Construction and Evaluation of a Home-made Spectrophotometric Semi-automated Flow Injection System

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Abstract: A Spectrophotometric semi-automated flow injection (FI) system was constructed from the components already present in our analytical laboratory. A model system based on FI determination of methylene blue and iron III were chosen for testing and evaluation the physical and chemical parameters of this system, respectively.

Keyword: Flow injection, Spectrophotometric, Methylene blue, Iron (III).

Introduction

Current advance in analytical chemistry rely on complex techniques as well as on the use of sophisticated instruments, the purchase and maintenance cost and difficult operation of which make the inaccessible to a great many scientists and research laboratories [1-2].

Flow injection analysis (FIA) is a major methodological innovation characterized by its simple basis, relatively inexpensive equipment handily operation and great capacity for achieving results that are excellent in view of the rapidity, accuracy and precision with which they are obtained [3-4].

The extreme versatility of this methodology makes it stand out from most new analytical techniques [5]. The majority of FI instruments were assembled from components found in laboratory or purchased piecemeal. This was because many researchers found challenge and joy in innovative design of their own systems and also became the commercially available system were quiet expensive [3].

The main purpose of this work is to constructe a spectrophotometric semi-automated flow injection system and evaluates the physical and chemical parameters of this system by determination of methylene blue and iron (III), respectively.

Experimental

All reagents used were analytical grade unless otherwise stated and all stock solutions were prepared in pure deionized distilled water which was used through this work.

Regents and Solution

Methylene blue solution

A stock of 10^{-2} M of methylene blue was prepared by dissolving 3.1999 g of methylene blue (FLUKA) in 1 liter deionized distilled water. The working and standard solutions were prepared by appropriate dilution of the stock solution.

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Iron III solution

A stock solution $2x10^{-3}$ M of iron (III) was prepared by dissolving 0.8080 g of iron (III) nitrate nonahydrate (BDH) in 1 liter of 0.1M perchloric acid. The working and standard solutions were prepared by appropriate dilution of the stock solution.

Potassium thiocyanate solution

The stock solution 0.5 M of potassium thiocyanate (Merck) was prepared by dissolving 48.5900 g of potassium thiocyanate in 1 liter of 0.1 M perchloric acid. The working thiocyanate concentrations were prepared by appropriate dilution of the stock solution.

Perchloric acid solution

A stock of 0.1 M perchloric acid (BDH) was prepared by dilution 22.43 ml with deionized distilled water to 500 ml.

Home-Made Semi-Automated FI System

Fig.1 shows the home-made semi-automated FI system. In order to maintain a stable relatively low pulsation and easy to control flow rate of the carrier/regent stream, bidirectional pump (Watson-Marlow USA) and eight roller Peristaltic pump (Auto-Analyzer п Dublin, UK) were used to propel the carrier/regent stream, the first one has the advantages of bidirectional and easy to change and control the flow rate but inherit the difficulties of using multi-channel, so, a peristaltic pump was used which flow rate can be controlled by the inner diameter of pump tubing and the rollers are long enough, so that several tubes can be handled simultaneously.

The sample volume was manually injected by a syringe through the medical canula then replaced with two-position injection valve loops which used in HPLC modified in the local private workshop to the specific volumes.

The absorbance of the coloured dye or the formed complex were measured at single beam spectrophotometer (Spectro SC / labomed, inc. USA) equipped with flow cell supplied (QS Hellma) with 450 µL volume and 10 mm path length. The flow cell holder and the top cover of the spectrophotometer were modified in order to let the tube pass through to keep it fit with the inlet and outlet of the flow cell. The signals were recorded by simens (Kompensograph) recorder with output sensitivity range (0.01-500 V) and speed control between (6-12 cm/h) and recorded peak height can be related to the sample concentration. Teflon tube 0.5 mm (i.d) was used for all of the rest manifold, mixing (Fig.2) and reaction (Fig.3) coils respectively.

The FI Procedures

The carrier stream was deionized distilled water run into the manifold (Fig.1) at flow rate 2 ml/min. A 100 µL as a sample volume of methylene blue was injected manually through the injection valve into the carrier stream. Mixing coil of 50 cm length was inserting for dilution the sample. The absorbance of the sample was measured at 660 nm [6] by a spectrophotometer equipped with flow cell. The recorded peak height can be related to the concentration of injected sample.

The above procedure was used for determination of iron (III), 0.I M thiocyanate concentration was used as a reagent stream, 20 µL of iron (III) as a sample volume and the absorbance was measured at 490 nm [7].

Results and Discussion

1. Determination of Methylene blue

Injection of a dye into non- reactive colourless carrier stream (deionized distilled water) in an
established technique for the physical characterization of flow injection system [1].

The FIA – Spectrophotometer manifold for the determination of methylene blue as a physical parameter was optimized by investigating the effect of various parameters which included, sample volume, flow rate and mixing coil length.

The peak height increased with increasing of injected sample volume between 20 – 120 µL as shown in (Table-1), maximum peak height was obtained in 120 µL, 100 µL was chosen to inject in subsequent experiments. The effect of flow rate was characterized over the range 0.23 – 3.6 ml/min, as shown in (Table-1), undistorted and great peak height sensitivity was obtained at flow rate 2.3 ml/min which was used in further investigation of conditions.

The effect of mixing coil length indicated that increasing of the coil length decrease the peak height (Table-1) which thought simply increasing mixing coil led to more dispersion in the system so, 50 cm as mixing coil length was used in subsequent work.

Under the established conditions a calibration graph for methylene blue was drawn as shown in Fig.4. It was linear over the range (1.0×10^{-5}- 4.0×10^{-5} M). The linear graph has a regression coefficient 0.9992 for 6 points. The detection limit (2 x noise) was (1×10^{-7} M) and the relative standard deviation (r.s.d) for 10 replicates of (3.5×10^{-5} M) methylene blue was 0.95 % as shown in (Fig.4C). The sample through put was 120 sample/ h and the dispersion coefficient in the system was 1.64.

2. Determination of Iron (III)

The manifold as shown in Fig.3 was used for FI spectrophotometer determination of iron (III), Potassium thiocyante was used as a reagent stream and the absorbance was measured at 490 nm. The following optimum condition had been obtained as chemical parameter for the home-made semi-automated FIA system.

The influence of potassium thiocyante concentration on peak height for 20 µL of 1×10^{-4} M iron (III) is shown in (Table-2), the peak height increase with increasing the concentration of potassium thiocyante which thought to be due to more complex formation, above 0.1 M the peak height was decrease. Thus 0.1 M potassium thiocyante was used in subsequent experiments.

Table-2 shows the effect of flow rate of reagent stream on peak height. The reaction between iron (III) and thiocyante was found to be instant when the stop flow method was used the peak height decrease with increasing flow rate. A 1.9 ml min^{-1} flow rate was chosen for subsequent work, because it achieved good reproducibility and rapid analysis.

The effect of sample volume is shown in (Table-2). The maximum peak height was obtained when 120 µL was injected, but the peak shape somewhat distorted, so 20 µL was injected in subsequent experiments in order to achieve higher samples throughput. Table-2 shows the effect of reaction coil length on 20 µL of 1 x 10 $^{-4}$ M iron (III) the flow rate was maintained at 1.9 ml/ min. The results showed that the peak height decrease with the increasing of the reaction coil length. These results arise because the complex formed instantly and increased coil length which leads to more dispersion in FIA system, so, 75 cm was chosen in the subsequent experiments.

The effect of percholric acid concentration on peak height was studied as shown in (Table- 2), 0. I M perchloric acid was found to give the maximum peak height.

Under the conditions established (Table-2) a calibration graph for iron (III) was obtained as shown in Fig.5.

It is linear over a range 0.2 x 10 $^{-4}$ M. The regression coefficient of the linear graph was 0.9992, detection limit (2 x noise) was 1 x 10 $^{-7}$ M and the r.s.d for nine replicates was 1.28 % as shown in Fig.5. The dispersion in the flow system was 3.14 and the samples throughput was 180 sample / h.

To evaluated the accuracy of the FI home-made system. A recovery experiments were performed on representive samples of methylene blue and iron (III). Addition standard methods were used for all of these determinations (Table-3) summarize all of these studies. The average recoveries were 99.76 % and 100.86 % for methylene blue and iron (III), respectively.

In order to establish the validity of this system the same batch of representive samples (Table-4) were analysed by classical method using also, the standard addition method to avoid all the possible interferences. The recoveries and r.s.d % were calculated as shown in (Table-3). The results were shown that satisfactory accuracy and r.s.d % were afforded by the home-made FI system.

Table-4 summarizes the average features of two commercial firms and the home-made FI system. A good agreement has been shown between the most of the features of commercial and the home-made instrument. The new commercial instruments are better than our system in many features such as compact and computerise but it cost a lot, our system is easy to use and maintains and cheaper. So, several applications of this FI system are being explored for determination albumin in serum [10], phosphate, silicate, nitrate and nitrite in water [11].

Table -1 Effect of various parameters on the analytical response for determination of methylene blue

Sample Volume (µL)	20.0	40.0	60.0	80.0	100	120		
Peak Height (mm)*	10.0	12.0	14.0	16.0	18.0	20.0		
Flow Rate (ml/min)	0.23	0.43	0.95	1.1	1.66	1.9	2.3	3.6
Peak Height (mm)*	9.0	10.0	14.0	15.0	18.0	19.0	20	20
Mixing Coil Length (cm)	25.0	50.0	75.0	100.0	125.0	150.0		
Peak Height (mm)*	19.0	18.0	14.0	9.0	8.0	7.0		

* Average of three peaks.

Table -2 Effect of various parameters on the analytical response for determination of iron (III)

Flow Rate (ml/min) Peak Height (mm)*	1.00 ١٦	1.50 ١٩	1.90 ٢٢	2.30 ٢٧	3.00 ٢٩	3.4 ٢٦	90 ٢٣
Sample Volume (µL) Peak Height (mm)*	20.0 ٢٥,٠	40.0 ٣٦,٠	60.0 ٤٠,٠	80.0 ٤٤,٠	100.0 ٣٩,٠	120 39.0	
Reaction Coil Length (cm) Peak Height (mm)*	25.0 ٣٣,٠	50.0 ٢٨,٠	75.0 ٢٥,٠	100.0 ٢٣,٠	125.0 ٢١,٠	150.0 ١٩,٠	
$[SCN^{-1}]$ M Peak Height (mm)*	1×10^{-2} 6.0	1.5×10^{-2} 8.0	1×10^{-1} 27.0	2.5×10^{-1} 15.0			
$[HClO_4]$ M Peak Height (mm)*	1.25×10^{-2} 22.0	2.5×10^{-2} 24.0	1×10^{-1} 31.0	2×10^{-1} 18.0			

* Average of three peaks

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Table-3 Determination of Methylene blue and Iron (III) in representative samples by a Home-made FI System and Manual spectrophotometric standard addition method.

No. and concentration (M) of Representative samples		FIA recovery ± r.s.d• %	Manual Spectrophotometric recovery ± r.s.d* %
Methylene blue Samples	Sample 1 1.0×10^{-5}	101 ± 0.95	95 ± 0.98
	Sample 2 2.5×10^{-5}	99.8 ± 0.85	96 ± 0.77
	Sample 3 4.0×10^{-5}	98.5 ± 0.80	103 ± 0.89
Iron (III) Samples	Sample 1 0.2×10^{-4}	98.90 ± 1.20	97.0 ± 1.100
	Sample 2 1.0×10^{-4}	101.20 ± 1.30	95.0 ± 1.00
	Sample 3 1.8×10^{-4}	101.50 ± 1.28	98.0 ± 0.93

• Five Replicates

* Three Replicates

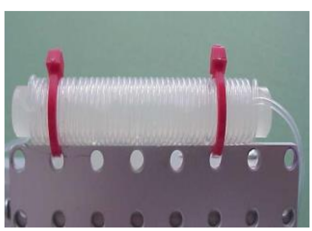
Table-4 Comparison of the operational characteristics for commercial FI systems and the Home-made FI system

Parameter	Commercial FI System*	Home-Made FI System
Response time (S)	5 - 60	5 - 60
Sample Through put / h	Up 300	Up 200
Start-Up (min)	Fast	Fast
Shutdown	Fast	Fast
Change of analysis	Quick	Quick
Sample and regent consumption (µL)	100 – 300	100 – 300
Cost	Expensive	Not expensive
Compact Design	Yes	No
Sample Volume	As low as 20 µL	20 µL
Data Acquisition	Peak height, area, width and peak to peak	Recorded peak height
Microprocessor and Computerized	Yes	No

* The average values of Teacator [8] and FIA Lab [9]

(b)

(c)



(f)

(d)

(e)

(g)

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Fig.1 (a) Home-made semi-automated flow injection system
(b) Peristaltic pump
(c) Injection valve
(d) Spectrophotometer
(e) Recorder
(f) Teflon reaction or dilution coil
(g) Flow cell with modified cell holder and top cove

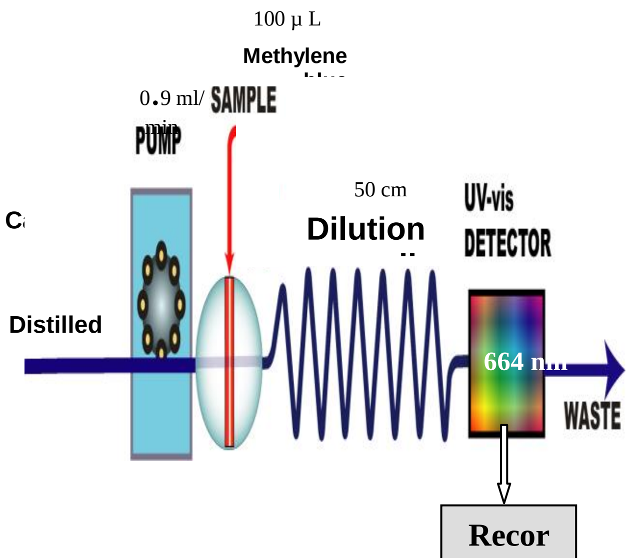
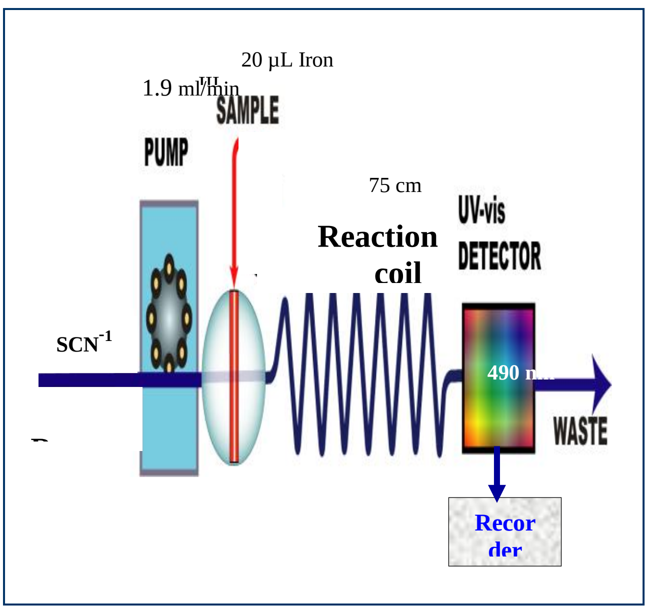


Fig. 2 Single stream manifold for determination … lue



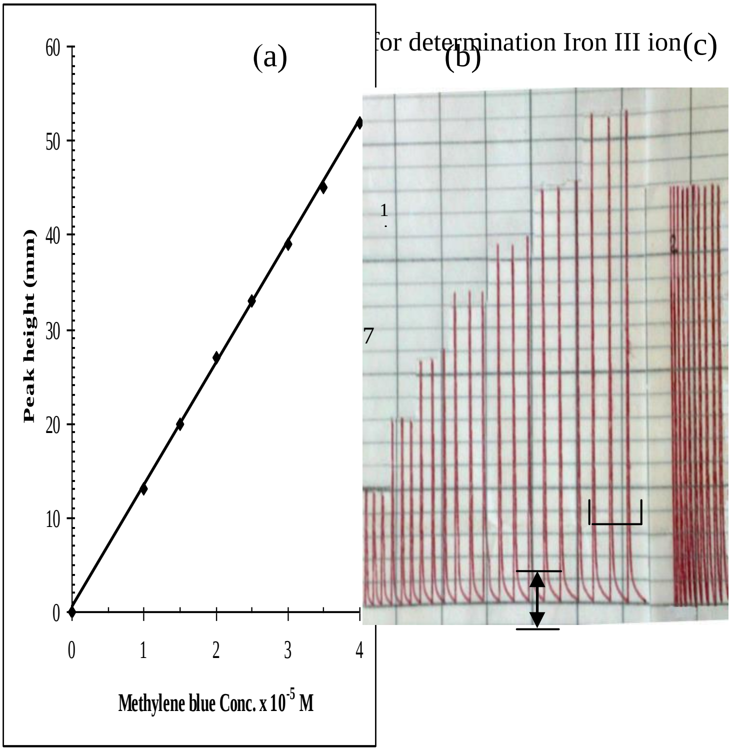


Fig. 4 (a) Corresponding calibration graph of methylene blue

(b) Peaks obtained by injected methylene blue standards in the corresponding concentration range.

(c) 10 replicate peaks of (3.5 x 10^{-5} M) standard methylene blue.

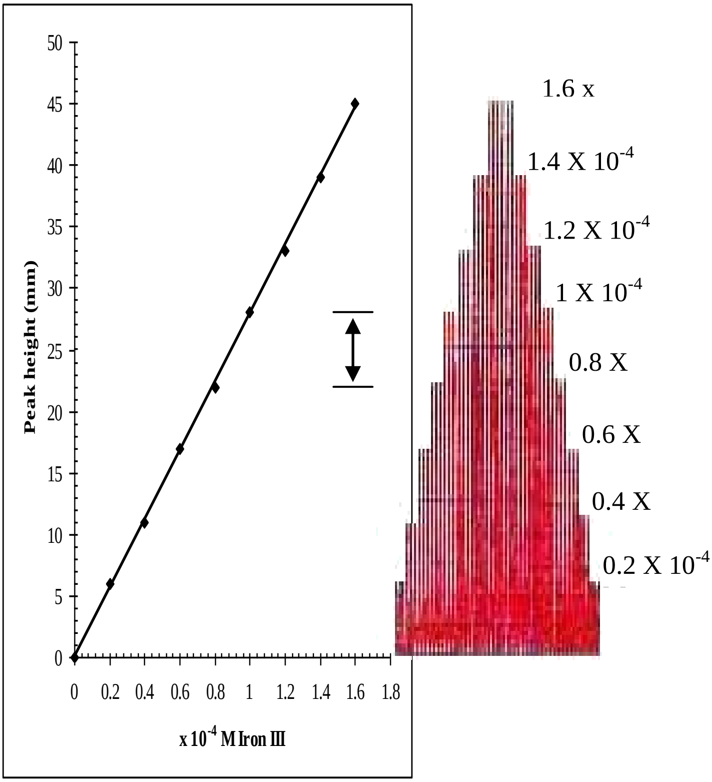


Fig. 5 (a) Corresponding calibration graph of Iron III ion
(b) Peaks obtained by injected Iron (III) standards in the concentration range shown above.

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بناء و تقييم نظام حقن جرياني محلي شبه آلي طيفي

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عباس دواس المالكي
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الخلاصة: تم بناء نظام حقن جرياني محلي شبه آلي / طيفي من الأجهزة الموجودة في مختبر الكيمياء التحليلية. حيث اعتمد اساساً على الحقن الجرياني لتقدير صبغة المثلين الزرقاء و الحديد الثلاثي والتي اختيرت لفحص و تقييم المتغيرات الفيزيائية و الكيميائية للنظام على التوالي. وتم تقيم الجهاز بمقارنته مع الأجهزة التجارية المتوفرة وكذلك بدراسة قيم الأسترجاع لنماذج قياسية من صبغة المثلين الزرقاء و الحديد الثلاثي على التوالي و مقارنتها بطريقة الاضافة القياسية الطيفية اليدوية.