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Determination of Some Nutrients by Home-made Siemi-automated Ion Chromatography System

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Abstract: ***This work demonstrates the application of semi-automated home-made IC system for determination of NO2-, NO2- and PO43- ions with simple, eco and simultaneous method in water , soft drink and pharmaceutical preparations. Linearity's were in the range 2.5 – 12.5 , 2.5 – 12.5 and 7.0 – 35 mg/L with regression coefficient 0.9950,0.9960 and 0.9990 , the ± RSD % equal to ± 0.45%, ± 0.56% and ± 0.51 %,the recoveries were the range 100% , 96% and 95% which were found by standard additions method , the sample throughput were 8.0, 6.0 and 3.0 sample/hour for NO2-,NO2- and PO43- ions respectively.***

Keywords: Semi-automated, IC system, Ion Chromatography

1. Introduction

The ideal method for analyzing anions,cations and polar substances is ion chromatography (IC) which is one member of large family of chromatography methods (1). It is a powerful analytical tool and a whole variety of ions in the sample can be analyzed in a single determination throughout a wide concentration range (2). IC methods is easy and helps to save time and reduce costs (3).

The pollution with nutrient has become major environment problem. The reasons for this have been markedly increased use of commercial cleaning preparation with phosphate are widely used as a detergent (4). nutrient, also, in aquatic systems may originate from natural sources such as the mineralization of algae, the dissolution of minerals, from anthropogenic point source discharges of sewage and industrial effluents and diffuse inputs from grazing and agricultural land (5).

Soft drinks are complex mixtures containing a variety of substances such as colouring compounds, flavouring agents, acidifiers, sweeteners, preservatives, and caffeine. The most common acidifier used in soft drinks is phosphoric which gives a tangy taste in the mouth (6). Phosphoric acid can also act as a preservative, keeping the contents of the bottle fresh (7).

Half of all drug molecules used in medicinal therapy are administered as salts (8- 9). So a number of counter-ions such as nitrate and phosphate, usually analyzed by variouschromatographic methods (10).

Salt formation is important in pharmaceutical and biopharmaceutical drug development to improve physicochemical properties of the drug. Approximately half of all drugs are formulated and administered as salts (11).. The different ions are used to alter the biological and physicochemical properties such as solubility, stability and dissolution rateIon chromatography (IC) with conductivity detection one of a variety of techniques used forcounter ion determination (12).

So, the main aim of this study to focus on using a home – made semi-automatedsuppressed IC system with conductivity detector for determination of nitrite, nitrate and phosphate ion in water, soft drink and pharmaceutical drugs.

2. Sampling and Storage

All different water samples were collected in clean polyethylene vessels. Soft drinks and pharmaceutical preparations were purchased from local markets. Analysis should be carried out as soon as possible after collection of the samples. If storage is necessary two drops of chloroform were added to the filtered sample before storage in freezing (13)

3. Experimental

All reagents used in this study were analytical grade unless otherwise stated which was previously described in details (14). During the analytical application with the home-made system, all stock and standard solutions were prepared in ultra-pure deionized water (18.2Ω-cm) resistivity at 25° C.To insure a very high purity of water this deionized water passes through a home-made unit containing four vessels cations,anions, mixed-bed resins and charcoals respectively (14) .

All deionizedwater, samples and solutions were passed through 0.45 µm membrane filter (Millipore –USA) before chromatographic analysis to avoid plugging the columns and to prevent damage of the flow system and the detector(15).

Also, during analytical application the mean of triplicates of symmetrical peaks heightcan be related to the sample concentration(16)

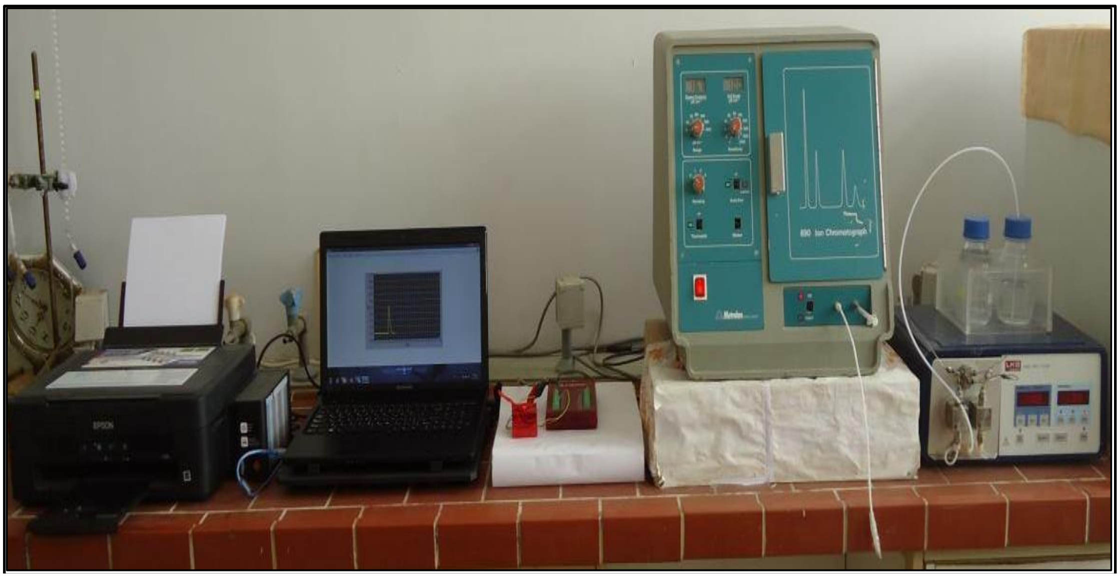


Figure 1: Home-made semi-automated IC system.

4. Instrumentation

All chromatographic measurements werecarry out by using a home-made semi-automated IC computerized system as shown in Figure 1, which was previously described in details (14).

All standards and samples were determined by using a semi-automated home-made IC suppressed conductivity computerized system, as shown in figure (1). The constructing system, which is adapted to determine anions and keep to be as simple as possible.

In order to maintain a stable pulse free and easily to control the eluent flow rate LKB pump was used to deliver the carrier stream with 1 ml min^{-}. The sample injection was carried out through a microinjection valve built-in (Metrohm 690 unit) with a fixed sample loop of 100 µL. The sample was pumping through the Dionex pacAS11-HC column, 250 x 4 mm, 5 µL. The separated ions were measured using Metrohm conductivity meter equipped with 1.5 µL platinized flow cell. The peaks height was recorded through data logger, personal computer and printer which ca n be related to the concentrations of the injected standards and samples.

5. Procedure

Under a temperature of 25 ^{o}C all chromatography experimentswere carried out by a home-made IC system, which consisting LKB pump C2150-HPLC pumping the eluent at 1ml/min. Samples or standards were injected with Metrohm electronic injection valve fitted with 100 µl loop in eluent of 0.1 mMNaOH .Dionex pacAS11-HC column , 250 x 4 mm , 5 µL was used as a separation column(17). A 15 cm x 1.5mm home-made suppressed column filled with Purolite with sulfonic group(H^+)and amber spherical beads (18) .Metrohm conductivity meter equipped with 1.5 µL platinized flow cell was used to measure the conductivity of the separated species. A data logger lab Jack-Ocean control/ Australia. Personal computer and printer were handling the data of the home made system. The peak height of a symmetrical peak is corresponding to standard and sample concentrations

6. Result and Discussion

1) Effect of Column type, elunt Concentration and Retention Time of separation ions

Ion Pac AS-11-HC analytical column, 5 µm 4 ×250 mm column was recommended as a suitable and efficient separation column for anions with 3 x 150 mm home-made suppressed column. Anions can be detect by using conductivity detector with eluntof 0.1mMNaOH solution, which can be freshly prepared. The Retention times (Rt) of the three anions were determined to each ion by obtained separate chromatogram for each ion independently and then mixed with other ions. Figure (2) shows NO_2 ion as example for this method which in spite of time consuming, but gives precisely R_t(Table1).

Figure 2D shows that the column has high efficiency to separated the three ions and the R_t ranged between 10-26 minutes for three ions shown in Chromatograms (Fig.2) and Sequence of three ions appearance.

2) Effected Column Temperature on the separation

The home- made IC system supply with Column temperature evaluating in the range 25-45^{o}C in five degree steps. As expected, increasing the column temperature decreased retention time and led to good baseline for the separation Chromatogram of the standards and samples. But due to difficulties of maintaining temperature stability in the constructed home- made IC system. So 25 ^{o}C was selected to be used in future work (19).

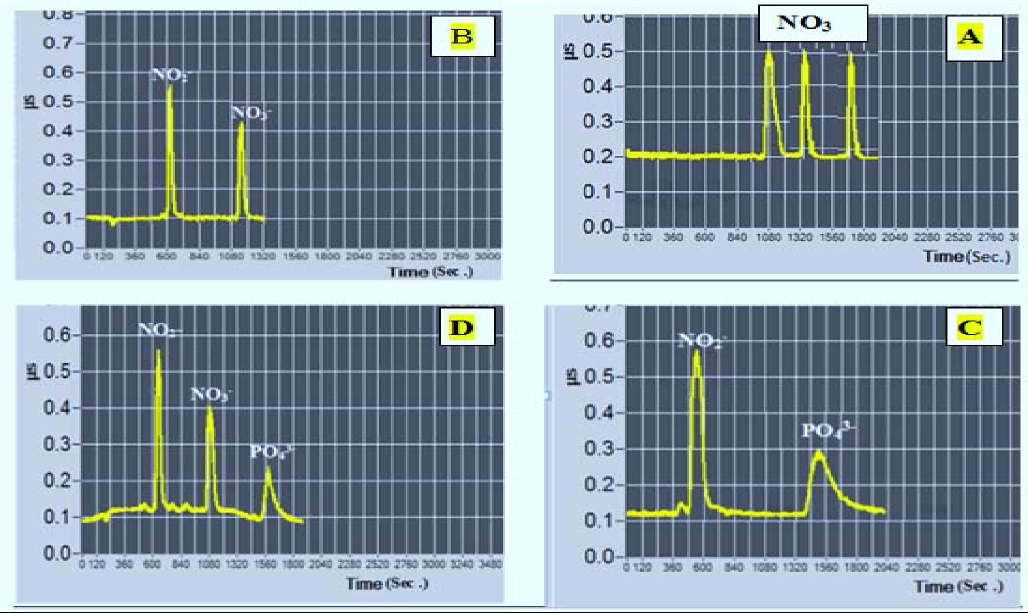


Fig 2. Determination of R_t of NO_3 and peak height A) NO_2 only B) NO_2 and NO_3 C) NO_2 and PO_4 D) NO_2 , NO_3 and PO_4

Table 1: Retention Time and Peak Height for NO_2^- , NO_3^- and PO_4^{-3} ions

Anion	Concentration ($\mu g\ ml^{-1}$)	Main t_R * (min)	± RSD%	Main Peak Height(mm)*	RSD%±
NO_2^-	**7.5**	**10**	**0.221**	**44**	**0.535**
NO_3^-	**7.5**	**20**	**0.190**	**30**	**0.502**
PO_4^{3-}	**21**	**26**	**0.231**	**19**	**0.505**

* Triplicates

3) Method performance (linearity, Reproducibility and Detection Limits):

Under the established conditions listed in Table 2, a method of the standard calibration was used to obtain the calibrations curve for NO_2^-ion, by plotting the concentration versus the peak height of asymmetrical peaks (Fig.3 and Table 3). Linearity was in the range 2.5 – 12.5 μg $ml^{-1}$for NO_2^- ion and a same manner was used for NO_3^- and PO_4^- ions which were in the range of 2.5-12.5 and 7.0 -35 μg ml^{-1}respectively (Table 2) .

Table 2: The optimum conditions for separation and determination of NO_2^- , NO_3^- and PO_4^{-3} ions

Parameters	Conditions
Separate Column	Ion Pac AS-11-HC Analytical Column; 5μm , 4×250 mm Dionex(P/N 052960)
Suppress column	15 cm × 1.5 mm
Detection System	Suppressed Conductivity detection
Isocratic Mobil phase	0.10mM NaOH
Test sample	Anions diluted in D.I Water 18.2Ω cm^{-1}
Back ground Conductivity	-0.3 μs cm^{-1}0.1
Flow Rate	1.0 mL min^{-1}
Temperature	25 °C
Pressure Background	190-200 Bar
Run Time	30 min
Injection Volume	100 µl

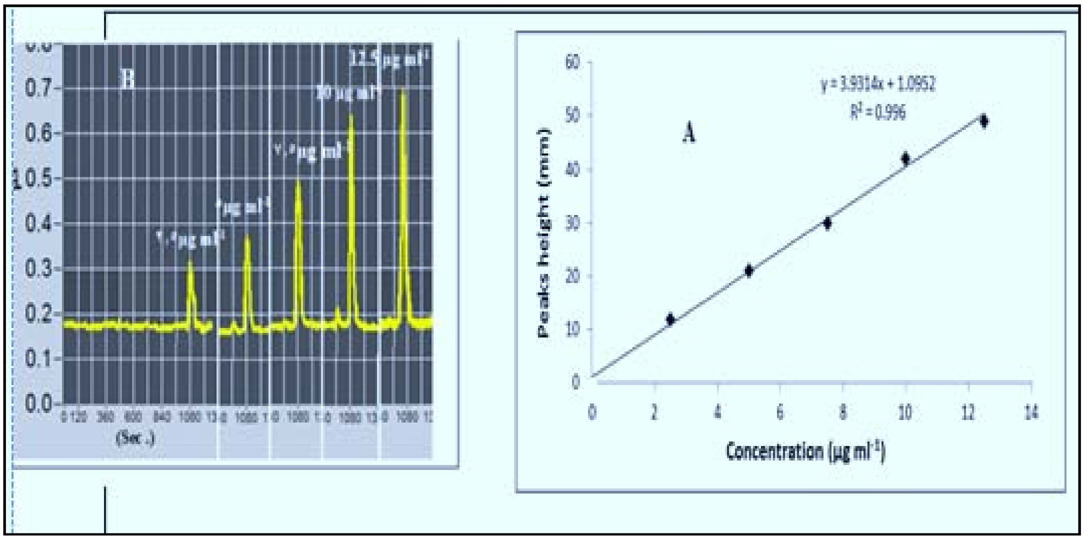


Figure 3: (A) Standard calibration graph of NO_2^- ion, (B) corresponding peaks

Table 3: Concentration of anions and corresponding peaks height

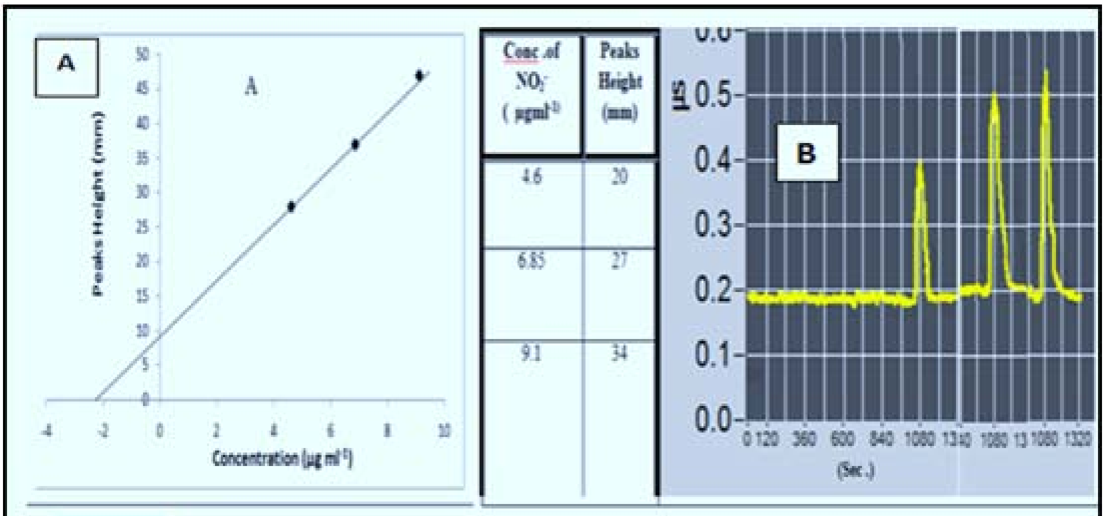
Peaks Height (mm)					Anions Standard (μg ml^{-1})					Anions
Levels						Levels				
5	**4**	**3**	**2**	**1**	**5**	**4**	**3**	**2**	**1**	
70	**58**	**44**	**28**	**13**	**12.5**	**10**	**7.5**	**5**	2.5	NO_2^-
49	**42**	**30**	**21**	-11 10	**12.5**	**10**	**7.5**	**5**	**2.5**	NO_3^-
32	**28**	**19**	**13**	**7**	**35**	**28**	**21**	**14**	**7**	PO_4^{3-}

Table 4 and Table 5 list regression statistics and numerical criteria of home-made IC system constructed for separation and determination of**NO_2^- , NO_3^-** and **PO_4^{-3}**ions. The R^2 were in the range of 0.9990 -0.9950 for calibration curves which clearly indicated an excellent value for the three ions. The values listed in table 4 and table 6 especially separation efficiency of the used column, peaks symmetry and resolution. Also clearly indicated that the conductivity IC system can be used to separated completely and determination of the three ions without any overlapping between the three peaks.

The values of essential statistical parameters such as (Ef-N, AS,Tr,α,Rs ,LLOQ,LLOD,DDT and MDL) which refer to successful and good extent of the proposed method for determination and separation of these ions by computerized home-made IC system (20).

Accuracy

To evaluate the accuracy of the home-made IC System. A recovery experiments were performed on three representative standards for the three ions. Standard additions method (Fig 4) was used for all of these determinations in order to avoid all the possible interferences (21). Table 4 summarized all of these studies. A good agreement between the results was obtained which clearly indicated that home-made system can be used for several applications.



Conc .of NO_2^- (μgml^{-1})	Peaks Height (mm)
4.6	20
6.85	27
9.1	34

Fig **4 A** **Calibration graph for NO_2^- by standard addition Method ,**
B. corresponding peaks.

Table 4: Ions recovery obtained by home-made IC and other method

Ions	Taken Conc. μg ml-^{1}	Found Conc. μg ml^{-1}	Recovery % RSD± %	Determination by other methods [9,10]	
				Found	Recovery % RSD± %
NO_2^-	7.5	7.5	± 100 **0.45**	7.4	**98.6 ± 0.54**
NO_3^-	7.5	7.2	± 96.0 **0.56**	**7.2**	**0.50** ± 96.0
PO_4^{3-}	21	20	± 95.2 **0.51**	**20**	**0.49** ± 95.2

Precision

Precision of method, reported as % RSD, was estimated by measuring repeatability (intra-day assay) for five replicate injections for representative concentrations of NO_2,NO_3 and PO_4 ions .The intermediate precision (inter-day variation) were also studied for two days using an intermediate concentration solution of these three ions . Table 5 Summarizes all of these studies. The average recoveries were in the range (95.2 - 100) which thought to be an acceptable result (22).

Table 5: Intra and inter-day precision and accuracy of standard analysts (n=5).

		Intra-day		Inter-day	
Ions	Taken conc. (μg mL^{-1})	Found (μg mL^{-1})	Recovery% ± RSD%	Found (μg mL^{-1})	Recovery % ± RSD%
NO_2^-	**7.5**	**7.5**	**100**± 0.45	**7.5**	**100** ± 0.48
NO_3^-	**7.5**	**7.2**	**96.0** ± 0.56	**7.3**	**97.3**± 0.52
PO_4^{3-}	21	**20**	**95.2**± 0.51	**20**	**95.2** ± 0.51

Application

The a home-made semi-automated IC system was applied successfully to separated and determination of **NO_2^-**,**NO_3^-** and **PO_4^-** in different types of samples .Table 6 lists some of these result . figure shows the chromatogram of the

Separation and determination of NO_2^- ,NO_3^- and PO_4^- , the peak resolution was quite satisfactory and easily to identify the separated anions from their retention times.

Table 6: Determination of NO_2^-,NO_3^- and PO_4^- in water , soft drink and pharmaceutical preparations by home-made IC system

Sample	Concentration µg ml^{-1}		
	NO_2^-	NO_3^-	PO_4^{3-}
Swage water of Al-Najabia power station	2.8	**5**	18.7
Shatt Al-Arab ,Grmat Ali	2.3	4.5	2.8
Pepise US Product	-----	-----	17
Baghdad soft drink	-----	-----	25
Calvitalis Syrup	------	-----	200
ISO Sorbidedinitratetablets	-------	40	-------

7. Conclusion

This work described a home- made semi –automated computerize suppressed ion chromatography IC System equipped with conductivity detector NO_2, NO_3 and PO_4 determination water, soft drink and pharmaceutical preparation. This developed method offers simple, inexpensive and needs only a very small volume of the sample. In spite of lack of sensitivity in this applications there is no need for high sensitivity since the pharmaceutical preparations and soft drink have a very high concentration.

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