sodium Phthalate at pH 5.40, Flow Rate 1 ml /min, 10µL Sample Loop, and Detection Wavelength 228nm.

In order to improve the peak shape of the analyzed anions and to get a stable baseline an organic modifier was examined, namely methanol, and study its effects on the capacity factors and peak shapes. The effect of adding organic solvent to the eluent on the capacity factor (Ḱ) of anions was studied using 0.15mM phthalate concentration at pH 5.40.The results are listed in Table (3-2). These values were also plotted as shown in Fig. (3-6).

Table (3-2): Capacity Factor (Ḱ) of Anions using Dionex Ion Pac AS4A Column [25×0.4(i.d) cm], Eluent 1.5×10^{-4}M Sodium Phthalate at pH 5.40 with Different Methanol Percentage.

Ions	*Capacity factor (Ḱ) with 0.15mM eluent concentration at pH=5.4 in different presence of methanol*					
	0%	*2%*	*4%*	*6%*	*8%*	*10%*
SCN^-	1.28	1.37	1.46	1.56	1.62	1.71
Cl^-	1.69	1.75	1.82	2.02	2.27	2.31
Br^-	2.07	2.41	2.63	2.97	3.23	3.61
NO_3^-	3.20	3.78	4.16	4.62	4.92	5.32
SO_4^{-2}	5.02	5.63	5.95	6.27	6.67	6.85

The methanol content has been found to affect the retention times of studied anions. In absence of methanol, the retentions of the separated ions were small so as the capacity factor (Ḱ) (1.28-5.02) for all analyzed anions compared to (1.71-6.85) at 10% methanol in 0.15mM phthalate at pH 5.40 as shown in Fig. (3-6) and summarized in Table (3-2). This can be explained by the relative decrease in the ionic strength of the eluent as the percentage of methanol increase and more decreasing in the polarity of the eluent. This increase in Ḱ was good enough to obtain a baseline separation for most of the analyzed anions. However, the peak shapes as well as the baseline of the analyzed peaks were well enhanced as shown in Fig. (3-7).

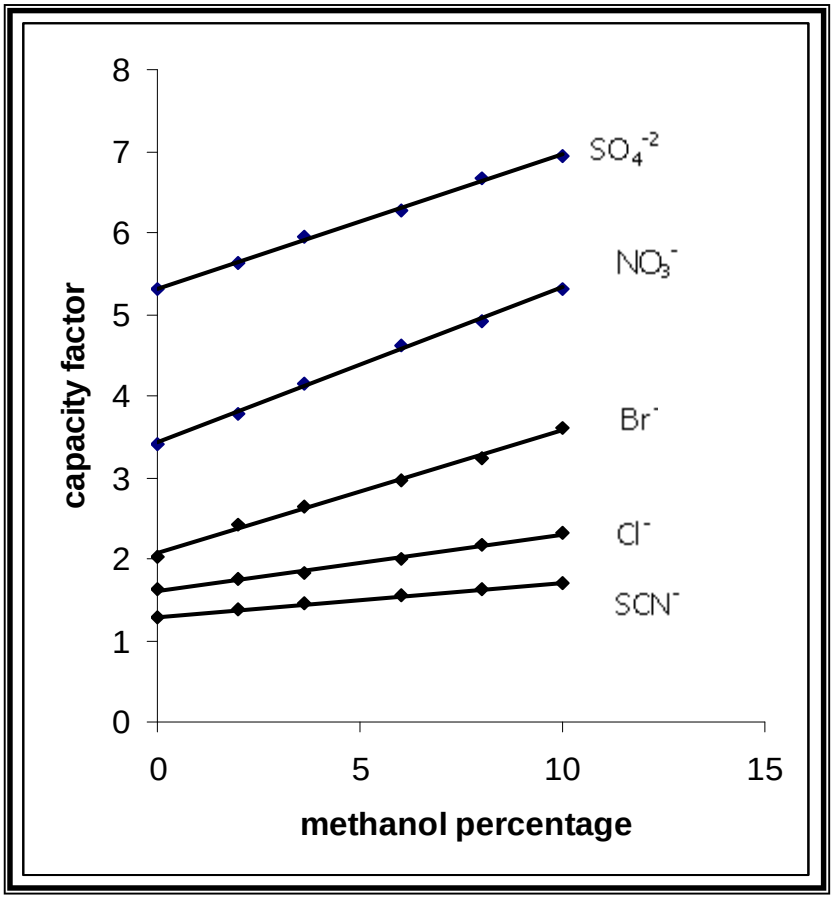


Fig.(3-6) :Effect of Organic Solvent on the Retentions of Anions with 0.15mM Eluent Concentration.

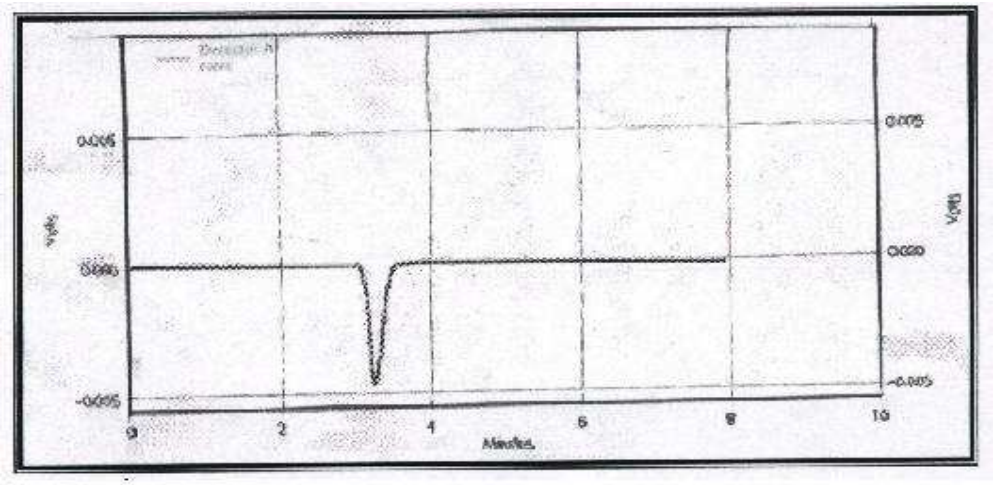


Fig. (3-7): Chromatogram of 1ppm Cl^-, Conditions was the same as in Fig. (3-6), except using 10% Methanol.

Consequently, we found that addition of 10% methanol to mobile phase provides good separation of the analyzed ions, although this was on the expense of the longer t_R. No attempt was made to use higher percentage of methanol due to column manufacture recommendation, which may cause swelling of the column packing material. Based on the above experiments, we have chosen 0.15mM phthalate solution in 10% methanol at pH 5.40, flow rate of 1.0 ml/min and detection wavelength of 228nm as eluent for IPC analysis of several inorganic anions that cannot be detected with UV or visible absorption.

3-2 Chromatographic analysis

Some singly and doubly charged anions at different concentrations were chromatographically analyzed at least three times with Dionex Ion Pac AS4A Column, using 10µL sample loop, with the optimized mobile phase eluent and 228 nm detection wavelength. The analyzed anions have given relatively sharp and symmetrical peaks as well as good detector response. The retention times for all analyzed anions were reproducible. The RSD in t_R were ranged from (0.218-1.534) % with an average of (0.736) %.The Ḱ 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 anions have produced good resolution between the peaks as shown in table (3-3).Consequently; the possibility of separating these anions in mixture has been achieved.

Table (3-3): Retention Times, Resolutions and Peak Asymmetries for some Anions using Dionex Ion Pac AS4A Column [25×0.4(i.d) cm], Eluent 1.5×10^{-4} M Sodium Phthalate at pH 5.40 in 10% Methanol, UV Detector.

sample No.	*Ion*	*Retention time, t_r (min)*	*Resolution, R_s*		*Peak Asymmetry at 10%*
1	*SCN^-*	2.31			0.84
2	*Cl^-*	2.63	*Cl^- /SCN^-*	1.23	1.10
3	*Br^-*	3.68	*Br^-/ Cl^-*	1.64	1.17
4	*NO_3^-*	6.42	*NO_3^-/ Br^-*	3.74	1.23
5	*SO_4^{-2}*	9.52	*SO_4^{-2}/ NO_3^-*	1.89	1.48

The peak asymmetries for the chromatographed anion were ranged from (0.84-1.48). The Resolution R_s ranged from (1.23-3.74) with an average value of (2.125) as listed in Table (3-3).

Mixtures of some anions were chromatographed using the optimized conditions are shown in Fig. (3-8) and (3-9). As mentioned before, in IPC, negative position peaks would be produced with this technique and that were the case in this work. This indicated that these peaks were for the anions that displaced the sites on the stationary phase that have been occupied by the flowing eluent giving a decrease in the baseline absorbance, which has been offset prior analysis. The first mixture contained **{**Cl^-, Br^-, NO_3^-, and SO_4^{-2}} while the other mixture contained **{**SCN^-, Br^-, NO_3^-, and $SO4^{-2}$**}** as shown in the following Figures:

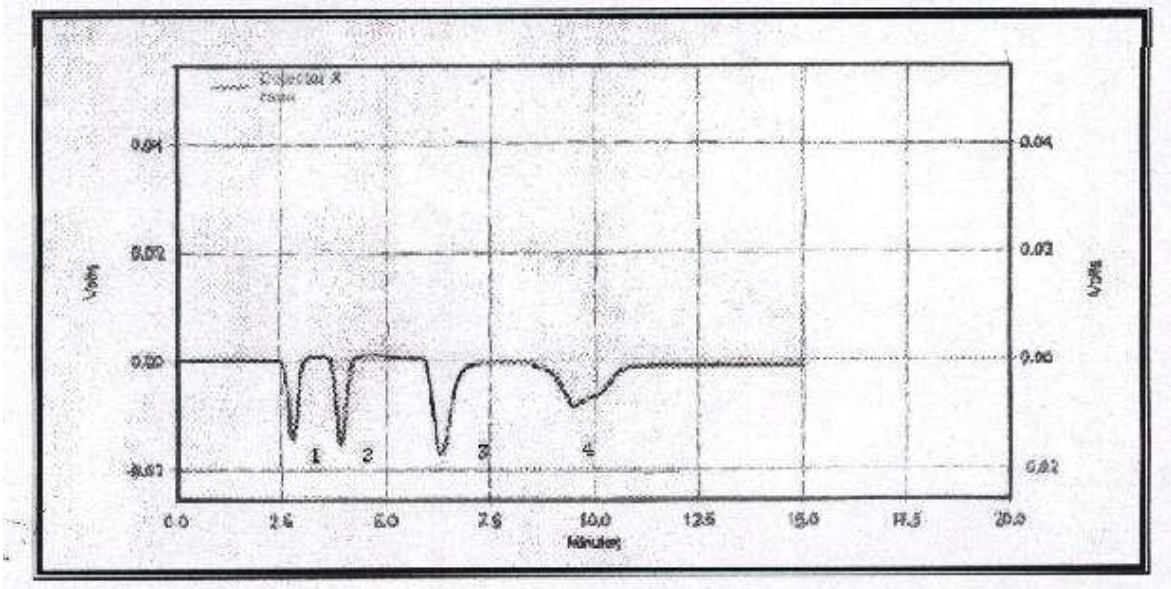


Fig.(3-8) :Chromatogram of a Mixture of four Anions (2ppm Cl^- (1),2.5ppm Br^-(2), 4ppm NO_3^-(3), 2ppm SO_4^{-2}(4)) using Dionex Ion Pac AS4A Column [25×0.4(i.d) cm]. Eluent 1.5×10^{-4} M Sodium Phthalate at pH 5.40 in 10% Methanol, Flow Rate 1 ml/min, 10µL Sample Loop, and Detection Wavelength 228nm.

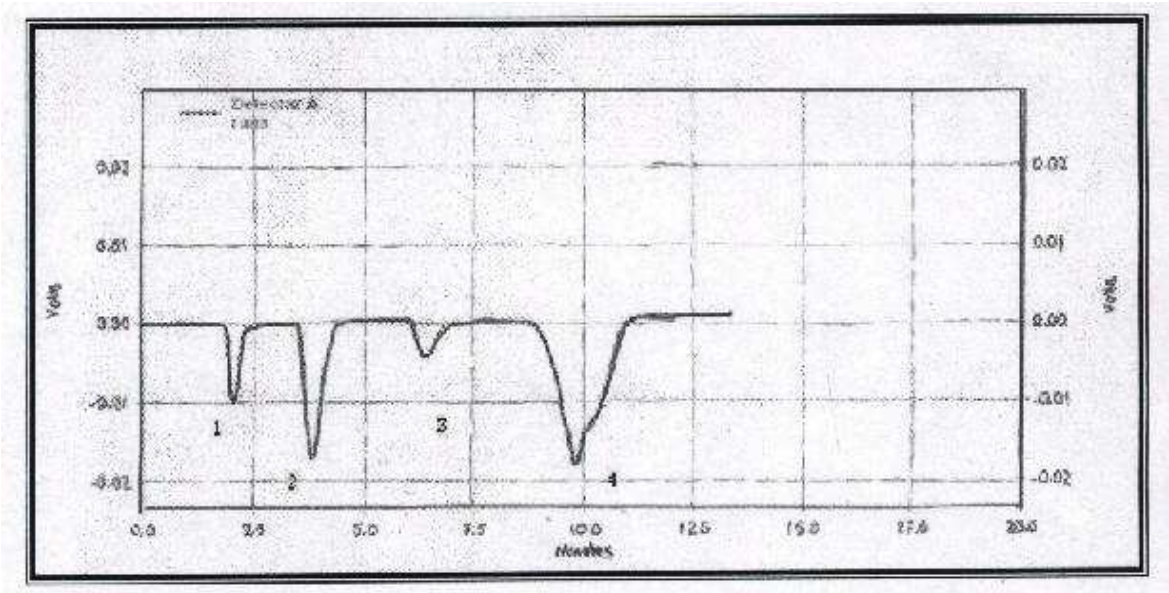


Fig.(3-9) :Chromatogram of a Mixture of four Anions (2.5ppm SCN^-(1), 4ppm Br^-(2), 2ppm NO_3^-(3), 5ppm SO_4^{-2}(4)), Conditions were the same as in Fig.(3-8).

The above two figures represent the separation of four anions which given symmetrical peaks (except for SO_4^{-2}) with good resolution in less than **15**min. However, a mixture of **(SCN^-, Cl^-)** cannot be separated easily because the small difference in retention time have resulted in co-elution together as shown in Fig. (3-10).

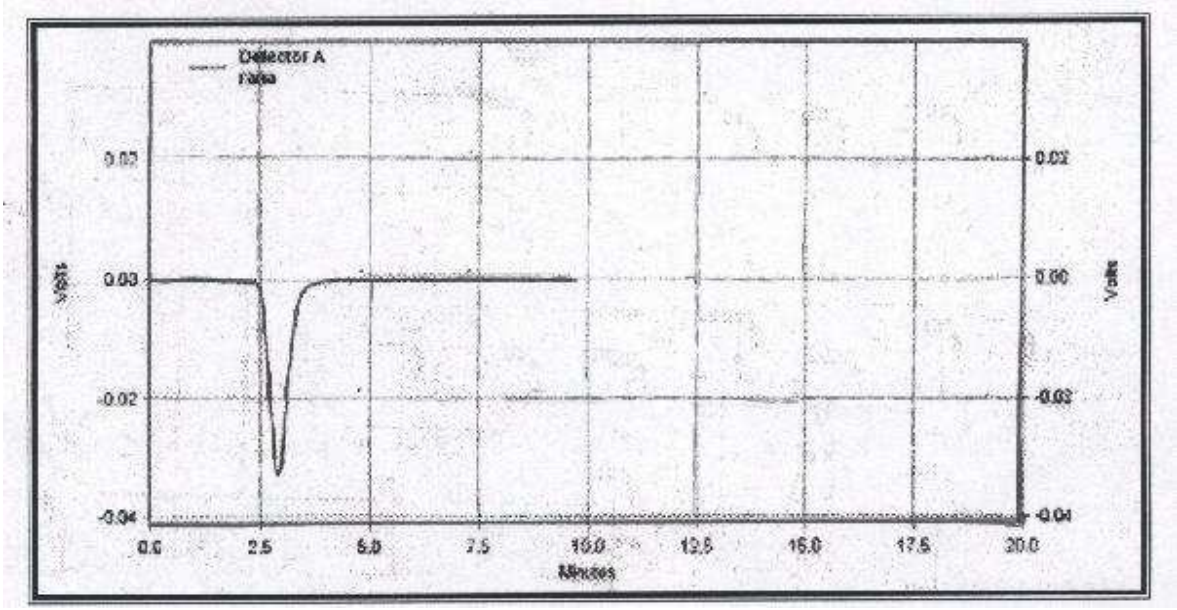


Fig. (3-10): Chromatogram of two Anions (Cl^- and SCN^-), Condition as in Fig.(3-8).

The reported chromatographic retention trend gave no precise sequence for anion elution but their elutions in general are [13]:

$$Cl^- < SCN^- < Br^- < NO_3^- < SO_4^{-2}$$

However, it have been reported that this trend might differ depending on the chromatographic conditions especially the kind and type of stationary and mobile phase. In this work the trend was:

$$SCN^- < Cl^- < Br^- < NO_3^- < SO_4^{-2}$$

The reported trend by Heckenberg and Haddad [51] was in the sequence (Cl^-, NO_2^-, Br^-, NO_3^-, I^- , and SO_4^{-2}) using 5mM potassium hydrogen phthalate at pH 4 with Vydac 302 column and IPD detection at 285nm.

Maki and Danielson [53] who used 0.15mM 1,5-naphthalenedisulfonate/ 10% acetonitrile as eluent and IC-PAK anion- exchange column (5cmx4.6mm(id)) with IPD at 280nm, have separated some ions in the sequence (Cl^-, Br^-, NO_3^-, SO_4^{-2}, and SCN^-). However, Gjerde and Fritz [38] have used 6.5 $x10^{-4}$ M potassium hydrogen phthalate at pH 4.4 as eluent and XAD-1 anion exchange as column; they separated the anion in (Cl^-, NO_3^-, SO_4^{-2}, and SCN^-) trend. Although, when using 1 $x10^{-4}$ M potassium hydrogen phthalate at pH 7.1 as eluent with the same column, they reported the trend (Cl^-, SCN^-, and $SO4^{-2}$) and when using 5 $x10^{-4}$ M, the trend was (Cl^-, NO_2^-, SO_4^{-2}, Br^-, and NO_3^-) with un-suppressed conductivity detection.

Jurkiewicz [47] has used 3.5 $x10^{-4}$ M 6,7-dihydroxy-2-naphthalene sulfonate as mobile phase and AS5 anion exchange column with IPD at 254nm, he obtained the following trend (Cl^-, Br^-, SCN^-, and SO_4^{-2}).

These different trends of elution may explain the difficulty of separating Cl^-, and SCN^- in this work. Therefore, it is important to run standard samples under certain conditions to obtain the correct trend for that analysis.

3-3 Quantitative analysis

Different concentrations of each analyte anions were 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. Calibration runs of some of the anions studied in this work were obtained are shown in Fig. (3-11) using IPD at 228 nm.

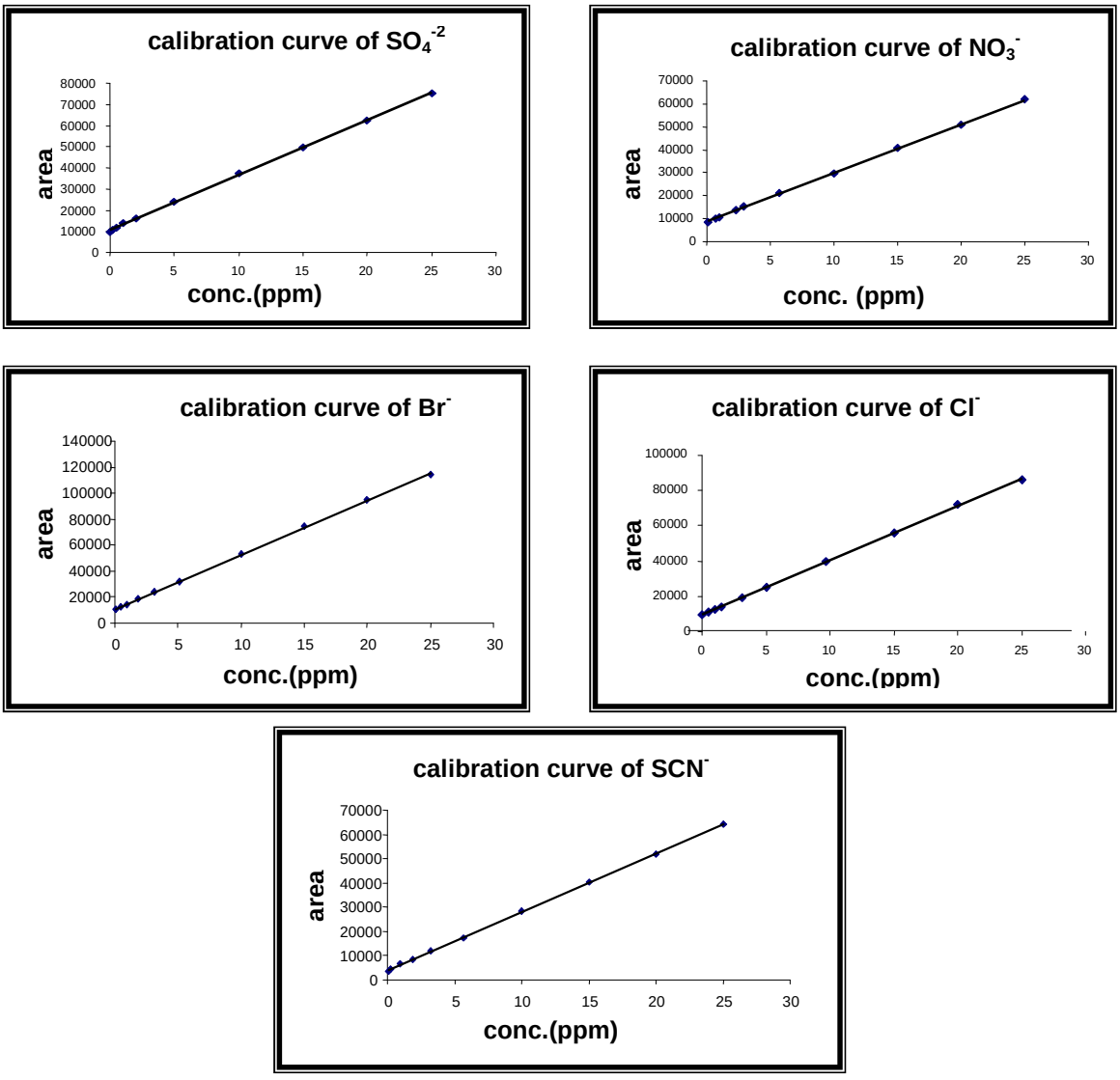


Fig. (3-11): Calibration Curves for some Anions using Conditions as in Fig. (3-8)

Linear calibration curves were obtained for all analyzed anions from at least 25ppm down to the detection limit of each anion. Table (3-4) lists the linear least square equations for the analyte calibration lines. The correlation coefficients were ranged from [0.9994-0.9999].

The detection limits were ranged from (0.1-0.2) ppm. The slope values showed relatively high values which ranged from (2117.8-4203.4) with an average value for the five anions of (2884.36) as shown in table (3-4).

Table (3-4): Linear Equation ,Regression R and Detection Limit for the Analyzed Anions using Dionex Ion Pac AS4A Column [25×0.4 (i.d) cm] ,Eluent 1.5×10^{-4} M Sodium Phthalate at pH 5.40 with 10% Methanol, Flow Rate 1ml/min ,Sample Loop 10µL,and Indirect Detection Wavelength 228nm.

Sample No.	*Ions*	*Linear equation*	*R*	*Detection limit (ppm)*
1	SCN^-	Y=2414X+3927	0.9998	0.15
2	Cl^-	Y=3086.4X+9597.2	0.9996	0.20
3	Br^-	Y=4203.4X+10526	0.9999	0.20
4	NO_3^-	Y=2117.8X+8740.3	0.9994	0.10
5	SO_4^{-2}	Y=2600.2X+10584	0.9998	0.15

Prepared standard mixture solutions of the analyzed ions 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 the linear equation. The recovery were ranged from 93.36% to 96.75% with an average of 95.14% the relative errors were ranged from (3.25-6.64) % with an average of 4.91% as shown in Table (3-5).

Table (3-5): Recovery and Percentage Relative Error for the Analyzed Anions using IPD method.

Ions	*Concentration injected (ppm)*	*Concentration Found(calculated)* (ppm)*	*% recovery*	*Relative Error %*
SCN^-	2.5	2.41	96.40	3.60
Cl^-	2	1.87	93.50	6.50
Br^-	2.5	2.38	95.20	4.84
NO_3^-	4	3.87	96.75	3.25
SO_4^{-2}	5	4.68	93.36	6.64

***using the liner equation for each ion.**

3-4 Conductivity study:

The same eluent with the optimum conditions was used to separate the same mixture with unsuppressed conductivity detection. Since most of the aromatic weak acid (or bases) salts have very low conductivity, therefore, they can be used to elute inorganic anions and detected directly because of having large conductivity than the eluent.

Different concentrations of each analyte anions were 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. Calibration runs were obtained are shown in Fig. (3-15) with conductivity detector.

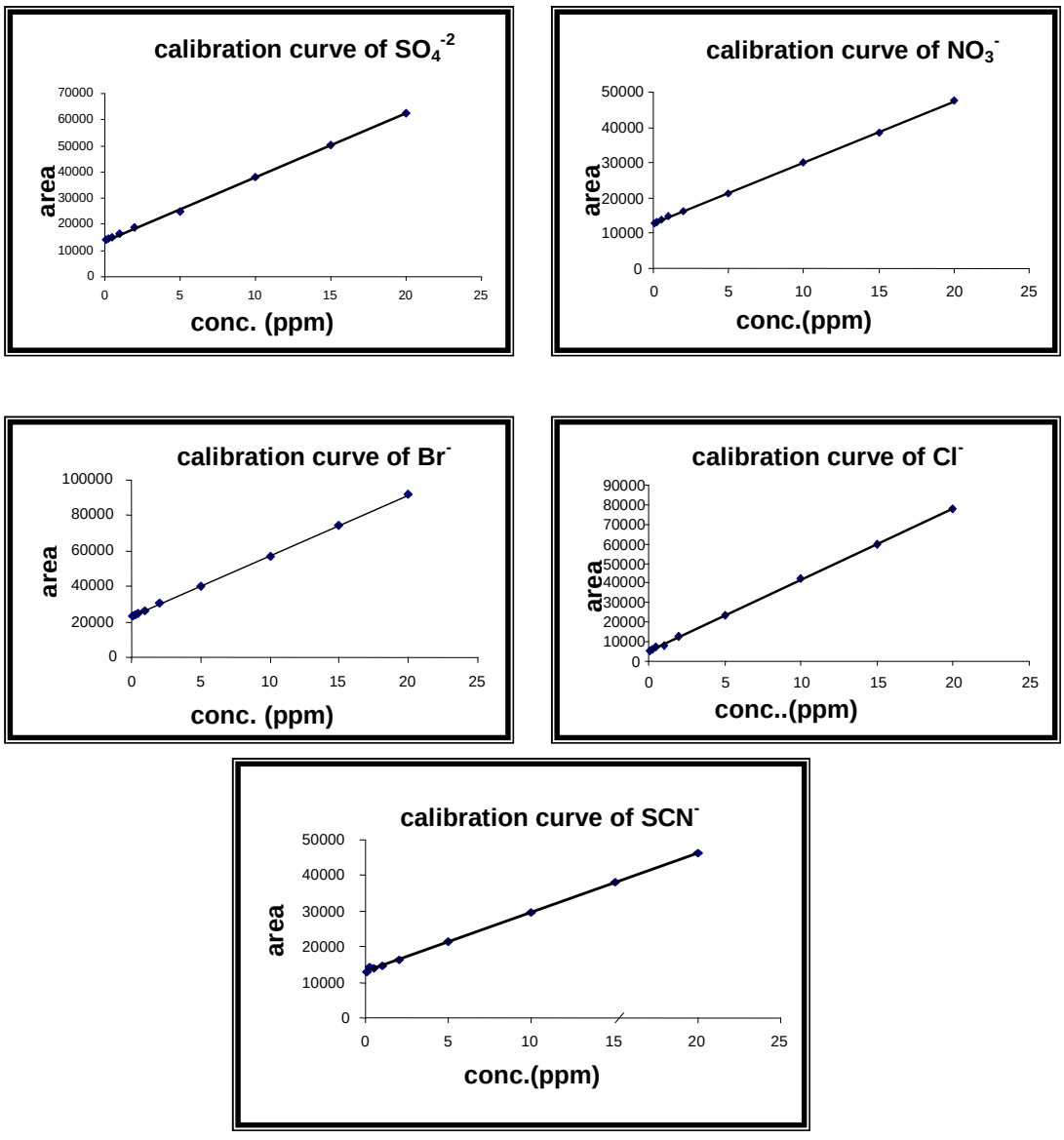


Fig. (3-12): Calibration Curves for some Anions using Conditions as in Fig. (3-8), except using Conductivity Detection.

Linear calibration curves were obtained for all analyzed anions at least 25ppm down to the detection limit of each anion. Table (3-7) lists the linear least square equations for the analyte calibration lines. The correlation coefficients were ranged from [0.9996-0.9999]. The detection limits were ranged from (0.15-0.25) ppm. The slope showed relatively low values (compared to IPC method) which ranged from (1659.3-3639.7) with an average value for the five anions of (2576.26) as listed in table (3-7).

Table (3-6): Linear Equation ,Regression R and Detection Limit for the Analyzed Anion using Dionex Ion Pac AS4A Column [25×0.4 (i.d) cm], Eluent 1.5×10^{-4}M Sodium Phthalate at pH 5.40 with 10% Methanol, flow rate 1mL/min ,Sample Loop 10µL, and Conductivity Detector.

Sample No.	*Ions*	*Linear equation*	*R*	*Detection limit (ppm)*
1	SCN^-	Y=1659.3X+13141	0.9999	0.20
2	Cl^-	Y=3639.7X+5206.7	0.9996	0.25
3	Br^-	Y=3422.6X+23116	0.9999	0.15
4	NO_3^-	Y=1721.8X+12892	0.9998	0.20
5	SO_4^{-2}	Y=2437.9X+13697	0.9997	0.15

The prepare standard solutions were injected for at least 3 times under the same condition and their concentration were calculated as described earlier using the linear equation.

The recovery were ranged from 89.00% to 96.80% with an average of 92.31% and relative errors ranged from (3.20-10.40)% with an average of 7.46% as shown in Table (3-8).

Table (3-7): Recovery and Percentage Relative Error for the Analyzed Anions using Conductivity Detector.

Ions	*Concentration injected (ppm)*	*Concentration Found(calculated)* (ppm)*	*% recovery*	*Relative Error%*
SCN^-	2.5	2.36	94.40	5.60
Cl^-	2	1.80	90.00	10.00
Br^-	2.5	2.42	96.80	3.20
NO_3^-	4	3.67	91.75	8.25
SO_4^{-2}	5	4.48	89.60	10.40

using the liner equation for each ion.

A comparison between UV-Visible detector and conductivity detection showed that the slope values for the five anions with conductivity detection were less than that using IPC detection. The detection limit values were also higher than with conductivity. The recovery and relative errors were lower and higher, respectively, with conductivity detection. This can be attributed to the influence of changing in temperature during analysis and from day to day and to the broader peaks obtained with conductivity compared to that of IPC method. We have some difficulties in separating the studied anions especially the first two, although partial separation of Cl^- from Br^- was obtained.

This may be due to the large relative conductivities of these anion, which caused overlapping and co-eluting peaks as shown in Figs. (3-12) and (3-13), respectively.

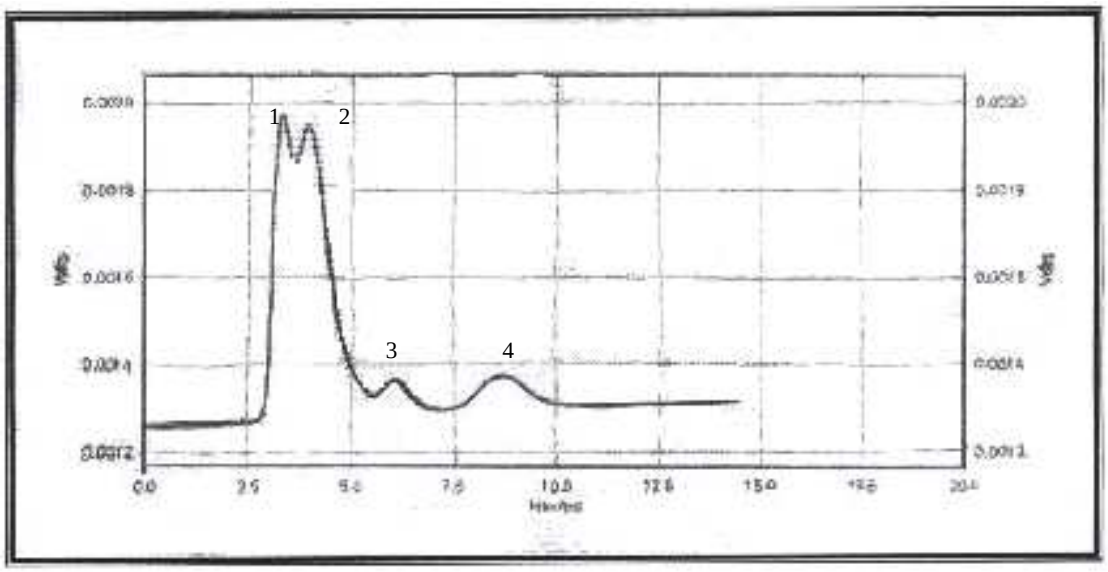


Fig.(3-13) :Chromatogram of Four Anions as(2ppm Cl^- (1),2.5ppm Br^-(2) , 4ppm NO_3^-(3), 2ppm SO_4^{-2}(4)), Conditions as in Fig.(3-8) except using Conductivity Detector.

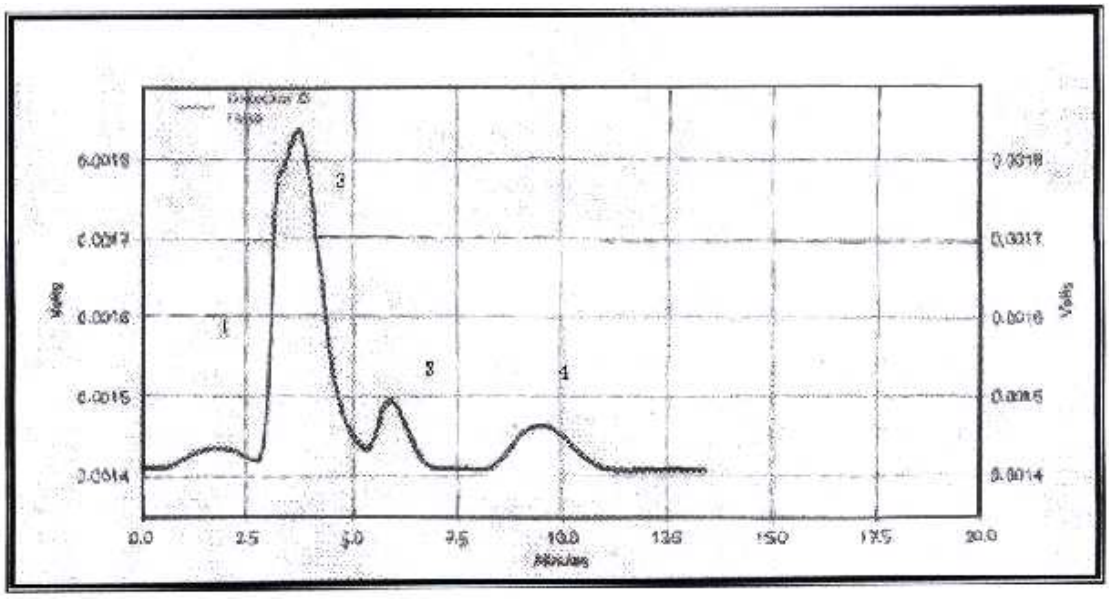


Fig.(3-14) : Chromatogram of Four Anions as (2.5ppm SCN^-(1),4ppm Br^-(2), 2ppm NO_3^-(3), 5ppm SO_4^{-2}(4), Condition as in Fig.(3-12).

However, the separation of some ions were possible, such as the case in the separation of mixture containing Cl^-, NO_3^- and SO_4^{-2} as shown in Fig. (3-14).symmetrical peaks were obtained, although they were broader which explained the difficulty in separation of more sample components.

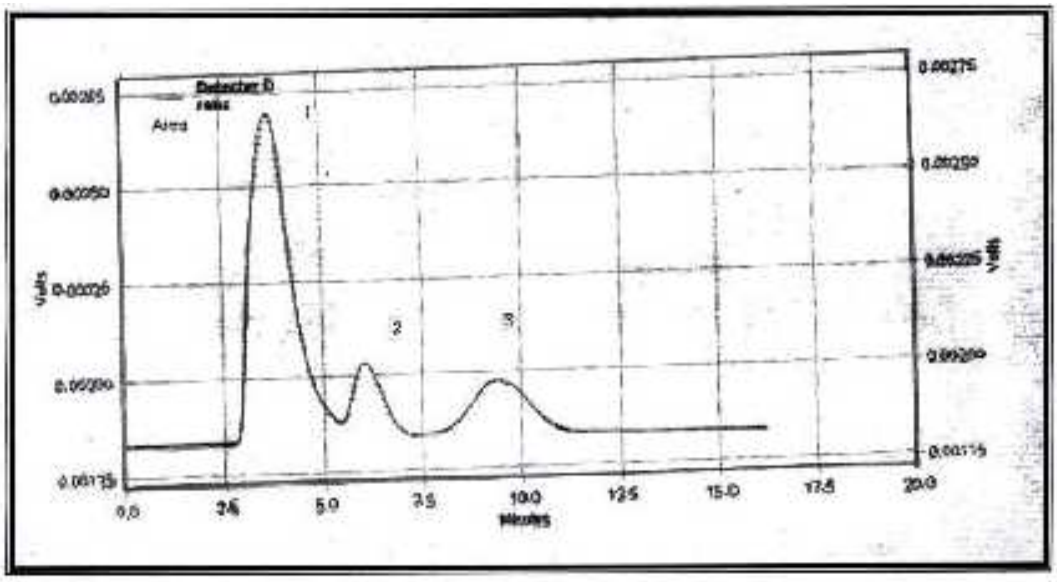


Fig.(3-15) : Chromatogram of a Mixture of Three Anions (3ppm Cl^-(1),4ppm NO_3^-(2), 5ppm SO_4^{-2}(3), Condition as in Fig.(3-12).

The peak asymmetries for the chromatographic anion were ranged from (0.74-1.35). The RSD in t_R were ranged from (0.333-1.056) % with an average value of (0.649) %. The Resolution R_s ranged from (1.05-2.32) with an average value of (1.562).Table (3-6) lists some of the chromatographic parameters for the analyzed anion.

Table (3-8): Retention Time, Resolution R_s, and Peak Asymmetry at 10% for Separated using Dionex Ion Pac AS4A Column [25×0.4(i.d) cm], Eluent 1.5×10^{-4}M Sodium Phthalate at pH 5.40 with 10% Methanol, Conductivity Detector.

sample No.	*Ions*	*Retention time, t_r (min)*	*Resolution, R_s*		*Peak Asymmetry at 10%*
1	*SCN^-*	2.36			0.74
2	*Cl^-*	2.71	*Cl^- /SCN^-*	1.16	1.15
3	*Br^-*	3.76	*Br^-/ Cl^-*	1.05	1.22
4	*NO_3^-*	6.54	*NO_3^-/ Br^-*	2.32	1.26
5	*SO_4^{-2}*	9.63	*SO_4^{-2}/ NO_3^-*	1.72	1.35

The above comparison showed that UV-detection was more suited for the analysis of these ions than conductivity detector.

2.1 Instruments and equipments:

The high performance liquid chromatograph used in this work was Shimadzu (Kyoto, Japan) which consisted of a system controller model SCL-10 AVP, UV-Vis detector model SPD-10AVP, 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), 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. Anion exchange column from Dionex Ion Pac AS4A column.

2.2 Chemicals:

The following materials and chemicals were used in this work; phthalic acid, sodium hydroxide, sodium sulfate, sodium nitrate, potassium thiocyanate, sodium chloride, potassium bromide, methanol (analar), hydrochloric acid. The above chemicals and reagent were obtained either from Fluka or BDH companies.

2.3 Preparation of mobile phase:

A 1.00 mM phthalate was prepared by 0.83 gram of phthalic acid in one liter deionized water and the pH of the solution was adjusted by adding sodium hydroxide to reach pH 5.40, then the volume completed to 1 liter.

2.4 Sample preparation:

Stock solutions of 1000 ppm of each of anions (SCN^-, CL^-, Br^-, NO_3^-, and SO_4^{-2}) were prepared by weighting (0.168, 0.165, 0.149, 0.137, 0.148) gram of each (KSCN, NaCl, KBr, $NaNO_3$, Na_2SO_4) respectively. Other standard solutions were prepared by subsequent dilution of the stock solutions .The solvent used to prepare these solutions was usually the same as the mobile phase employed for their separation. Mixtures of two or more of the above analyte were also prepared by mixing the appropriate volumes of the stock solutions.

2.5 Analysis of samples:

All prepared standard solutions and their mixtures have been chromatographically analyzed on the anion exchange column with sodium phthalate at pH 5.40 as eluent at optimum flow rate 1ml\min with UV-Visible or conductivity detectors.

Conclusion:

For both indirect photometric and unsuppressed conductivity 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 diverse ionic species using conventional HPLC equipment with UV detector.

Phthalate eluent was used for the separation and detection of the small singly charged and multicharge of common inorganic anions,such as (SCN^- ,Cl^-,Br^-,NO_3^-,and SO_4^{-2}) with capacity factors (ќ) were ranged from (1.71-6.85) using single anion exchange column. The peaks were symmetrically shaped and well resolved with an average value of R_s (2.125). The RSD in t_R was averaged 0.736%. The average recovery was (95.14) %, with relative error percentage averaged was (4.91) % with IPC .But unsuppressed conductivity detection given peak a symmetry ranged from (0.74-1.35), and the average value of R_s (1.562). The RSD in t_R averaged a value of 0.649%. The recovery was (92.31) %, and the relative error percentage averaged (7.46) %.

Quantitative chromatographic analyses of four anions were performed using UV detection at 228nm and conductivity detector. Both detectors have given a liner calibration curves from their detection limits to at least 25ppm .A mixture of(Cl^-,Br^-,NO_3^-,and SO_4^{-2}) and(SCN^-,Br^-,NO_3^-,and SO_4^{-2}) have been base line separated using IPD technique , in less than 15 minutes.

Chapter One

Introduction

Chapter Two

Experimental

Chapter three

Results

And

Discussion

References

Future work

We suggest the following for future work

1. Using other anion exchanger column from different suppliers for comparisom.
2. Use gradient elution which is possible with IPD.
3. With the above trying to saparate the large and multicharged inorganic and organic anion.
4. Apply the same technequice for the separation of cations.

Refrences

1. Robert L., Pecsok L. and Donald S., "Modern Methods of Chemical Analysis", 2nd edition, John Wiley and Sons, New York, 85-92 (1986).
2. Veronika R. Meyer, **"**Practical High-Performance Liquid Chromatography"**,** 1st edition, John Wiley and Sons , New York, 25-27 (1988).
3. Gazdag M., Szepsi G. and Faban-Varge K., *J. Chromatogr***., 454**, 107 (1988).
4. Gennaro M., Angelino S. and Gianotti V., *Analusis*, **27**, 742-748 (1999).
5. VanLeeuwen J., Buydens L., Vandeginste B., Kateman G., Shoenmakers P. and Mulholland M., *Chem. and Intell. Lab. Syst***., 10,** 337-347 (1991).
6. Neil D.Danielson, "Encyclopedia of Physical Scince and Technology"; by Academic prees Inc, **7**, 342 (1987).
7. Peter E. Jackson, "Encyclopedia of Analytical Chemistry"**,** John Wiley and Sons Ltd , Chichester, 2779-2801 (2000).
8. Daniel C. Harris,"Quantitative Chemical Analysis", 6thedition , by W.H.Freeman and Company , New York ,555-556, 608, 645-649 (2003).
9. Kenneth A. and Judith F., "Contemporary Instrumental Analysis", by Prentice Hall, Inc., New Jersey , 586-587, 630-635 (2000).
10. Douglass A. Skoog, "Priciple of Instrumental Analysis", 3rd edition , by saunders College Publishing, adivision of Holt, Rinehart and Winston, New York and london, 725-730 (1985).

11. Snyder Kirkland, "Introduction to Modern Liquid Chromatography", 2nd edition, John Wiley and Sons, New York ,110-121 (1979).
12. Ptyde A. and Gilbert M., "Applications of High Performance Liquid Chromatography", Chapman and Hall, London, 121-127 (1979).
13. James M. Miller, "Chromatography Concepts and Contrasts " by Johan and Sons, Drew university Madison , New Jersey, 178-181 (1988).
14. Douglass A. Skoog, "Priciple of Instrumental Analysis", by saunders College Publishing, adivision of Holt, Rinehart and Winston, Inc. , 730-738 (1998).
15. Frank A. Settle, "Hand Book of Instrumental Techniques For Analytical Chemistry" , by Pratice Hall PTR 204 (1997).
16. James M. Miller, "Chromatography", 1st edition, John Wiely and Sons , New York , 85-87 (1988).
17. Roger M. Smith, "Gas and Liquid Chromatography in Analytical Chemistry", 1st edition , John Wiley and Sons , New York, 50-55, (1988).
18. http://www.googel.com/ *Ion Chromatography.*
19. Telpchak M., *J.Chromatographia* ,**6**, 234 (1973).
20. Rieman W. III and Walton H.F, "Ion Exchang in Analytical Chemistry", paragon, press Oxford 98 (1970).
21. Small H., Stevens T.S, and Bauman W.C, *Anal.Chem.*, **47**, 1801 (1975).
22. Gabbott, Dawkins, Lioyd , *J. Chromatogr.*, **145**, 452 (1988).
23. Sambi A., *Analytical Chemical Act.*, **199**, 295 (1994).

24. Pekka V., Timo K. and Sari P., *J. Sep. Sci.*, **24**, 141-147 (2001).
25. Frenzel W., Schepers D. and Schulze G., *Anal. Chem. Acta*., **277**, 103-11 (1993).
26. Woods C. and Rowland A., *J. Chromatogr.*, **789**, 287-299 (1997).
27. Vera P., Pavel C. and Karel S., *Electrophoresis*, **24**, 1883-1891 (2003).
28. Vohra S.K, *Amer. Lab*, **5**, 66 (1981).
29. Samuelson R., Opea A. and Osteryoung J., *J. Anal. Chem.*, **52**, 2215-2216 (1980).
30. Kenneth P. , *General Chemistry Laboratory*, (1993).
31. Stevens T.S, Jewelt G.L and Bredeweg R.A, *Anal. Chem.*, **54**, 1206 (1982).
32. Edward S. and Yeung E.S, *Acc. Chem. Res.*, **22**, 125-130 (1989).
33. Fritz J.S,Gjerde D.T and Becker R.M, *Anal. Chem.*,**52**,1519-1522 (1980).
34. Hisakuni Sato , *Analytica Chimica Acta.*, **206,** 281-288 (1988).
35. Okada T. and Kuwamoto T., *J. Chromatogr.*, **284**, 149 (1984).
36. Cassidy R. and Kuwamoto T., *J. Chromatogr. Sci* . , **21**, 454 (1983).
37. Gjerde D.T, Fritz J.S and Schmuckler G., *J.Chromatogr.*,**186**,509 (1979).
38. Gjerde D.T and Fritz J.S, *Anal. Chem.* , **53**, 2324 (1981).
39. Fritz J.S and Gjerde D.T, "Ion Chromatography", Huthig , 2nd edition, New York, 3-4, 63-88, 139-155 (1987).
40. Small H., "Ion Chromatography " , Plenum press, 67-69, 177-186, 196-201 (1989).
41. Bello M.A and Gonzalez A.G, *Analusis*, **27**, 97-100 (1999).

42. Schmuckler G. and Brenman L., *LC-GC Int.*, **5**, 36-38 (1992)

43. Reve R.N, *J. Chromatogr.* , **177**, 393 (1979).

44. Cortes H.J, *J. Chromatogr.*, **243**, 392 (1982).

45. Small H., Miller T.E., *J. Anal. Chem.*, **54**, 462-469, (1982).

46. Maki S.A and Danielson N.D, *Journal of Chromatography*, **542**, 101-113 (1991).

47. Jurkiewicz K., *J. Liq. Chromatogr.*, **12(11),** 2145 (1989).

48. Rapsomanikis S. and Harrison R., *Anal. Chem. Acta.*, **199**, 41-47 (1987).

49. Papp E.J, *J. Chromatogr.*, **402**, 211-220 (1987).

50. Sato H.P, *Anal. Chem.*, **62**, 1567 (1990) .

51. Heckenberg A.L and Haddad P.R , *J. Chromatogr.*, ***299***, 301 (1984).

52. Wilson S.A and Yeung E.S, *Anal. Chim. Acta.*, **157**, 53 (1978).

53. Maki S.A and Danielson N.D, *Anal. Chem.*, **7**, 63 (1991).

54. Maki S.A and Danielson N.D, *J.chromatogr.*, **28**, 537 (1990).

55. Maki S.A, Ibn Al-Haitham, *Journal for Pure and Applied Scince*,**2**,9(1998).

Republic of Iraqi
Ministry of Higher Education
And Scientific Research
Al-Nahrain University
College of Science
Department of Chemistry

Separation and detection of some anions by Ion Chromatography using indirect photometric and conductivity methods

A thesis
Submitted to the College of Science
Al-Nahrain University
In partial fulfillment of Requirements
For the Degree of Master of Science in
Chemistry

By
Rana Abd Hammza Al- Hasnawe
B.Sc. (Al-Nahrain University 2002)

August 2005 ***Rajab 1426***

Supervisor certification

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

Assistant Professor

Dr. Shahbaz. A. Maki

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

Head of the Department of chemistry
College of Science
Al-Nahrain University

Examining Committee's Certification

We The Examining Committee, Certify That We have Read This Thesis and examined the Student in It's Contents and that, According to our Opinion, is Accepted us a thesis for the Degree of Master of Science in Chemistry.

Signature:
Name:
Title:
(Chairman)

Signature:
Name:
Title:
(Member)

Signature:
Name:
Title:
(Member)

Signature:
Name: Dr.Shahbaz.A.Maki
Title: Assistant Professor
(Member/ Advisor)

I hereby, certify a bone the decision of the examining committee.

Signature:
Name: Dr. Laith Abd Al-Aziz
Title: Assistant Professor
Address: Dean of College of Science
Date:

ACKNOWLEDGEMENT

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

I would like to express my deepest thanks to my respected Supervisor Dr. Shahbaz A. Maki for his great help and assistance provided during this work.

Sincere thanks are also to the Dean of the college of science and the head of department of chemistry and the staff members of the department of chemistry.

Finally, sincere thanks and deep respect to my father, my mother and my sisters, as well as my Uncles.

I am especially grateful to my friends Mutaz , Noor for their help in justifying this thesis.

Finally to all my friends …… I present my thanks.

RANA

2005

Summary

Anion exchange chromatography with both indirect photometric detection (IPD) and unsuppressed conductivity detection modes were used for the separation and detection of inorganic anions. Salt of phthalic acid has been used as an eluent for several anions both with IPD and conductivity detection. Phthalate possess ion-exchange capability, chromatographic selectivity, and large molar absorptivity.

Phthalate has shown good chromatographic performance toward the analyses of some anions such as (Thiocyanate, Chloride, Bromide, Nitrate and Sulfate) using conventional HPLC equipment with either indirect UV or conductivity detectors. The analysis of these anions using $1.5x10^{-4}$M phthalate at pH 5.40 in 10%methanol, AS4A Ion Pack column, 1 mL/min flow rate was achieved. IPD at 228 nm and conductivity detection.

Chromatogram of a mixture containing for anions such as (Cl^-, Br^-, NO_3^-, SO_4^{-2}) and other mixture containing four anions such as (SCN^-, Br^-, NO_3^-, SO_4^{-2}) have given well separated peaks. The capacity factors (ќ) for the analyzed anions were ranged from (1.71-6.85), peak symmetries was ranged from (0.84-1.48), and resolution with an average value of 3.758 were obtained, which indicate a good chromatographic performance. The RSD in t_R averaged 0.736%. The average recovery was 95.14 %, and the relative error percentage was averaged 4.91 % with IPD. However, using unsuppressed conductivity detection, the peak symmetry was ranged from (0.74-1.35), and an average value of resolution of 1.949. The average value of recovery was 92.31 %, and the relative error averaged 7.46 % using conductivity detection.

Calibration curve for all analyzed anions were linear from their detection limits at least 25 ppm. The correlation coefficients for the linear calibration curve were ranged from [0.9994-0.9999] with both detection techniques. The detection limit was ranged from (0.1-0.2) ppm using IPD detection compared to (0.15-0.25) ppm with conductivity detection.

Contents

List of Tables

List of Figures

List of Abbreviations

LG	**Liquid chromatography**
GC	**Gas-chromatography**
HPLC	**High performance liquid chromatography**
MPa	**Macro Pascal**
HETP	**High equivalent theoretical plates**
LSC	**Liquid solid chromatography**
IEC	**Ion exchange chromatography**
IC	**Ion chromatography**
id	**Internal diameter**
UV	**Ultra violate**
IPD	**Indirect photometric detection**
IPC	**Indirect photometric chromatography**
RI	**Refractive index**
ppm	**Part per million**

Supervisor certification

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

Assistant Professor

Dr. Shahbaz. A. Maki

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

Graduate Committee Deviser

Dr. Ayad S. Hameed

Head of Department

Of Chemistry

Table (3-1): **Some Chromatographic Parameters of Anions Using Dionex Ion Pac AS4A Column (25×0.4 (i.d) cm), with Different Eluent Concentration.**

Ion	*Capacity factor (Ḱ) and separation factor(α)and retention time(t_R) at different eluent concentration (mM)*														
	1.00			*0.50*			*0.25*			*0.15*			*0.05*		
	t_R	Ḱ	α	t_R	Ḱ	α	t_R	Ḱ		t_R	Ḱ	α	t_R	Ḱ	α
SCN^-	1.14	0.80		1.36	1.08		1.53	1.16		2.31	1.28		2.63	1.39	
Cl^-	1.36	0.98	1.22	1.56	1.34	1.24	1.87	1.51	1.3	2.63	1.69	1.32	3.47	1.80	1.29
Br^-	1.87	1.23	1.25	2.15	1.69	1.26	2.38	1.90	1.2	3.68	2.07	1.22	4.21	2.27	1.26
NO_3^-	2.64	2.05	1.67	3.27	2.68	1.58	4.57	2.93	1.5	6.42	3.20	1.54	__*	___*	__*
SO_4^{-2}	3.52	3.58	1.75	4.36	4.33	1.61	5.28	4.64	1.5	9.52	5.02	1.57	__*	___*	__*

* Not measured because they were not detected clearly.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم
قسم الكيمياء

فصل وتعيين بعض الأيونات السالبة بواسطة كروماتوغرافي الأيون باستخدام طرق التحليل الضوئي غير المباشر والتوصيلية

رسالة
مقدمة الى كلية العلوم- جامعة النهرين
وهي جزء من متطلبات نيل درجة الماجستير في الكيمياء

من قبل
رنا عبد حمزة الحسناوي
بكالوريوس ٢٠٠٢ (جامعة النهرين)

آب ٢٠٠٥ **رجب ١٤٢٦**

بِسم الله الرحمن الرحيم

قَا لوُاْ سُبَحنكَ لآ عِلمَ لَنَا إلاَّ مَا عَلَّمتَنَا إنَّكَ
أنَتَ آلعِلِيمُ آلحَكِيمُ

صَدَقَ الله آلَعظِيَم

سورة البقرة (٣٢)

الإهداء

إلى الشمس التي أضاءت طريقي

أمي

إلى منبع علمي و مغذ ي عقلي
إلى من روى بعرقه شجرة تحصيلي

أبي

إلى أحباب قلبي و مسا ندي شدتي

أخواتي

إلى من كا نت لي سندا في كل لحظة و ثانية

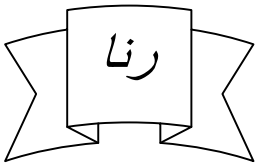
نور

إلى مهجتي في الحياة

زملائي وزميلاتي كافة

و إلى كل من له معزة في قلبي

أهدي ثمرة جهدي



الخلاصة

تم في هذا البحث استخدام طرق كروماتوغرافيا التبادل الأيوني بواسطة التحليل الضوئي الغير مباشر(IPD) والتوصيلية دون استخدام المثبط لفصل وتعين الأيونات السالبة اللاعضوية. استخدام ملح حامض الفثالك كناقل لعدة أيونات سالبة بكلتا الطريقتين. يمتلك الفثاليت قابلية تبادل ايوني، أنتقائية كروماتوغرافيا وامتصاصية مولارية عالية.

اظهر الفثاليت أداء كروماتوغراف جيد اتجاه تحليل بعض الأيونات السالبة مثل ثايوسيانيت، الكلوريد، البروميد، النترات، والكبريتات باستخدام جهاز الكروماتوغرافيا السائل عالية الكفاءةHPLC بواسطة كاشف الأشعة فوق البنفسجية الغير مباشر أو كاشف التوصلية. تم تحليل هذه الأيونات السالبة بأستخدام الفثاليت بتركيز ٠,١٥ mM وفي pH 5.40 في ١٠% ميثانول، مع عمود التعبئة الأيوني دايونكس (AS4A)، بمعدل سرعة جريان 1mL/min ، مع IPD بطول موجي ٢٢٨ nm وكاشف التوصلية دون استخدام المثبط .

أعطى فصل الكروماتوغراف لمزيج من أربعة أيونات سالبة (SCN^- , Br^-, NO_3^-, SO_4^{-2}) ومزيج آخر يحتوي على (Cl^- , Br^-, NO_3^-, SO_4^{-2}) قمم منفصلة. عامل السعة $\acute{K}$ للأيونات السالبة المحللة من ١,١٧ إلى ٦,٨٥، قمة التناظر من ٠,٨٤ إلى ١,٤٨، وقابلية الفصل بمعدل ٢,١٢٥ التي أعطت كفاءة كروماتوغرافيا جيدة.

الانحراف المعياري النسبي لزمن الاحتجاز بمعدل ٠,٧٣٦ %. نسبة المسترجع بمعدل ٩٥,١٤ % ونسبة الخطأ النسبي بمعدل ٤,٩١ % بواسطة IPD.بينما استخدم كاشف التوصيلية دون استخدام المثبط، ، قمة التناظر من ٠,٧٤ إلى ١,٣٥، وقابلية الفصل بمعدل ١,٥٦٢.

الانحراف المعياري النسبي لزمن الاحتجاز بمعدل ٠,٦٤٩ نسبة المسترجع بمعدل ٩٢,٣١ % ونسبة الخطأ النسبي بمعدل ٧,٤٦ %.كانت منحنيات المعيارية لكل الأيونات السالبة المحللة ممتدة من حدودها الكشفية إلى اقل من ٢٥ ppm .

ووصلت معاملات الارتباط لمنحنيات المعيارية الخطية من (٠,٩٩٩٤-٠,٩٩٩٩) ووصلت حدود الكشف من (٠,١-٠,٢) ppm باستخدام طريقة IPD مقارنة بطريقة كاشف التوصيلية التي وصلت إلى (٠,١٥-٠,٢٥) ppm .

معلومات شخصية

الاسم :- رنا عبد حمزة الحسناوي

الكلية :- العلوم

القسم :- كيمياء

الدرجة :- ماجستير

اسم البحث :- فصل وتعيين بعض الأيونات السالبة بواسطة كروماتوغرافي الأيون باستخدام طرق التحليل الضوئي غير المباشر والتوصيلية.

الأستاذ المشرف :- دكتور شهباز احمد مكي (أستاذ مساعد، كيمياء تحليلية، كلية العلوم، جامعة النهرين).

العنوان :- بغداد / حي الرسالة / محلة ٨٥١ / زقاق٣٠ / دار ٢٨

الموبايل :- ٠٧٩٠٢٤٨٢٩٣٠

تاريخ المناقشة :- الاربعاء ٢١ /٩ /٢٠٠٥

1.1 Chromatography:

Chromatography is a technique used for separation of components in a sample by distribution of its compounds between two phases; one is stationary and the other mobile phase [(1)]. The stationary phase may be solid or liquid supported on a solid or a gel, and may be packed in a column or spread on an inert materials as in thin layer chromatography.

The mobile phase may be a gaseous, as in gas chromatography "GC" or liquid as in liquid chromatography "LC" [(2)].The separation mechanism in GC involved two kinds of interactions, one between solute molecules themselves and the other between the solute and the stationary phase [(3)]. However, in LC there are three kinds of interactions solute-mobile phase, solute- stationary phase as well as solute-solute interactions. The main aims of chromatographic methods are to achieve best resolution between the compounds of the mixture, minimum detection limit, and short analysis time [(4,5)]. The chromatographic methods can be classified as shown table (1-1). This classification is based on the typed stationary-phase and equilibrium involved in separation.

Table (1-1): Classification of Chromatography Techniques [4, 5]

General classification	*Stationary phase*	*Type of equilibrium*	*Specific method*
Liquid chromatography LC mobile phase liquid	Liquid adsorbed on a solid	Partition between immiscible liquids	Liquid-liquid or partition
	Organic species bonded to a solid surface	Partition between liquid and bonded surface	Liquid bonded phase
	Solid	Adsorption	Liquid-solid or adsorption
	Ion exchange resin	Ion-exchange	Ion-exchange
	Liquid in interstices of polymeric solid	Partition/sieving	Size-exclusion
Gas chromatography GC mobile phase: gas	Liquid adsorbed on a solid	Partition between gas and liquid	Gas-liquid
	Organic species bonded to a solid surface	Partition between liquid and bonded surface	Gas-bonded phase
	Solid	Adsorption	Gas-solid
Supercritical-fluid chromatography (SFC) mobile phase : supercritical fluid	Organic species bonded to a solid surface	Partition between supercritical fluid and bonded surface	

1.2 Liquid chromatography:

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. There are two types of LC, classical and high performance liquid chromatography (HPLC).

Classical LC uses long column approximately 50x 2cm packed with stationary phase of large particles size (50-250 ㎛ m 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. Liquid chromatography techniques can be used for the determination of ionic species as well as other compounds [6,7].

However, HPLC uses high pressure to force solvent through closed columns containing very fine particles stationary phase that give high-resolution separations. The HPLC systems consist of a solvent delivery system, a sample injection valve, a column, a detector, and read out 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 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 3-10㎛ m 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 [8]. 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 a related to the relative retention of the solute (t_R) compared with the width of its peak (W).

$$N=16 (t_R/w)^2 \text{ ---------- (1-1)}$$

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 [(9)]. 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 [(10)].

Resolution (R_s) is the efficiency of a chromatographic system; it defines the degree of separation between two peaks [(9)]:

$$R_s = ½ (t_{R2}-t_{R1}) / (W_2-W_1) \text{ ---------- (1-3)}$$

Where t_{R2} and t_{R1} are the retention times of peaks 1 and 2, $W_1$and W_2 are the widths of the peaks at the base line, respectively. The larger resolution is better the separation.

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 further proper control of the composition of mobile phase can lead to better separation and high column efficiency. Other advantages of liquid chromatography methods that many detectors used in HPLC are non-destructive, thus facilitating sample recovery and providing the opportunity for subsequent spectro-analytical and other studies [(11)].

HPLC has been used for analysis of wide variety of pharmaceutical products, body fluid samples, and environmental sample [(12)].

1.3 ***Normal phase chromatography:***

Normal phase is used in the conventional sense to mean system in which the stationary phase is more polar than the mobile phase [(10)]. The stationary phases in normal phase chromatography are mostly an inorganic polymer that have a large number of pores of molecular size (several nm to tens of nm), makes their surface areas large. The two most common materials are hydrated silicon-oxygen (silica or silica gel), and hydrated aluminum-oxygen polymers (alumina) [(9)].

The effectiveness of separation depends upon adsorbent, which should have large surface areas of uniform size. Bonded phase appear to be slowly replacing traditional solid silica and alumina in normal-phase LC, although silica and alumina still find wide spread use[10].

1.4 ***Reversed phase chromatography:***

In reversed phase chromatography, the stationary phase is less polar than the mobile phase. 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 $-C_{18}H_{37}$. 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 used for reversed phase chromatography is composed of organic polymer beads. A typical polymer is a resin composed of polystyrene and divinyl benzene.

Reversed phase is quite popular since the peaks in reversed phase separation tend to be narrow and symmetrical and the adsorption/desorption equilibrium reaction tend to be fast [9]. Separation in reversed phase chromatography is due to the different binding properties of the solute present in the sample as result of the different in their hydrophobic properties. Manipulating the hydrophobic properties of the mobile phase can control the degree of solute molecules binding to the reversed phase medium. The separation of solutes that vary only slightly on their hydrophobic properties is readily achieved [13].

1.5 *Modes of LC:*

The modes of liquid chromatography 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 adsorptions, partition, ion exchange, and other types of chromatography.

1.5. I Adsorption chromatography:

Adsorption of liquid solid chromatography (LSC) is the original form (the normal phase mode) of LC first introduced by Tswett at the beginning of twentieth century [(14)]. A solid stationary phase and a liquid or gaseous mobile phase are used, solute is adsorbed on the surface of the solid particles, the more strongly solute is adsorbed; the slower it travels through the column [(8)].

LSC is principally carried out using polar stationary phase such as silica or alumina. It involves no partition of the sample solute in the stationary phase [(15)]. Instead of the polar group of each solute interacts through primarily hydrogen bonding forces with the polar sites of the stationary phase. Therefore, careful adjustment of polarity of the mobile phase for selective competition on the polar sites is needed for reproducible separation [(16)].

1.5. II *Partition chromatography:*

A high-boiling liquid stationary phase is bonded to a solid surface[(8)]. The interaction and separation in partition chromatography (reversed phase mode) are based on non-polar stationary phase.

The retention of an analyte depend 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[17]. The liquid stationary phase bonded chemically to an inert solid surface, the main advantage of this bonded stationary phase is its quite stability, which cannot be easily removed or lost during use [18].

1.5. III *Ion exchange chromatography:-*

Ion exchange chromatography (IEC) was the first of various LC methods used widely under modern LC conditions [13]. The Ion exchange chromatography used for the separation of ionic species includes ion interaction, ion exclusion, and chelating chromatography, in addition to reversed-phase separation of metal complexes [19].

Ion exchange chromatography is carried out with packing that possess charge bearing functional groups, the most common retention mechanism in simple ion exchange of sample ions X and mobile phase ions Y with charged group R of stationary phase:-

$$X^- + R^+Y^- \longleftrightarrow Y^- + R^+X^- \ldots\ldots\ldots\ldots \text{(Anion exchange) ---- } [a_1]$$

$$X^+ + R^-Y^+ \longleftrightarrow Y^+ + R^-X^+ \ldots\ldots\ldots\ldots \text{(Cation exchange) ---- } [a_2]$$

For anion exchange chromatography, as shown in {eq. (a_1)}, the sample ion X^- is in competition with the mobile phase ion Y^- for the ionic sites R^+ of the ion exchanger. In cation exchange chromatography {eq. (a_2)}, sample cations X^+ compete with the mobile phase ions Y^+ for the ionic sites R^- of the ion exchanger.

Sample ions that interact weakly with the ion exchanger (in the presence of competing mobile phase ions) will be weakly retained on the column and elute early in the chromatogram. Samples ions that interact strongly with the ion exchanger will be retained more strongly and elute later [13]. The types of functional groups of the anion-cation exchange chromatography (ion chromatography) shown in Table (1- 2):-

Table (1-2): Types of Functional Groups in Ion –Exchange Chromatography

CATIONIC EXCHANGERS	*ANIONIC EXCHANGERS*
Sulfonic acid $-SO_3^-H^+$	***Quaternary amine-*** $N(CH_3)_3^+OH^-$
Carboxylic Acid $-COO^-H^+$	***Quaternary amine-*** $N(CH_3)_2(ETOH)^+OH^-$
Phosphoric acid $PO_3^-H^+$	***Tertiary Amine –*** $NH(CH_3)_2^+OH^-$
Phosphoric acid $HPO_2^-H^+$	***Secondary Amine–*** $NH_2(CH_3)_2^+OH^-$
Phenolic $-O^-H^+$	***Primary amine*** $-NH_3^+OH^-$

Many Ion exchangers for LC consist of a polymeric stationary phase with ionic functional groups (e.g. $-SO_3^-$ groups for cation exchangers and $-N(CH_3)_3^+$ groups for anion exchangers) [20].

In the stationary phase, the ions are immobilized and it travel 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 optimized by many parameters including type of functional group of the stationary phase, and concentration and characteristics of eluent ions [(21)].The first two parameters determined by the design of the ion exchange column and usually optimized for anions, or cations. The other parameters adjusted by the analysis.

The variable number of functional group sites in the stationary phase known as the capacity and it usually expressed as the number of equivalents per column or equivalents per weight 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" [(22)] 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 treated similarly using an appropriate counter ion to liberate the OH^- or Cl^-, which then titrated with standard acid or Ag^+, respectively [(20)]. 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[(6)].Ion exchange chromatography is quite flexible, in that various other mechanisms of separation invoked, although these used frequently in modern LC. The technique being used for the analyses of samples of biochemical interest, for the measurements of the additives such as vitamins and preservatives in foods and beverages, for various active ingredients in medical formulation, for drugs and their metabolites in serum and urine, for residue analyses in food raw materials, and for many other separation problems [(13)].

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 [(13)].

Modern ion exchange chromatography began with a report by Small et al. [(21)] 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 cations including sodium, ammonium, potassium and calcium[(22)]. The key element was their development of a device later known as suppressor column to lower the background conductometric signal resulting from the ionic mobile phase while enhancing the conductometric signal from analyte ions [(23)].

The selectivity of an IC separation altered by changing the stationary phase; however, this approach is expensive. Changing the mobile phase composition is much easier[(24)]. A drawback to this approach is that a charge in a type and concentration of the eluent will not alter the separation selectivity, if the analyte have the same charge as the eluent ion [(25,26)] and it is difficult to optimize the selectivity when utilizing mutable compounds. The IC selectivity also influenced by temperature [(27)].

1.6. Detection system:

The commonly used detectors for HPLC are UV-Visible, Refractive index (RI), Fluorescence, Conductivity, and electrochemical as well as other hyphenated detectors. However, the detectors used for the detection of ionic species are Conductivity, UV-Visible detectors.

1.6. I Conductivity detection:

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 [28-29]. This system consists of suppressed.

The suppressed conductivity detection is based on the use of a two column 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 also as being packed with small polymer beads, these beads carry acidic protons (H^+) on their surface of polymer [30] as shown in Fig.(1-1). The suppressor column, 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.

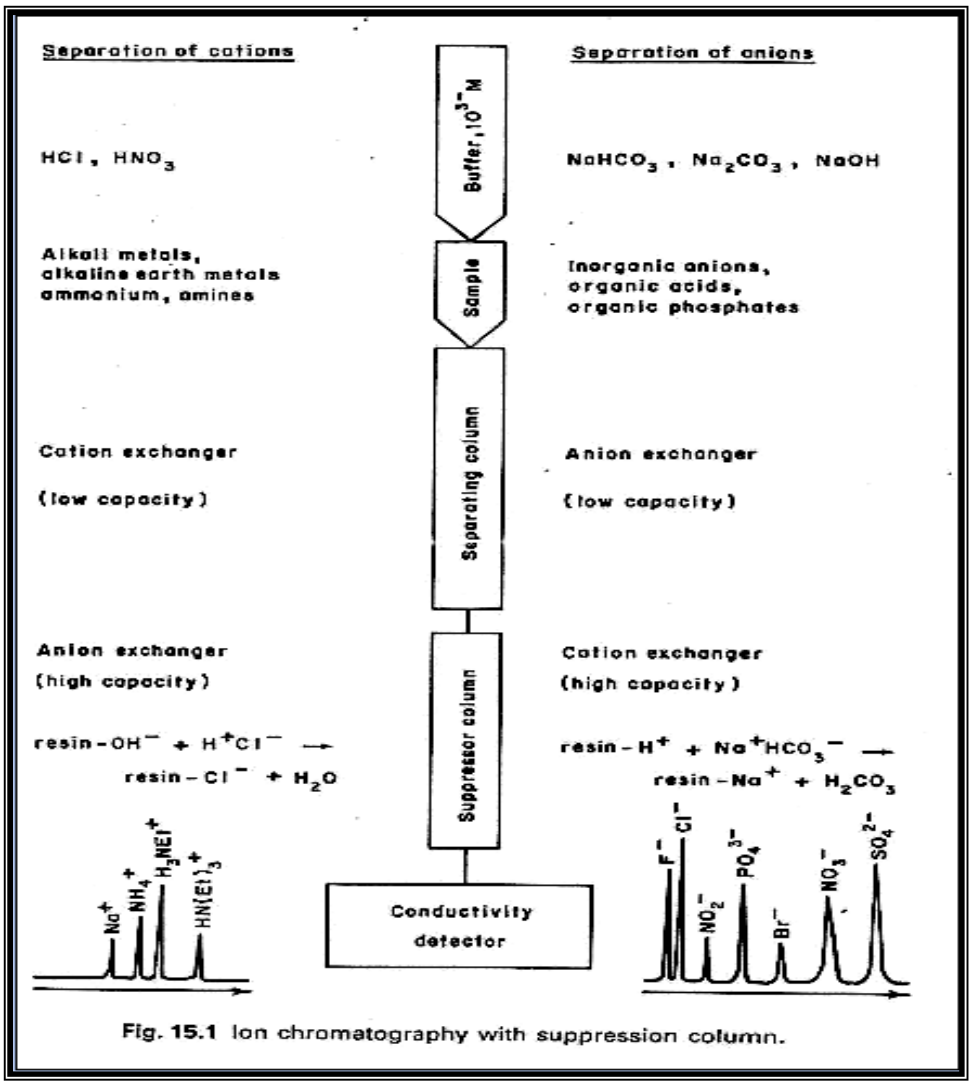


Fig. (1-1): Ion Chromatography with Suppression Column.

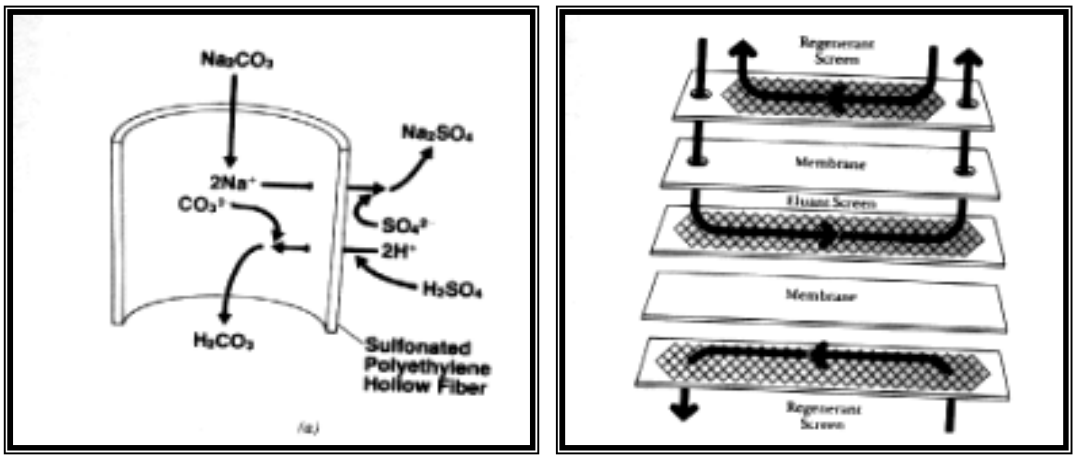
The use of a suppressor column is not without problems-eventually the resin becomes exhausted and needs to regenerated, which is inconvenient. Also, the slightly ionized carbonic acid produces a small continuous base line conductance signal, so that the vacancy peak from the sample injection is detected 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 hallow fiber.

B. a sandwich.

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



(A) **(B)**

Fig. (1-2): Design of Suppressor Column for Ion Chromatography: (A) Hallow Fiber, (B) Membrane Sandwich.

The first problem has been involved using continuous (membrane sandwich), flowing suppressor streams that contact the chromatographic stream through a porous membrane. The hallow-fiber ion exchange tubing packed with plastic beads to decrease the internal volume and zone spreading has been used [13, 31]. 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, was passed through a cation ion- exchange 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 [17].

$$NaOH + H^+R^- \longrightarrow HOH + R\text{-}Na^+ \quad \text{(eluent)}$$

$$NaCl + H^+R^- \longrightarrow H^+ + Cl^- + R\text{-}Na^+$$

$$NaNO_3 + H^+R^- \longrightarrow H^+ + NO_3^- + R\text{-}Na^+$$

If the solutions of sodium carbonate or bicarbonate used as the eluent, they converted in to the weakly ionized carbonic acid and the conductivity 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 removed from the solution [30].

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

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

In unsuppressed IC, the second column is not used [17]. Detection is not 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 normally not considered an indirect detector even though it dose function in that mode in single-column IC. Sensitivity transfer in the indirect mode has been demonstrated with good results [32-33].

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 hydroxide [34, 35] and salicylate [34, 36] as eluent in anion chromatography. The former has a large equivalent conductivity and the latter has a strong eluting ability, which leads to a low level of background conductivity.

Gjerde et al. is the first used direct conductivity detection in (1979) [37]. 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 found necessary to ensure reproducible chromatographic performances [37].

Gjerde and Fritz [33, 38] found that the sensitivity of conductivity detection increased when weak acid eluent, such as benzoic acid, used in anion chromatography. This effect 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 produced depending on the equivalent conductance of the analyte samples and the pH of the mobile phase, which determined the ionic form of the analyte [39, 40]. 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 [41, 42].

1.6. II UV-Visible Detection:

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 [43].

Many inorganic cations and anions do not have significant absorption in UV-Visible range of the spectrum; therefore, direct detection cannot be used. However, there are cases where the ions detected directly by their UV detection in the (185-220) nm range [18].

Cochrane and Hillman [44] have reported the separation of nitrate and nitrite as examples of ions that detected 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 [44].

The UV/VIS detector may also used in an indirect mode. Small and Miller [45] reported this approach, as a detection technique for ionic species. Indirect photometric detection (IPD), in which transparent sample ions eluted with a light-absorbing ion.

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 separation them [46].

The eluent in (IPD) should possess several characteristic such as ion exchange capability, ion-exchange selectivity and a large molar absorptivity [45].The most common (IPD) eluents used are benzoate, phthalate, sulfobenzoate, and salicylate [47, 48]. These eluents have showed good separation profiles for many analyte anions with reasonably low detection limits [49, 50]. However, the elutions of analyte ions will 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 found to be important to ensure reproducible elution and retention times [46, 51]. In this technique, the ion exchange column first equilibrated with the light absorbing eluent preferably at a very low concentration. The resultant high background absorbance signal offset electronically. A decrease in the background signal observed as the non-UV absorbing analyte ion eluted from the column [47, 48]. Negative analyte peaks obtained instead of the conventional positive peaks, as shown Fig. (1-3):

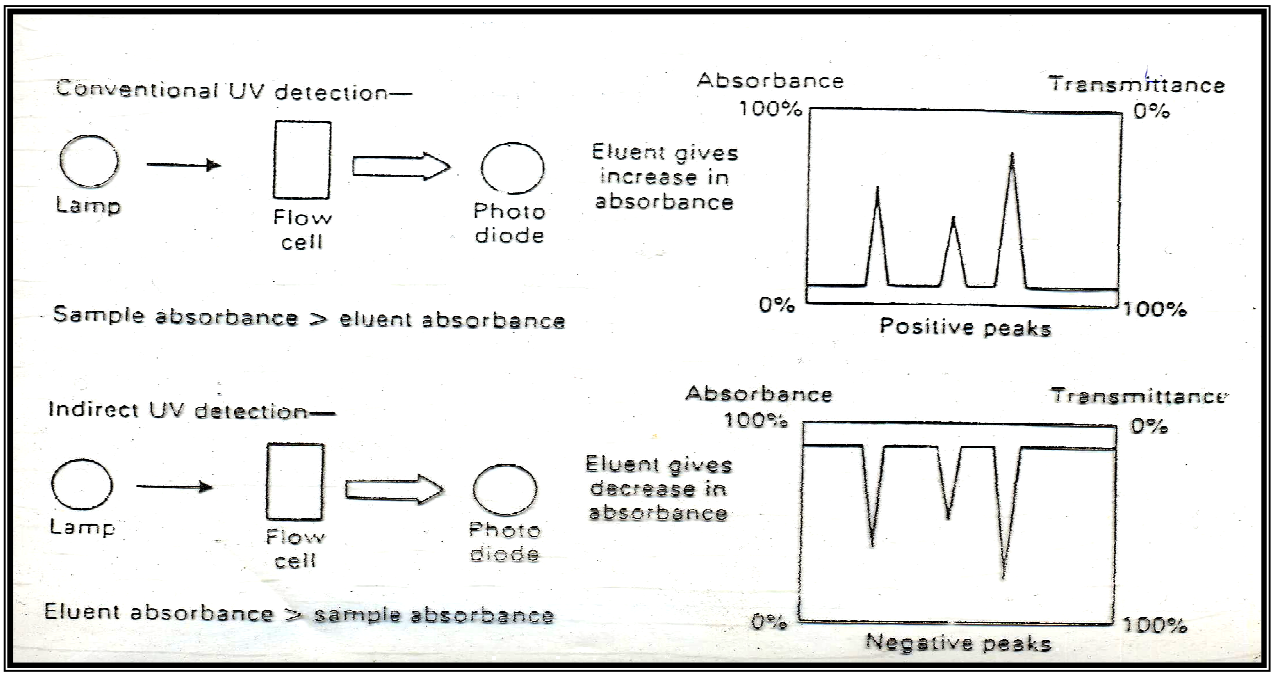


Fig.(1-3): Comparison between Conventional and Indirect Photometric Chromatography

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:

$$S=C_s\epsilon_s+ (C_E-C_s)\ \epsilon_E-C_E\epsilon_E=Cs\ (\epsilon s - \epsilon_E) \text{ ---------- (1-5)}$$

Where Cs and C_E concentrations of the sample and eluent ions, respectively[48]. ϵ_s and ϵ_E are the molar absorptivities for the sample and eluent ions, respectively. Since the molar absorptivity of the sample ions assumed zero, eq. (1-5) becomes:

$$S \ \alpha\ Cs\ \epsilon_E \text{ ---------- (1-6)}$$

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 consider as well. The base-line noise related to the background signal, which controlled by the eluent concentration:

$$\text{Noise (N)}\ \alpha\ C_E \text{ ---------- (1-7)}$$

It follows from equations (1-6) and (1-7) that the signal-to-noise ratio (S/N) is equal to:

$$S/N \ \alpha\ C_s\epsilon_E / \ C_E \text{ ---------- (1-8)}$$

This relationship shows that a small signal can seen using a very dilute eluent with large molar absorptivity [47, 48].

The signal –to noise ratio (S/N) 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 [46,52].

Maki and Danielson [53,54] had 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^-, SO_4^{-2}, SCN^-, and I^- in less than 18 min. with detection limit 0.4-1ng for all anions. Changing the mobile phase to naphthalendisulfonate 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 commercial anion exchange column has been reported recently [55].These three eluents required no pH adjustment with detection limit for example for chloride 0.04ng and 0.1ng with conductivity and indirect photometry respectively [43].

1.7 Aim of the work:

Aim of this work is to analysis of a wide variety of anions ranging from small singly charged ones to large and multicharge type by indirect photometric detection (IPD) and compared with unsuppressed conductivity detection using phthalate 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.