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Determination of Calcium by new sequential injection unit using a chemical dye

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Abstract : This study includes the design sequential injection system for determining Calcium ion containing valve is designed locally and this Method is quick, highly accurate and cheap. The SIA system based on the reaction of Calcium with Eriochrome Black T at pH (9.5-11.5)with an absorption maximum at 542 nm. Various parameters (physical and chemical) affecting the determination have been investigated such as flow rate, reaction coil length, volume of reagent (Eriochrome Black T), volume of sample, concentration of Eriochrome Black T. The calibration curve was prepared and the dispersion coefficient, repeatability, interferences and application were studied. The linear range was (0.05-10 mg/L) at sampling rate of 125 sample per hour, the detection limit (0.0084 mg/L) . Relative standard deviation for (10 mg/L), n = 3 were found (0.0545%). Dispersion coefficient was also measured for the method.
Keywords : Sequential Injection, Eriochrome Black T, Calcium.

Introduction

Sequential injection analysis(SIA) the third generation of flow injection analysis[1], it was born in 1989, at laboratories of the center for process analytical chemistry (CPAC) at the university of Washington[2].this method removes the problems happen on in classical on-line column pre-concentration systems, improves the overall operational efficiency and yields the strength necessary for routine assays and analyze a large number of samples in an accurate, reproducible and quick way[1,3]. SIA is becoming an important tool for the automation of chemical procedures[3]

Calcium is the fifth most abundant element in earth's crust and is always found in ionic form in compounds[4]. Calcium is one of 21 elements known to be essential to humans and its very important component of healthy diet and a mineral necessary for life. Its requirements as adequate intakes (AI) rather than as recommended dietary allowances (RDA)[5].the average adult body contains approximately 25000mmol [1kg], It plays a key role in skeletal mineralization[6].Calcium has many important uses such as exocytosis, neuropterans miller release and muscle contraction[7].Calciumdeficiency cause osteoporosis[8], poor dental and poor blood clotting but over- retention from Calcium can cause hyperkalemia impaired kidney function[9] and calcium deficiency due to lead exposure in children can cause central nervous system disorders[10].

There are several sources of calcium most important Omega-3 fatty acids such as EPA help increase levels of calcium in the body, deposit calcium in the bones ,and improve bone strength[11].

Calcium requirement is dependent on the state of calcium metabolism, which is regulated by three main mechanisms: intestinal absorption, renal reabsorption, and bone turnover. These in turn are regulated by a set of

interacting hormones, including parathyroid hormone (PTH), ionized calcium itself, and their corresponding receptors in the gut, kidney, and bone[9].

Calcium, iron, magnesium and zinc are essential micronutrients necessary for the growth and development. The disturbances in the micronutrient status will lead to the development of periodontitis and diabetic complications[12].

Eriochrome Black T (EBT) was a reddish-brown powder is dissolved in hot water or slightly soluble in ethanol and acetone, and is used in revealing interactions formation complexes[13],the molecular weight (439.381 g/mole) and partial formula is ($C_{20}H_{12}N_3O_7SNa$), and a scientific name: [Sodium salt of 1- (1-hydroxyl -2-naphthylazo)-5- nitro-2- naphthol-4- sulfonic acid][14].Use Eriochrome Black T to determination many items such as manganese, lead, cadmium, copper, indium, Gallium, zinc, aluminum and cobalt[15]andituseas indicator[16].

Calcium determination by a number of ways, including using the method of flow analysis system based on fluorescence microdetectors[17],using catalase enzyme electrode[18],usingmicrowave induced plasma-atomic emission spectrometry[19],usingautomated spectrophotometric[20],usingPlastrumtestudinis by HPLC–ELSD[21], using laser-induced breakdown spectroscopy[22]andUltra-sensitive Flow Injection Analysis[23].

Experimental

Apparatus

Spectrophotometer Labomed.in G single beam, USA, and a spectrophotometer Shimadzu UV-1700 spectrophotometer, Analytical balance sensitive Denver Instrument, Recorder Pen Siemens C 1032, Hitter thermal Ardeas 51, peristaltic pump Germany, Ismatic, files Interaction with the radius of 0.5 mm, homemade valves, pipes load of Teflon, flow cell volume of 450 µL, pH meter.

Chemicals [24]

(1) The standard stock of 1000 mg/L Calcium prepared by dissolving 3.6767gof calcium chloride dehydrate $CaCl_2.2H_2O$, in water and dilute the solution with distilled water to 1 liter.
(2) 0.2% of Eriochrome Black T were prepared by dissolving 0.2 g of Eriochrome Black T in 100 ml of Methanol in volumetric flask .
(3) Buffer solution of prepared pH(9.5-11.5), dissolve 60 g of ammonium chloride NH_4Cl in water , add 120 ml of conc. ammonia solution, and dilute the solution with water to 1 liter.

Results and Discussion

Determination of the wavelength for maximum absorption

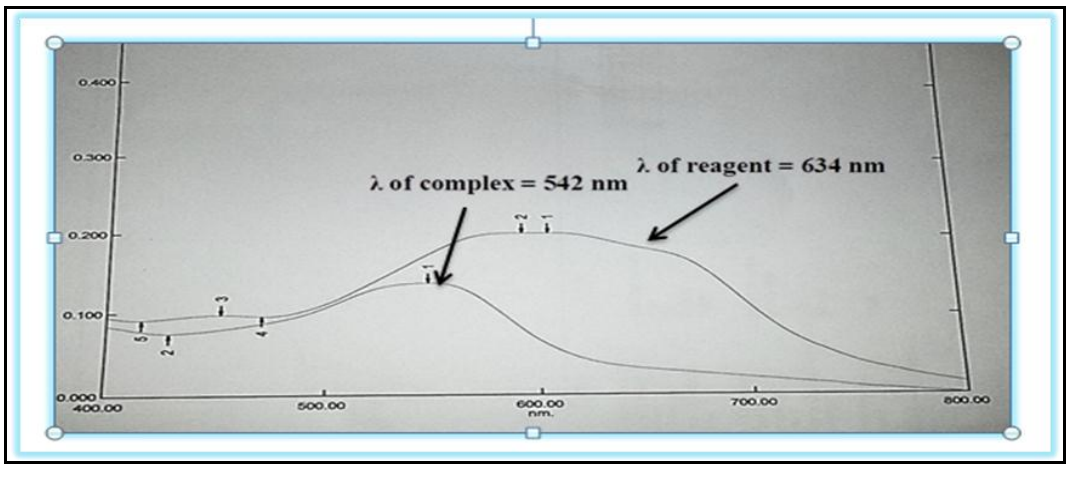

Fig. 1: UV-Vis spectroscopy for Calcium complex with EBT.

Ultraviolet visible spectroscopy was used to determine the optimum conditions for the complex formation and the result are shown in Fig. 1. The λ_{max} of complex was**542** nm[24].

Design of SIA Units

This research included design of a new valve from cheap and obtainable materials, this valve consists of four secondary valves, each secondary valve has three aperturesand These valves have the lever control of the direction in which the chemical reaction, as shown in Fig. 2 and the work of the new valve in a two steps, areLoading step and injection step and shown in Fig.3. The new valve is inserted with the pump and UV-visible detector and signal recorder for the design of a new system sequential injection to estimate the calcium ion and Fig.4illustrate the new designs of the system.

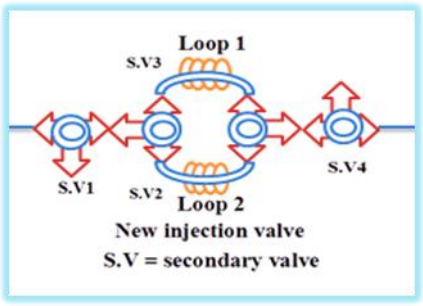

Fig. 2: New injection valve

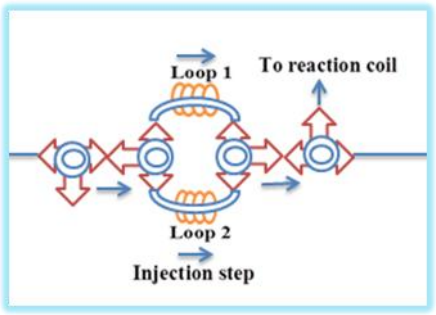

Fig.4:injection stage

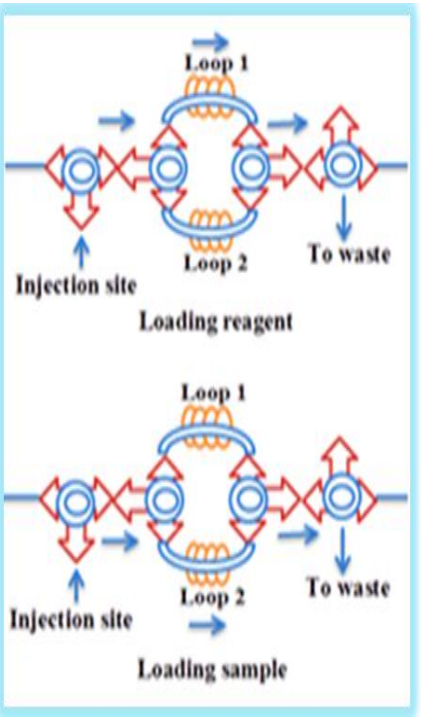

Fig.3:Loading stage

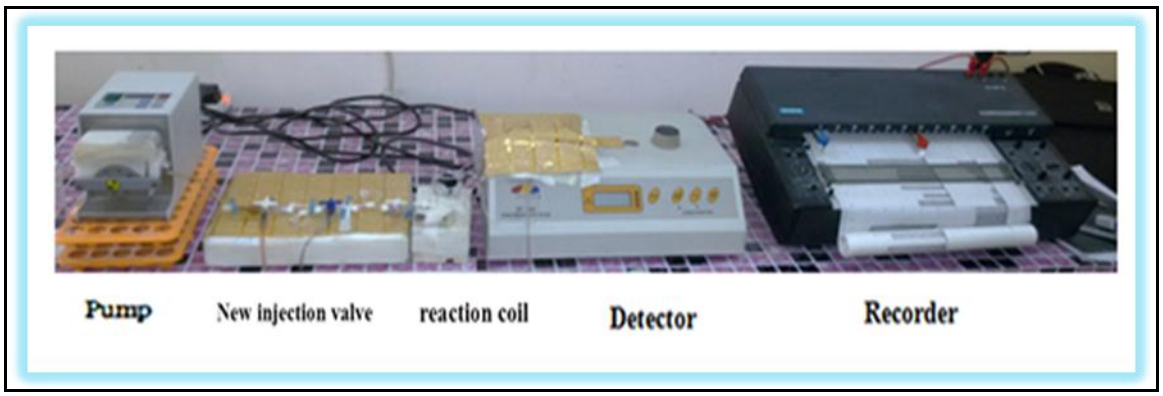

Fig.5: New design of SIA unit

The different factors affecting the unit have been investigated and chosen for a final method evaluation. The following results allow the operator to choose different operation conditions. All unit depend on a new valve is homemade, this valve using in SIA system.

Physical parameters

Effect of the flow rate

The effect of the flow rate on the peak height was studied in the range of 1.0-6.5 mL min^{-1}(Table 1and Fig. 6).Lower flow rate cause doublet[25], the flow rate was 5.6 ml min^{-1} as optimum rate after that the height of peak was reduced due to decreased the sensitivity of measurement in high flow rate because the reaction was no completed when increased the flow rate[26].

Table 1: Effect of the flow rate on the peak height at; Ca conc. = 10 ppm , R. C.L (reaction coil length)= 30cm, EBT conc. = 0.0005%, sample volume (L_1) = reagent volume (L_2) = 78.50µL.

RSD%	SD	Mean	Peak height mm			Flow rate ml/min
0.3592	0.0503	14.0033	14.0500	13.9500	14.0100	1.0000
0.4358	0.0721	16.5600	16.4800	16.6200	16.5800	2.5000
0.1636	0.0378	23.0966	23.0800	23.1400	23.0700	3.5000
0.0648	0.0200	30.8300	30.8500	30.8100	30.8300	4.6000
0.0748	0.0351	46.9233	46.9200	46.8900	46.9600	5.6000
0.0752	0.0300	39.8800	39.8800	39.9100	39.8500	6.5000

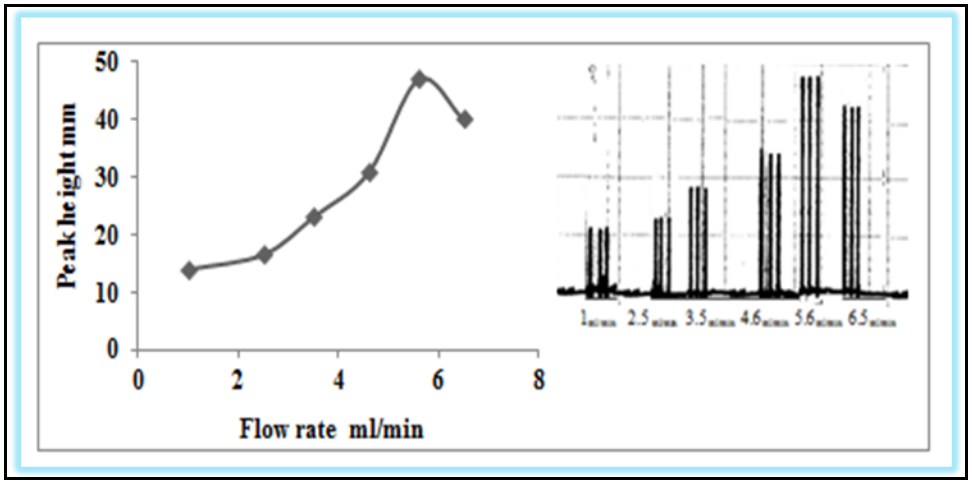

Fig. 6: Change of peak height with flow rate in SIA unit.

Effect of the reaction coil length

Table 2 and Fig. 7 shows effect of the reaction coil length on the peak height in the range (10-40) cm it was seen that the suitable reaction coil length was 30 cm, since it provided the greatest sensitivity[27] and when reaction coil length increase the chemical reaction will complete.

Table 2: Effect of the reaction coil length on the peak height; Ca conc. = 10 ppm, flow rate(5.6 mL min^{-1}), EBT conc. = 0.0005%, and sample volume (L1) = Reagent volume (L2) = 78.50µL.

RSD%	SD	Mean	Peak height mm			Reaction coil length cm
0.1906	0.0556	29.1700	29.1200	29.2300	29.1600	10
0.1042	0.0400	38.3600	38.3600	38.4000	38.3200	20
0.0748	0.0351	46.9233	46.9200	46.8900	46.9600	30
0.0947	0.0305	32.1833	32.2100	32.1900	32.1500	40

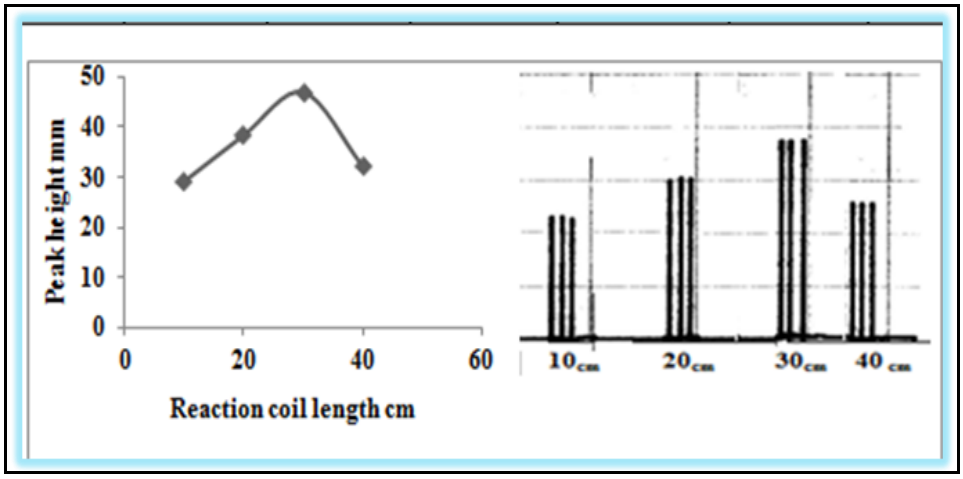

Fig. 7: Change of peak height with the reaction coil length in SIA unit.

Effect of the EBT volume

The influence of the EBT volume on the peak height was investigated by injecting different volumes (78.5- 157.0)µL. The peak height increased to the maximum at (78.50) µL and after that volume, the peak height decreased. So (78.50) µL was chosen for further work as shown in (Table 3 and Fig. 8).

Table 3: Effect of the volume EBT on the peak height at ; Ca conc. = 10 ppm, flow rate=5.6 ml min^{-1}, EBT conc. =0.0005%, sample volume (L1) = 78.50µL and R.C.L (reaction coil length) = 30 cm.

RSD%	SD	Mean	Peak height mm			Reagent volume µL
0.0748	0.0351	46.9233	46.9200	46.8900	46.9600	78.5000
0.1321	0.0416	31.4966	31.5100	31.5300	31.4500	117.750
0.2263	0.0451	19.9266	19.9700	19.9300	19.8800	157.000

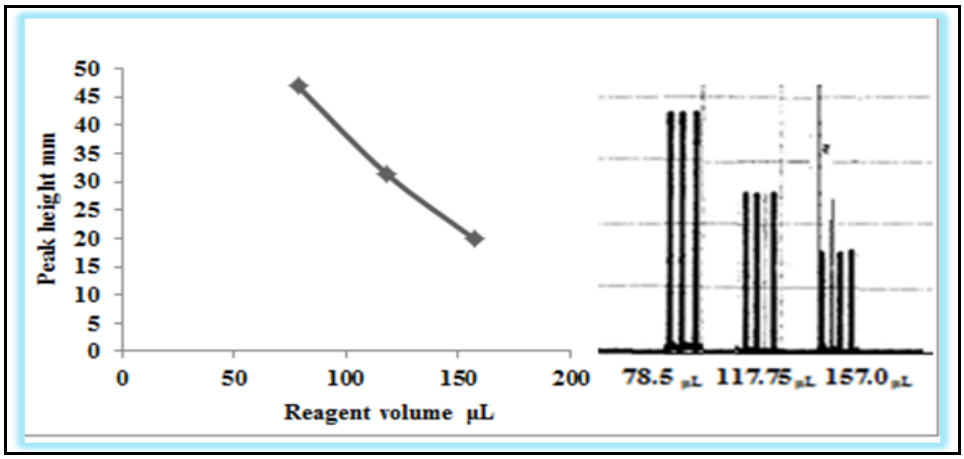

Fig. 8: Change of peak height with the reagent volume in SIA unit.

Effect of sample volume

The influence of the various volume (39.25-117.75 µL) of sample was observed sample volume that exhibited the greatest peak height was found to be 78.50µL and it was chosen as the optimum (Fig. 9 and Table 4).

Table 4: Effect of the reagent volume on the peak height at ; Ca conc. = 10 ppm, flow rate=5.6ml min^{-1}, EBT conc. =0.0005%, reagent volume (L_1) = 78.50µL and R.C.L (Reaction coil length) = 30 cm.

RSD%	SD	Mean	Peak height mm			Sample volume µL
0.0748	0.0351	46.9233	46.9200	46.8900	46.9600	78.5000
0.1316	0.0451	34.2533	34.2500	34.3000	34.2100	117.750
0.0882	0.0251	28.4333	28.4600	28.4100	28.4300	157.000

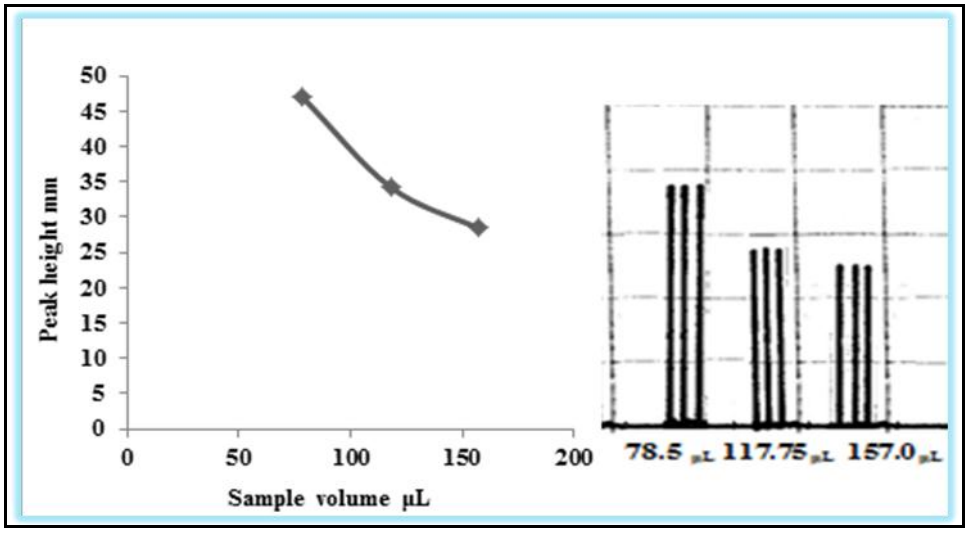

Fig. 9: Change of peak height with the sample volume in SIA unit.

Chemical parameters

Effect of the reagent concentration

The reagent concentration was varied in the range (0.0001-0.005%) in order to maximize the peakheight. Table 5 and Fig. 10 show the effect of reagent concentration on the peak height of the calcium ion. The maximum peak height was obtained with 0.005% reagent and after that concentration, the percentage of complexity decrease, the 0.005%reagent was chosen for further work.

Table 5: Effect of the reagent concentration on the peak height at ; Ca conc. = 10ppm ,flow rate=5.6 ml min^{-1} , R.C. L(Reaction coil length) = 30 cm, sample volume (L_1) = reagent volume (L_2) = 78.50µL .

RSD%	SD	Mean	Peak height mm			reagent Conc.%
0.3869	0.0751	19.4066	19.3200	19.4400	19.4600	0.0001
0.0748	0.0351	46.9233	46.9200	46.8900	46.9600	0.0005
0.0953	0.0650	68.2233	68.2900	68.1600	68.2200	0.0010
0.0392	0.0361	91.9400	91.9000	91.9700	91.9500	0.0050

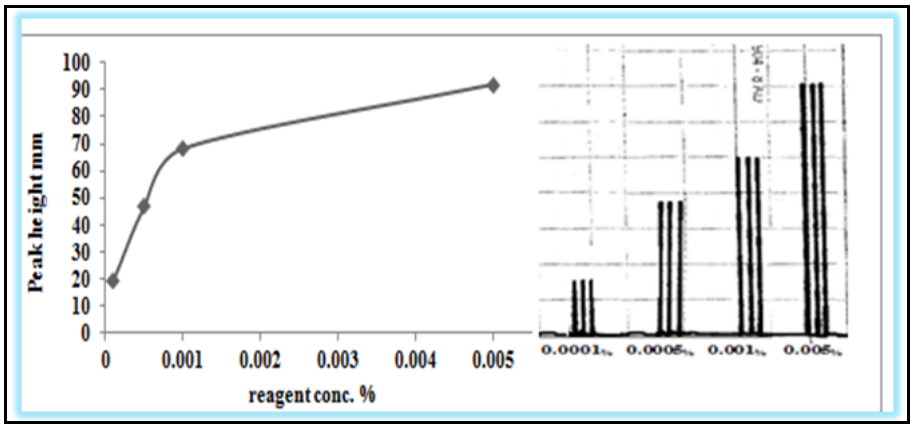

Fig. 10: Change of peak height with reagent concentration in SIA unit.

Calibration curve in SIA method

Calibration curve was prepared at the optimum conditions of complex ion and change in the Ion concentration (Table 11. Fig.6). The calibration curve is linear in the range of (0.05-10) mg/L and the detection limit is 0.0084mg/ml .

Table 6: Effect of the concentration of Ca conc. with peak height at; flow rate=5.6ml min^{-1},R.C.L (Reaction coil length) = 30 cm, sample volume (L_1) = reagent volume (L_2) = 78.50µL, EBT conc.=0.005% and reagent peak height = 24.96 mm.

RSD%	SD	Mean*	Peak height mm			CaConc.$_{mg/l}$
1.0253	0.0351	3.4233	3.4200	3.3900	3.4600	0.0500
0.9345	0.0400	4.2800	4.2400	4.2800	4.3200	0.1000
0.7489	0.0503	6.7166	6.6700	6.7100	6.7700	0.5000
0.2606	0.0244	9.3600	9.3900	9.3300	9.3600	1.0000
0.2145	0.0450	20.9733	20.9300	20.9700	21.0200	3.0000
0.1665	0.0556	33.4000	33.3900	33.4600	33.3500	5.0000
0.1503	0.0602	40.0266	39.9700	40.0200	40.0900	6.0000
0.1004	0.0458	45.6133	45.6600	45.6000	45.5700	7.0000
0.0937	0.0472	50.3566	50.3200	50.3400	50.4100	8.0000
0.1287	0.0750	58.2733	58.2700	58.3500	58.2000	9.0000
0.0539	0.0361	66.9800	66.9400	67.0100	66.9900	10.0000

*** removal the reagent peak height from all peaks height.**

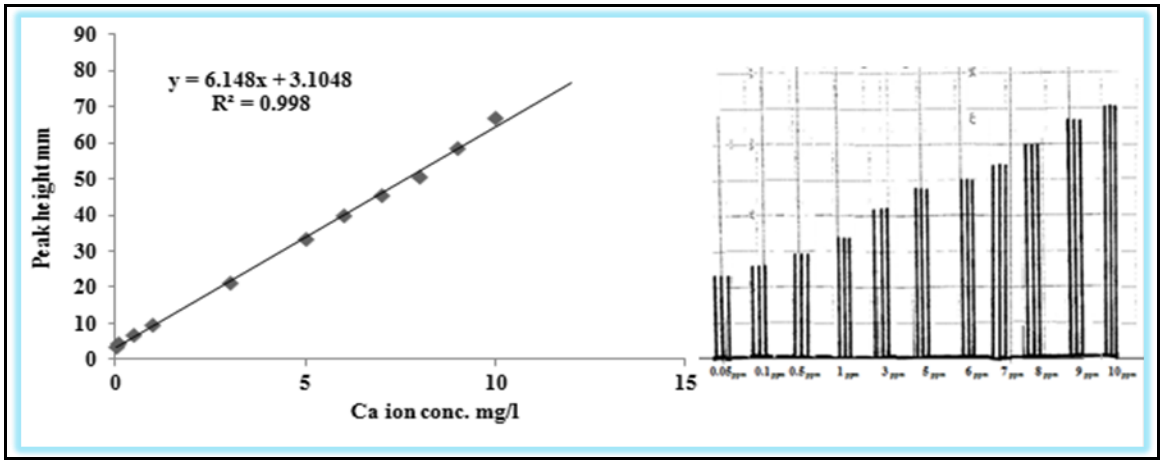

Fig. 11: Calibration curve of Ca ion with EBT in SIA Unit.

Repeatability

Repeatability was established through re-injection the same concentration of Calcium complex. Theconcentration of Calcium ion was 5ppm and 0.005% from EBT. Fig.12 shows the repeatability of Calcium complex. The efficiency of the proposed SIA unit for the determination of Calcium was reflected from the results of repeatability and the detection of limit was (0.0084 mg/L) depend on the repeatability results as (Table 7 and Fig.12) .

Table 7: Repeatability for 5 ppm of Calcium in SIA unit.

No. of peak height	1	2	3	4	5	6	7	8	9	10	Mean	SD	RSD%
Peak height mm	58.3600	58.3700	58.3600	58.3600	58.3700	58.3700	58.3700	58.3700	58.3600	58.3600	58.3640	0.0052	0.0089

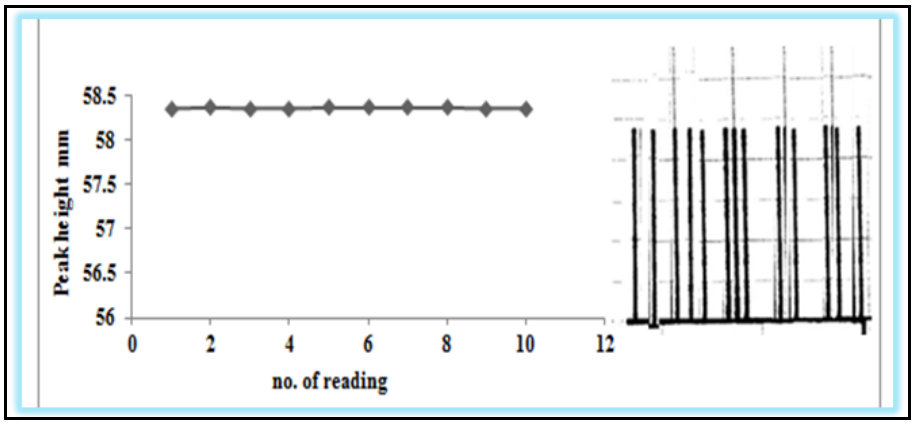

Fig. 12: Repeatability of Calcium for 5 ppm with EBT.

Study of the dead volume

Experiment was conducted to study the dead volume, shows the efficiency of innovative design. Wherever, the dead volume is small, it means best and accurate results. Two experiments were done (i) in the first, the reagent was injected in one loop and in the another inject loop H_2O instead of sample Ca ion and there was response estimated about 24.96 mm and (ii) in the second experiment sample was injected and in another loop H_2O instead of reagent and there was no response, then injected Ca ion andreagent in order to complex formation and measured peak height. The result shown in Fig.13.

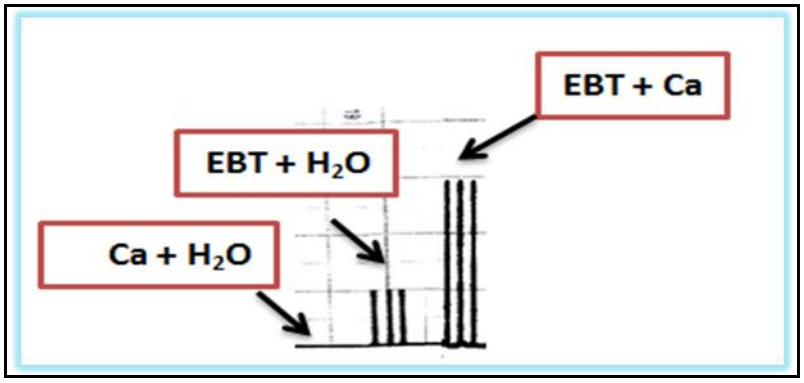

Fig. 13: Study of the dead volumein SIA unit.

Determination of dispersion

To measure the dispersion value in different sample zones of (3 and 10)ppm Calcium ion for SIA, two experiments were carried out.In the first experiment, after mixing of reactants (EBTand Calcium ion) that passes through manifold unit gives continuous response; this indicates non-existence of dispersion effect by convection or diffusion. This measurement represents (H^o). The second experiment includes injecting different Concentration of Ca ions (3 and 10 ppm) for SIA and signal recorded as H_{max} . The valuedispersion (D) from this experiment can be calculatedfrom this equation:

$D=\frac{H^\circ}{Hmax}$ these values fall in limit state of dispersion (Table 8).

Table 8: Determination of dispersion of Ca ion in SIA Unit.

Dispersion (D) $D=H^0/H_{max}$	Response mm		Mg concentration mg/L
	H_{max}	H^0	
1.1160	45.9333	51.2633	3
1.1123	91.9400	102.2700	10

Fig. 14: Determination of dispersion of Ca ion in SIA Unit.

Effect of Interfering ions

The effect of seriously interfering cations (Na^+, Al^{3+}, Fe^{3+},Cu^{2+}, Mg^{2+}, Zn^{2+} , and Ni^{2+}) and anions (Cl^-, CO_3^{2-} ,F^-,CH_3COO^- and $SO4^{2-}$) for determination of Calcium at was studied. The concentrations of interfering ions was in 15 and 50 ppm .The evident was not interferences with Calcium complex expect Mg^{2+},Fe^{3+} at 50ppm were interference .This interfering ion of Fe^{3+} can be removed by using masking agent where the interfering ion was masked by adding 3.0 drops from 100 ppm of F^- ,This interfering ion of Mg^{2+}can be removed by using masking agent where the interfering ion was masked by adding 12.0 drops from 100 ppm of EDTA[19].

Applications

Has been taking various samples contain calcium, Calcium has been determined by EBT As noted in the Table 9 , the method characterized by high precision, which were represented by a recovery.

Table 9: Applications SIA method for determination Calcium.

1- Pharmaceutical samples			
Sample	**The take value for sample in SIA ppm**	**The found value in SIA ppm**	**Recovery%**
Calcicare	10.0000	8.7894	87.8940
Calcium	10.0000	8.9376	89.3768
Meravit	10.0000	9.3862	93.8624
Calcium tap	10.0000	9.4223	94.2236
2- Water samples			
Sample	**The take value for sample in SIA ppm**	**The found value in SIAppm**	**Recovery%**
Rawdatain	36.0000	35.0252	97.2924
Al-Waha	2.0000	2.2262	111.3100
Fadak	22.0000	21.1433	96.1059
Nawar	15.0000	14.8774	99.1834

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