Designing and constructing a micro flow injection system (μFIA) for phosphate determination in soft drinks

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Abstract: Lab. made micro flow Injection system (µFIA) was designed which supplied with two microcontroller UNO and Mega types. These two microcontroller (Arduino) were provided with home- made software programs.The first one, type UNO used to control home- made mini peristaltic pump to drive reagents to detector. The other one was Mega type which was used as a data-logger to manipulate and recording the results as peak height corresponding the concentration by using Microsoft Excel 2010 program. The diameter of the tubes was 0.2mm i.d which used in all parts of manifolds The proposed µFIA manifold was used for phosphate determination in soft drinks samples which supplied from local market. The best conditions for the determination of phosphate by Murphy method was studied. linearity, detection limit, r.s.d% were 5-30 µg/ml,0.09 µg/ml and 0.48% respectively. The phosphate concentrations in the different soft drink samples were in the rang 250-825 µg/ml by using the standard addition method in order to eliminate all expected interferences. The home -made Semi- automated micro-flow injection analysis (µFIA) system was applied successfully for phosphate estimation with simple ,high sensitive and accurate .

1. Introduction

Phosphorus is one of the elements which is vastly dispersed in nature and very vital dietary mineral . It has a good affinity for oxygen therefore, it is not found in a free state which found in different oxides states [1,2]. Some foods and beverage contain phosphorus foe examples, eggs, milk, , fish, bread , meat, and soft drinks. Where phosphorus amount in soft drinks are smaller than other foods, for instance, a cup of milk has a 250 mg of phosphorus while a 12-ounce can of Pepsi contain just over 50 mg [3,4]. various additives were added to soft drinks with phosphoric acid [5].Phosphate detection based on molybdenum blue method which formed a firstly molybdophosphoric acid complex which produced from the reaction of ortho phosphate and molybdate in acidic solution. Then the complex was reduced ascorbic acid to form the molybdenum blue. The intensity of the molybdenum blue complex is calculated at specific wavelength spectrophotometrically and the concentration of phosphate is measured from the corresponding intensity of the complex color [6,7,8]. The concept of flow injection (FI) was introduced in pioneering paper by Ruzicka and Hansen [9] It's have many applications in many aspects like biological, chemical, clinical, drugs, industrial, food and environmental analysis [10-13].There are many various forms of flow injection analysis included merging zone, split-loop injection, stopped flow, gas diffusion and reverse flow injection [14,15]. It has been described for some aspects as diluting and transported the sample automatically in a various flow systems to eliminate interference in the measurement step and reducing reagents and samples consumption to micro liters.[16,17].Micro flow injection system is now used in chemical ,biological, pharmaceutical analysis and other fields because low reagent consumption, miniaturizing the sample,

waste storage, simple procedure in addition to high speed, precision and accuracy [18,19]. Minimizing the flow analysis system can be achieved by constructing a manifolds with diminutive detectors and transporting the carrier stream with large hydraulic device [20,21]. Micro syringes and micro controlling pump to displacement the peristaltic pumps [22] and micro tubes was used in all parts of the manifold. [23,24] These lead to miniaturized the flow injection apparatus with micro diameters tube [25]. In this work the diameter of tubes was 0.2 mm i.d supplied from Technicon Instruments corporation which used in all parts of manifolds. Two home- made microcontroller software programs were used .The first one type UNO used to control home- made peristaltic pump to propel carrier stream and reagents to detector. The other one was Mega type which was used as data-logger to manipulate and recording the results as peaks height corresponding the concentration by using Microsoft Excel 2010 program. The optimum conditions of FIA procedure were investigated for determination of phosphate in soft drinks samples which supplied from local market.

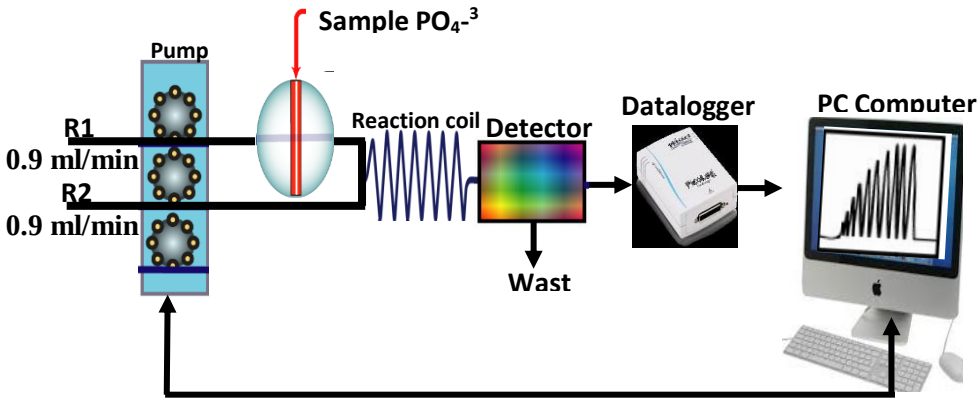
2. Experimental

2.1 Chemicals

Analytical grade reagents and deionized water were used through this work. A phosphate standard solution (100 µg/ ml) was prepared by dissolving 0.1432g of dried (at 110C°) Potassium dihydrogene phosphate in 1L water. A 0.25 %w/v Ascorbic acid solution was prepared by dissolving 2.5 g in 1L of water containing 10 ml of glycerin to increase the stability of the formed complex [26]. A solution of Ammonium Molybdate (6×10^{-3} M) was prepared by dissolving 7.4154g in 1L of 0.4 M nitric acid.

2.2 Micro – flow injection manifold

Fig. 1 shows the micro Flow injection manifold which used for phosphate determination in soft drinks samples. It is consist a two home- made microcontroller software programs. The first one type UNO used to control home- made peristaltic pump with variable speed in the rang (0.4-5 ml/min) to propel carrier stream and reagents to detector. The other one was Mega type which was used as data-logger to manipulate and recording the results as peaks height corresponding the concentration by using Microsoft Excel 2010 program. The PO_4^{-3} sample (30µL) was injected through injection valve supplied with variable loops (20-50 µL).The reaction coil was made from 0.2 mm i.d tube (supplied from Technicon Instruments corporation) in order to minimize consumption of reagents. Detector was spectrophotometer (SPECTRO SC,Labomed,Inc,USA) which supplied with 7 µL flow cell.



R1= 0.004M Ammonium Molybdate, R2= 0.2%W/V ascorbic acid

Figure 1: µFIA system for phosphate determination in soft drinks

3. Results and Discussion

To achieve high sensitivity, linear calibration range and economy of reagent consumption, the effect of various physical and chemical parameters were examined. The influence of total flow rate on signal of 10 µg/ml phosphate in the range (1.4-5 ml/min) was showed in Fig.2.It was found that 1.8 ml/min of total flow rate was the best so, it is used in subsequent work . When the manifold in Fig.1 was used, the signal (peak height) of 10 µg/ml phosphate increased almost parabolically with increasing of injected sample volume (Fig.3) between 20-50 µl so,30µL was chosen in subsequent experimental due to the peaks over that somewhat distorted. Also, to obtain high sample throughput and good precision [27] . The effect of Reaction coil length on peak height of 10 µg/ml phosphate in the range (20-60 cm) was indicate in Fig 4, therefore, 30 cm reaction coil length was selected in order to avoid the tailing in peaks over the 30 cm length , to increase the sample throughput and minimize consumption of reagents [28] . The effect of Ammonium molybdate and ascorbic acid concentrations over the range (0.002-0.006M) and (0.05-0.25%w/v) respectively were showed in Fig.5 and 6. It was recorded that 0.004M for ammonium molybdate and 0.2%w/v for ascorbic acid were chosen for subsequent work, these concentrations were chosen due to avoid the disturbances in peaks shape and baseline and providing high sensitivity [29,30].

Table 1: The optimum conditions for µFIA

parameters	values
Internal diameter of tubes (i.d)	0.20 mm
Total flow rate	1.8 ml/min
Sample volume	30µL
Reaction coil length	30cm
Ammonium molybdate Concentration	0.004M
Ascorbic acid concentration	0.2%W/V

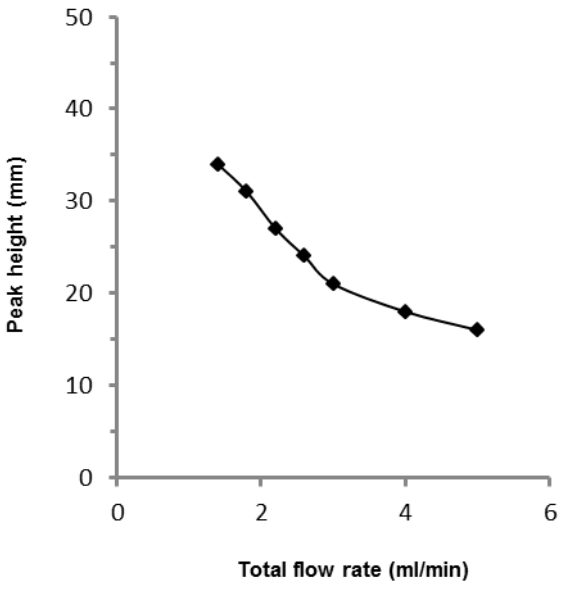


Figure 2. Total flow rate influence

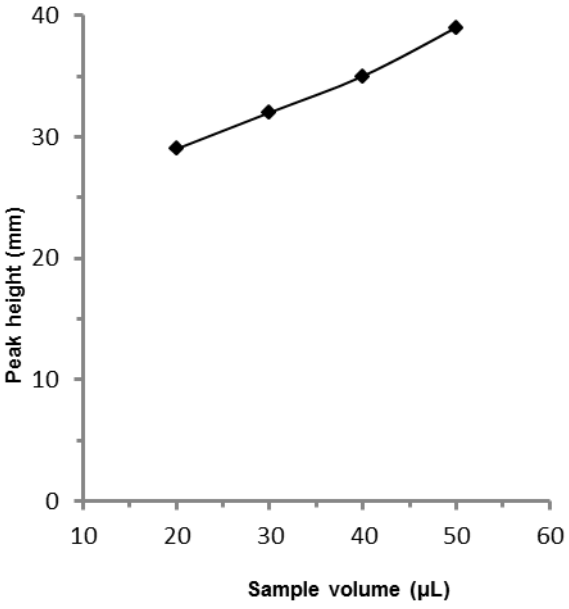


Figure3. Effect of sample volume

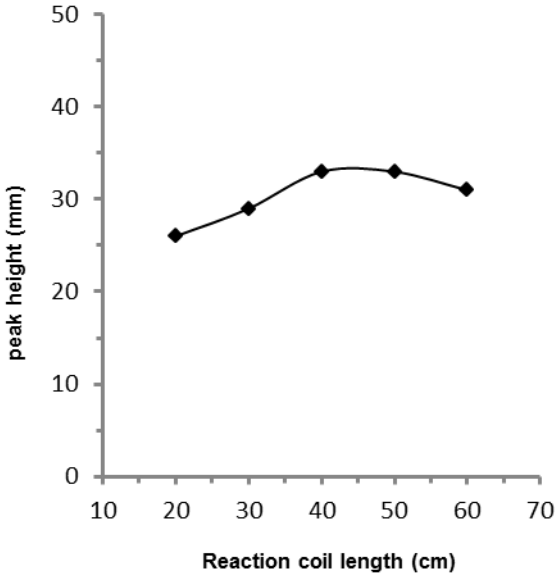


Figure4. Reaction coil length influence

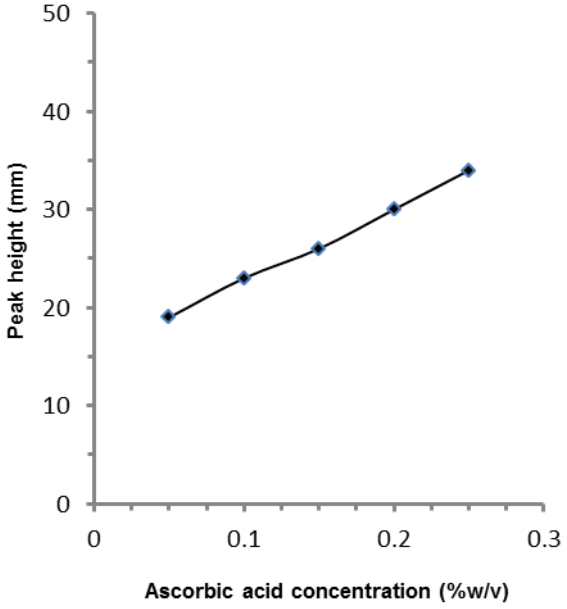


Figure5. Effect of Ascorbic acid conc.

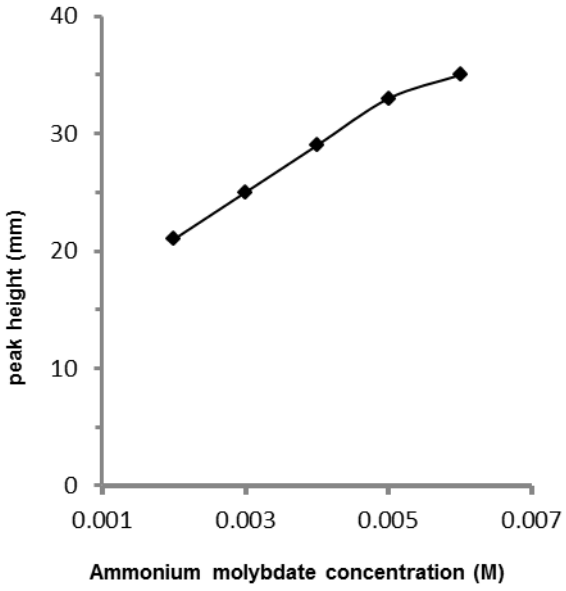


Figure 6. Effect of Ammonium molybdate Concentration

3.1 Standard calibration graph

Under the established conditions which listed in table 1, the linearity of phosphate detection was found in the range 5-30 µg/ml .Fig.7 shows the calibration graph and the recording peak height for series of phosphate standard. The equation y=2.6571+0.2857 give the relationship between the peak height and the concentration where y and x are the peak height and the concentration of PO_4^{-3} respectively. The Regression coefficient for six points , r.s.d% for ten replicates and the detection limit (DL) were 0.9999 , 0.0375% ,0.09 µg/ml respectively. The dispersion factor in flow manifold was 1.25 (Fig. 8) and the sample throughput could be 120 sample/h.

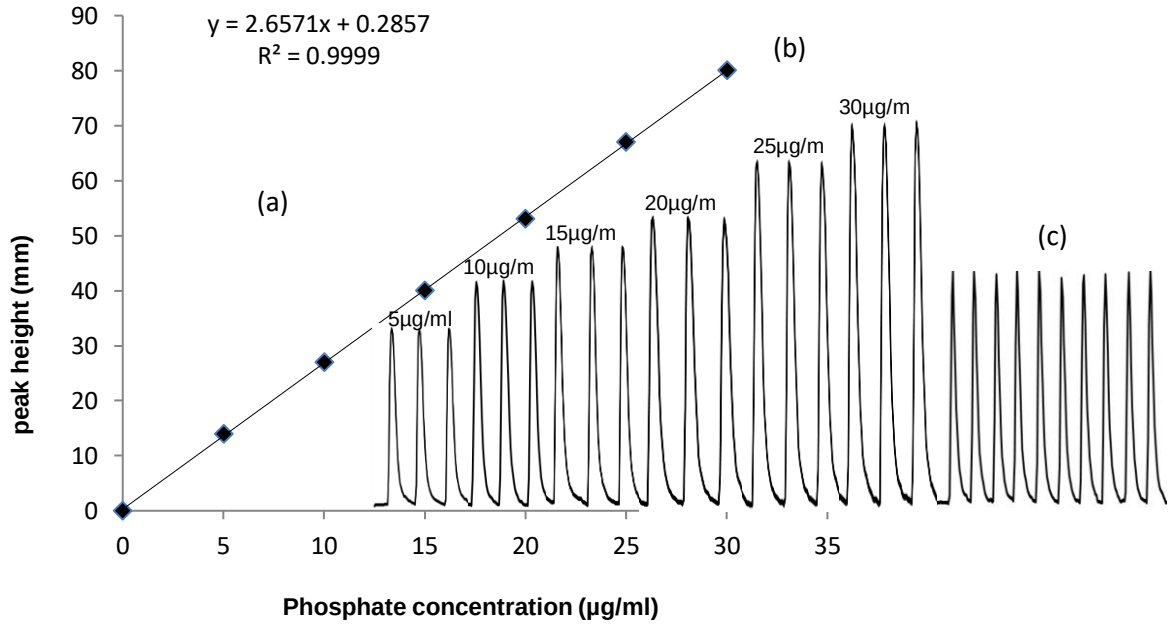


Figure7. (a). Concentration of phosphate (ppm) (b). corresponding peaks (c) Ten injections of 10 µg\ml phosphate.

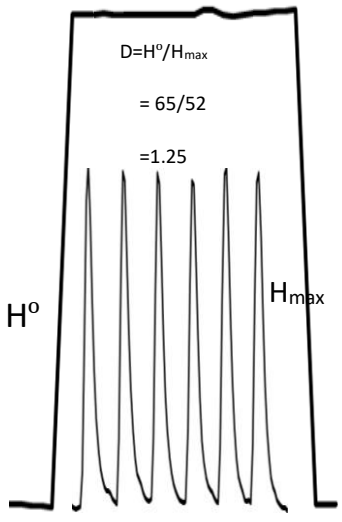


Fig 8: The dispersion in flow system

4. Analytical applications

Different samples of soft drinks were supplied from local market. The pH of all samples were measured by pH meter and the results over the range (2.17-2.72). The phosphate concentrations of soft

drinks samples were analysed by micro-flow injection analysis (µFIA). The concentrations of phosphate in the samples were determined by standard method addition obtained using standard solutions by add 1 ml of sample to each standard solutions are shown in Fig.9 and measured spectrophotometrically at 660 nm. The accuracy test was carried out by matching the results of our lab. made system with Murphy classical methods Additionally, the micro flow injection method is simple, inexpensive, rapid, and has a high sensitivity. and consume a small amounts of samples and reagents.

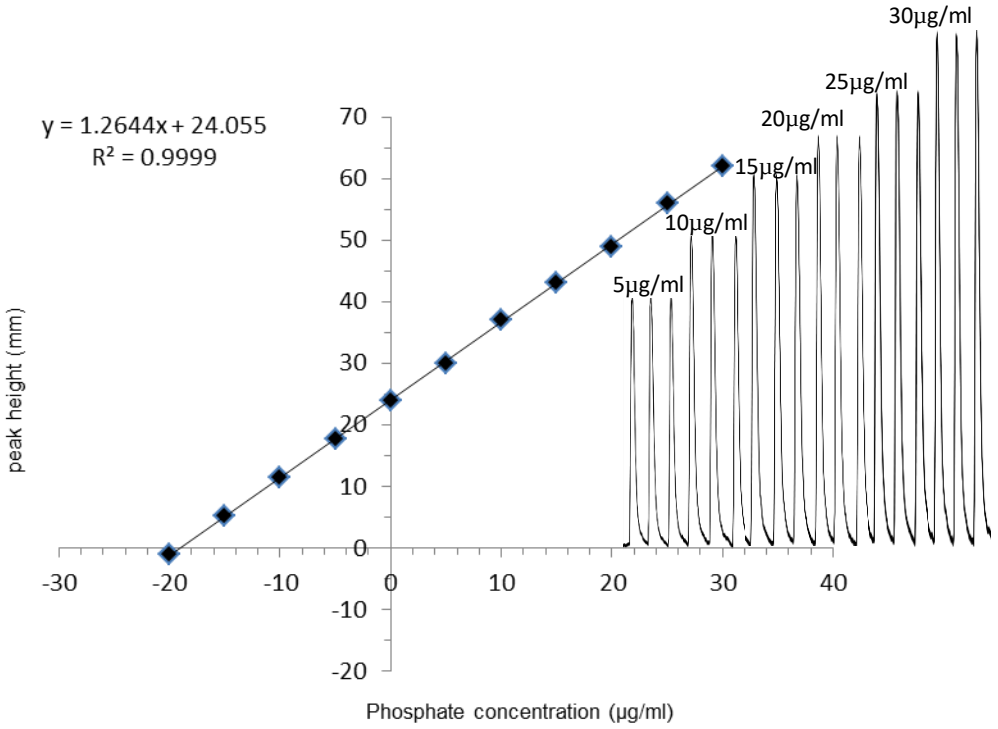


Figure 9. Phosphate concentrations detection by standard method addition

Table 2. Concentrations of phosphate in soft drinks samples

Sample No.	Trade mark	volume	Company name	pH	Standard method addition (µg/ml)	Classical method (µg/ml)
1	Pepsi	330 ml	Baghdad-Iraq	2.17	800±0.0850	780±0.0543
2	coca cola	250 ml	Alwaha-babel-Iraq	2.23	500±0.0776	550±0.0473
3	RT cola	250 ml	Belad-Iraq	2.17	825±0.0853	800±0.0437
4	Pepsi (plastic)	1.75 L	Baghdad-Iraq	2.17	600±0.0954	630±0.0263
5	Coca cola(plastic)	750 ml	Alwaha-babel-Iraq	2.19	600±0.0965	570±0.0342
6	Coca cola(glassy)	250 ml	Babel-Iraq	2.18	250±0.0769	225±0.0523
7	Coca cola(plastic)	450 ml	Kirkuk-Iraq	2.72	612±0.0983	640±0.0438
8	Al-Reem(glassy)	200 ml	Al-Reem- Baghdad	2.17	500±0.0786	550±0.0231
9	RC cola (glassy)	200 ml	Dijla- Baghdad-	2.33	575±0.0932	550±0.0165

5. Conclusion

The home- made micro flow injection system which was designed and constructed in our laboratory was simple, fast , inexpensive , accurate and reproducible and applied successfully for phosphate in soft drinks samples.

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