

Home-Made Micro Valve for Determining Malachite Green Dye by Flow Injection Analysis

Amal Saadoon Majeed¹, Ahmed Saleh Farhood²,
Luma Ahmed Mohammed Ali^{2,*}, and Dakhil Nassir Taha²

¹Department of Basic Science, College of Nursing, University of Kufa, Kufa, Iraq

²Department of Chemistry, College of Science, University of Babylon, Babylon, Iraq

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ABSTRACT

The research is included studying and designing micro flow injection system which is characterized with rapidity, simplicity, and low cost for the determination of green malachite dye. The study of flow rate of carrier stream, repeatability, dispersion coefficient, and calibration graph are conducted. It is found that the optimum conditions for the determination of mentioned dye are flow rate 4.200 mL/min, sampling rate 102 sample/h, limit of detection 0.05 ppm, linear range (0.05-18.00) ppm with linearity ($R^2 = 0.9700$), RSD is 0.355, the repeatability for seven successive injections is studied for the two concentrations 5 ppm and 12 ppm, and the dispersion coefficient values are 1.73 and 1.28 at the two concentrations 2 ppm and 9 ppm respectively.

Keywords: μ flow injection; green malachite dye; home-made micro valve

ABSTRAK

Penelitian ini mempelajari dan merancang sistem injeksi aliran mikro yang lebih unggul dalam kecepatan, kesederhanaan, dan biaya yang murah untuk penentuan pewarna malachite hijau. Studi laju alir arus larutan pembawa, pengulangan, koefisien dispersi, dan grafik kalibrasi telah dilakukan. Ditemukan bahwa kondisi optimum untuk penentuan zat warna tersebut adalah laju alir 4,200 mL/menit, laju sampling 102 sampel/jam, batas deteksi 0,05 ppm, kisaran linier (0,05-18,00) ppm dengan linearitas ($R^2 = 0,9700$), RSD=0,355, pengulangan untuk tujuh injeksi berturut-turut untuk dua konsentrasi 5 ppm dan 12 ppm, dan nilai koefisien dispersi adalah 1,73 dan 1,28 pada dua konsentrasi masing-masing 2 ppm dan 9 ppm.

Kata kunci: injeksi aliran mikro; pewarna malachite hijau; katup mikro

INTRODUCTION

Flow injection is considered as a powerful technique in the analytical chemistry field according to the classification of International Union of Pure Applied Chemistry IUPAC in 1994 [1]. New concept of continuous flow injection analysis had been patented in 1975 by Ruzicka and Hansen in Denmark, and Stewart in USA simultaneously as recorded in the literatures [2-8]. Flow injection technique is characterized with high analytical capabilities in terms of very small volumes of sample and reagent, high sampling rate, low limit of detection, and extent range of concentrations [9]. Flow injection system consists of the pump for inspiration of carrier solution, injection sample and reagent unit, reaction coil, detector, and the recorder of response [10-13].

This technique based on using trace amounts of both sample and reagent with high reproducibility. It considered the easiest method to conduct the most

important chemical processes like mixing, addition of reagents, dilution, and extraction. Flow injection technique got the better of the other classical analytical methods which suffered from many problems during the chemical analysis. FI technique operates within closed system which in turn helps get rid of external conditions affecting the reaction system. Therefore; FIA is widespread in many fields [14-15]

A novel approach of microsystem was described for the first time in 1984 by Ruzicka and Hansen. The microsystem was a design of integrated micro conduits with miniature of potentiometric or optical detectors and integrated gas-diffusion or ion-exchange units [16]. Microfluidics is a term used to describe the microsystems at the beginning of 1990s. In microfluidics the diameters of channels decreased down to tens of micrometers and this integrated circuit paved way for the fabrication of a complete Lab-on-a-chip or micro total analysis system (μ TAS). Microfluidic system consists of thin piece of glass or polymeric plate

* Corresponding author.
Email address : alnakashluma@Yahoo.com

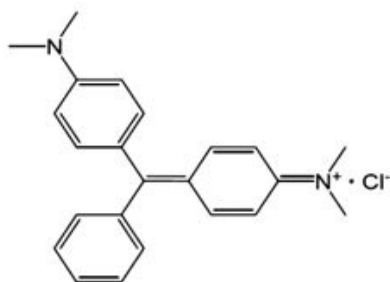


Fig 1. Chemical structure of Green Malachite Dye

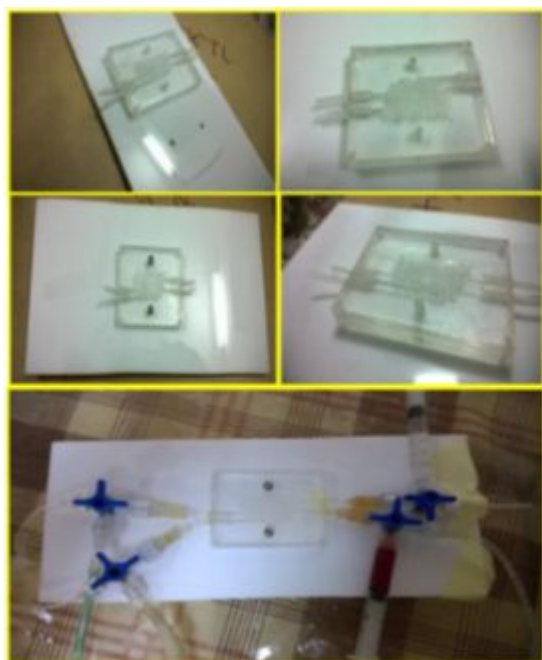


Fig 2. Stages of designing the microfluidic chip with asymmetrical & interfered conduits

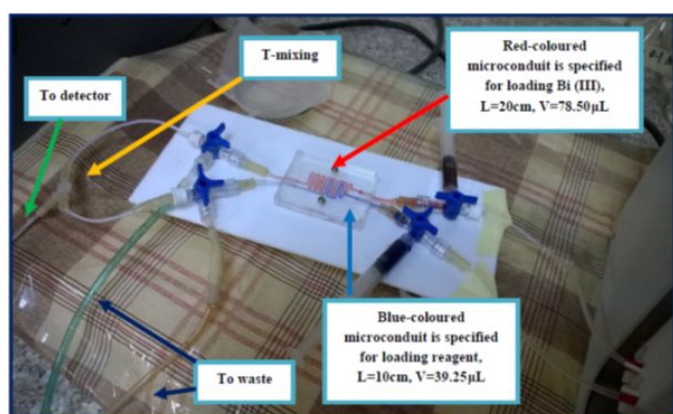


Fig 3. Asymmetrical & interfered micro conduits of the new micro valve

which has the dimensions of few centimeters and provided with micro channels [17-19].

Green Malachite Dye $C_{23}H_{25}ClN_2$ (M. wt=364.9) is an organic compound which artificial green dye, Triphenylmethane dye, used in the color industry, as well as used as anti-microbial in aquaculture, the dye silk materials, paper, leather fibers, and it may be used as anti-bacterial treatment [20-23]. Fig. 1 shows the chemical structure of Green Malachite Dye.

The aim of the present work is design a new micro valve with two interfered micro conduits as attempt for miniaturizing and minimizing the consumption volumes of reaction components towards greener analytical chemistry. The method combines the advantages such as rapidity, low cost, wide range of calibration graph, and satisfactory limit of detection.

EXPERIMENTAL SECTION

Materials

The chemicals were of analytical-reagent grade and used without further purification. Distilled water was used to prepare all solutions. A stock solution of 100 ppm of the dye is prepared by dissolving 0.01 g of Green Malachite Dye then the volume is completed to 100 mL with distilled water in volumetric flask. Working solutions were prepared daily by dilution of stock solutions with distilled water.

Instrumentation

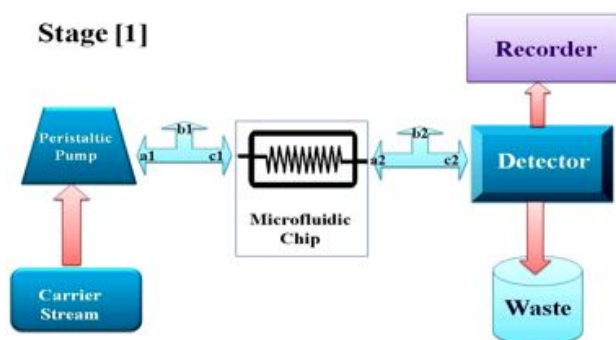
A peristaltic pump (ismatic, Germany), the homemade microfluidic chip, UV-Visible spectrophotometer (Apple), flow cell (450 μ L, Helmma), kompensograph (C 1032 Siemens, Germany), and Teflon tubing throughout of i.d. 1 mm are used.

Procedure

Fabrication of micro valve

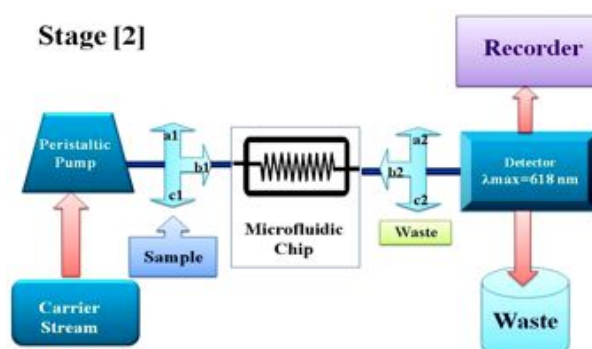
The designing of innovative microfluidic chip with high efficiency and superiority was from available and cheap raw materials. The implementation was by using appropriate alternatives. The stages of designing the new microfluidic chip are showing in details in Fig. 2.

The microfluidic chip contains two conduits with lengths of 10.0 and 20.0 cm with volumes 39.25 and 78.50 μ L. The two conduits with volumes 39.25 and 78.50 μ L are specified for loading Green Malachite dye respectively. The conduits of the dye are merged outside the microfluidic chip and mixed via T-mixing (Fig. 3). The dimensions of microfluidic chip are 6.0 cm \times 4.5 cm. The control process of injection and loading can be implemented by 3-way sub-valves, which are fixed outside the chip [24].



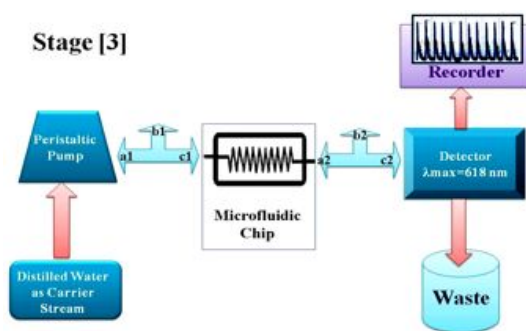
The directions a1, c1, a2, and c2 are opened, but the directions b1 and b2 are closed

Fig 4. Passing of distilled water into the micro flow injection system



The directions c1, b1, b2, and c2 are opened, but the directions a1 and a2 are closed

Fig 5. Injection of Green Malachite Dye into the microconduit



The directions a1, c1, a2, and c2 are opened, but the directions b1 and b2 are closed

Fig 6. Inspiration of sample segment with helping of carrier stream toward the detector and recording of signal

Table 1. Calibration of pump speed

Speed of Pump (rpm)	Flow Rate (mL.min ⁻¹)
30	1.2
40	1.5
50	2.1
60	2.5
70	3.0
80	3.7
90	4.2

Table 2. Effect of flow rate on the response

Flow Rate (mL.min ⁻¹)	Response (cm)
1.2	7.6
1.5	8.1
2.1	9.2
2.5	9.4
3	9.4
3.7	9.3
4.2	10.6

The work stages

The determination of Green Malachite Dye was conducted through three stages, including the passing of distilled water into the home-made injection system and

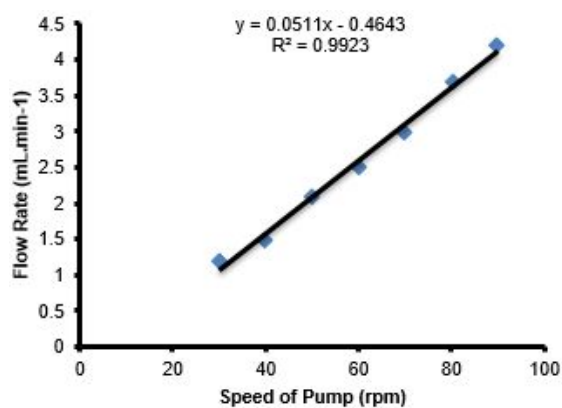


Fig 7. Relationship between speed of pump and flow rate

second injection of Green Malachite Dye into the micro conduit and then the dye is pumped with helping of carrier stream of distilled water, as illustrated in the Fig. 4, 5 and 6.

RESULT AND DISCUSSION

Calibration of Pump Speed

The different values of pump speed is calibrated to convert these values and their unit from round/min (rpm) to mL/min (mL.min⁻¹), as shown in Table 1 and Fig. 7.

Optimum Flow Rate

Flow rate effects on the dilution of solution and conversely affects the response, therefore; flow rate is considered as very important factor. The effect of flow rate is studied at $\lambda_{\max} = 618$ nm and the concentration 12 ppm, the results in Table 2 shows the effect of flow rate on the response. The response increases with increasing of flow rate from 1.2 mL.min⁻¹ to 4.2 mL.min⁻¹.

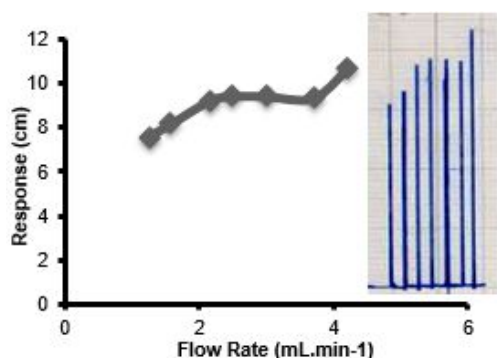


Fig 8. Relationship between response (cm) and flow rate (mL.min⁻¹)

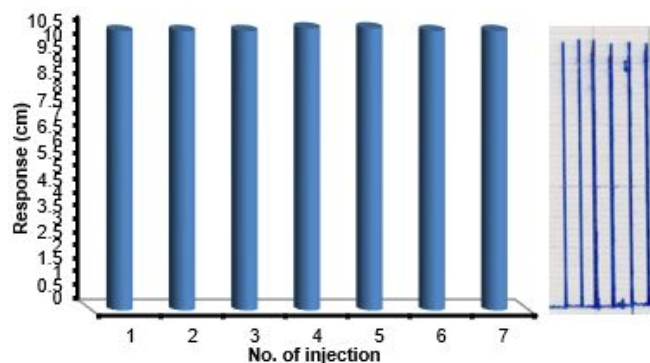


Fig 9. Repeatability at 12 ppm

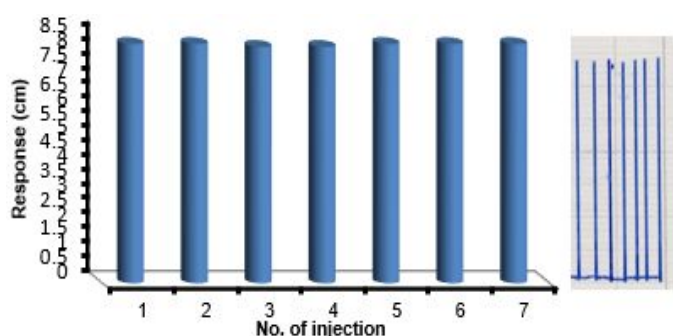


Fig 10. Repeatability at 5 ppm

Table 3. Repeatability values and measured response at two different concentrations

No. of injection	Response (cm) at 5 ppm	Response (cm) at 12 ppm
1	8.2	10.5
2	8.2	10.5
3	8.1	10.5
4	8.1	10.6
5	8.2	10.6
6	8.2	10.5
7	8.2	10.5
SD	0.045	0.048
RSD	1.556	0.465

Table 4. Dispersion coefficient values

Conc. (ppm)	Response (cm) without dilution H ^o	Response (cm) with dilution H ^{max}	Dispersion coefficient D
9	13.5	10.5	1.28
2	3.3	1.9	1.73

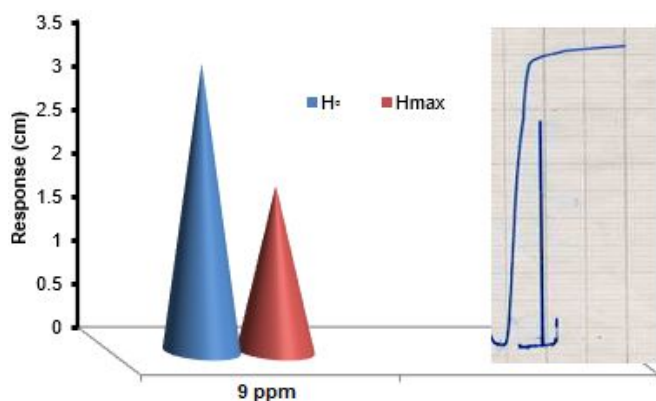


Fig 11. Dispersion coefficient at 9 ppm

The optimum flow rate is 4.2 mL.min⁻¹ according to Fig. 8.

Repeatability

Repeatability is the successive injection of the same concentration of the sample under the same

optimum conditions. The purpose of this study is to prove the precision of injection and consequently the efficiency of the new system [25-26]. The effect of repeatability is studied at λ_{max} = 618 nm and flow rate 4.2 mL.min⁻¹, the given results in Table 3 shows the values of repeatability and the measured response at the two concentrations 5 ppm and 12 ppm. The number of injections are 7 (n = 7) according to the Fig. 9 and 10.

Dispersion Coefficient

The coefficient of dispersion (D) is the most popular experimental parameter able to measure the degree of dilution of the sample from injection point until its passage before the detector. The dispersion coefficient (D) is easily calculable by using the following equation [27].

$$D = H^o / H^{max} \tag{1}$$

where H^o: peak height without dilution outside the FIA system; H^{max}: peak height with dilution inside the FIA system.

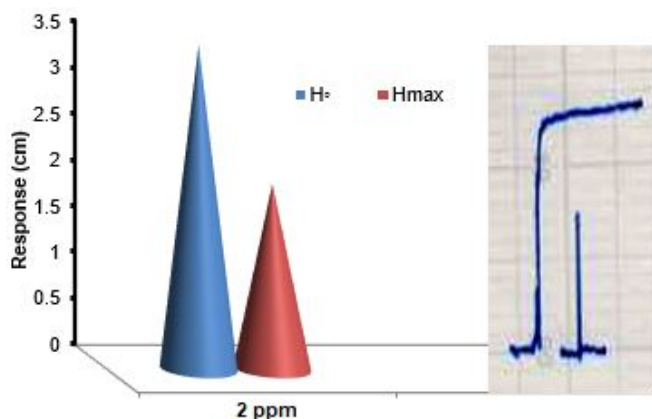


Fig 12. Dispersion coefficient at 2 ppm

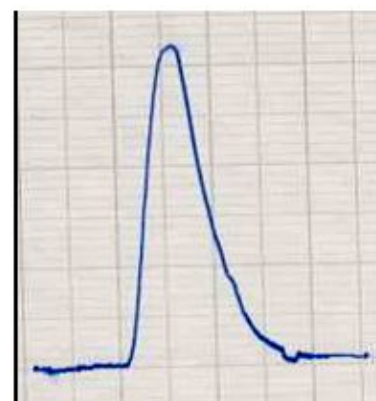


Fig 13. Zero dead volume

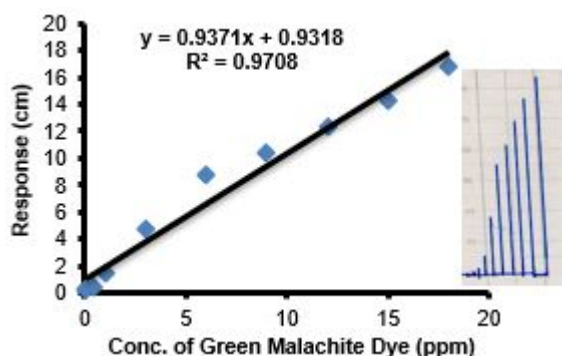


Fig 14. Calibration graph for Green Malachite Dye by using micro valve

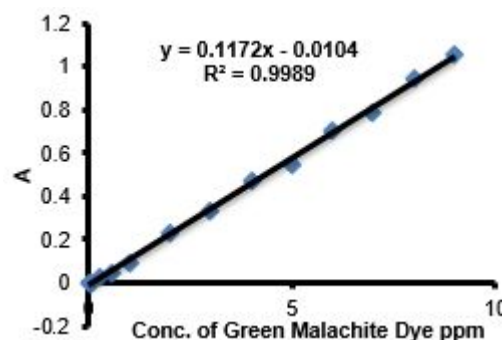


Fig 15. Calibration graph for Green Malachite Dye by spectrophotometric method

Table 5. Calibration graph for Green Malachite Dye by μ FIA method

Seq. No.	Conc. of Dye (ppm)	Response \bar{Y} (cm) $n=3$
1	0.05	0.2
2	0.10	0.3
3	0.50	0.5
4	1.00	1.5
5	3.00	4.6
6	6.00	8.8
7	9.00	10.5
8	12.00	12.4
9	15.00	14.2
	18.00	16.9

The effect of dispersion is studied at $\lambda_{\max} = 618$ nm and flow rate $4.2 \text{ mL}\cdot\text{min}^{-1}$ for the two concentrations 9 ppm and 2 ppm. The dispersion values are calculated according to the equation (1) and the results are in Table 4 and illustrated through the Fig. 11 and 12.

Dead Volume

Dead volume is the remaining volume in the injection valve after sample injection and recording its response. If there is a remaining volume a response will

appear without injection of the sample, in this case the dead volume not equals zero. The home-made micro valve has zero dead volume (Fig. 13) because the measured response starts from zero base line ascending to the maximum of the peak then descending again to the base line during the washing. This process is repeated after every new injection of the sample to show the efficiency of the micro valve.

Sampling Rate

The numbers of analyzed samples that can be made in a time are studied at fixed previously optimum conditions. The time of reaction is calculated per hour by using stopwatch found that the time of the measured response starts from zero base line ascending to the maximum of the peak then descending again to the base line requires 35 sec. Therefore; the sampling rate is 102 samples per hour.

Calibration Graph

μ Flow injection method

At $\lambda_{\max} = 618$ nm and flow rate $4.2 \text{ mL}\cdot\text{min}^{-1}$, the effect of changing the dye concentration is studied. It is

found that the sensitivity of response increases from 0.2 cm to 16.9 cm with increasing of the concentration from 0.05 ppm to 18.00 ppm with linearity $R^2=0.9708$ (see Table 5 and Fig. 14).

Spectrophotometric Method

The calibration graph for Green Malachite Dye is determined at $\lambda_{\max} = 618$ nm spectrophotometrically. It is found that the linear range 0.01-9.00 ppm with limit of detection 0.01 ppm and linearity $R^2 = 0.9989$, according to the results in Table 6 and Fig. 15.

Statistical Analysis [28-29]

The analysis of variance (ANOVA) and student 't' test, is conducted and the results are according to the Table 7 and 8 by using Table Curve 2d v5.01 Systat Software. All measurements are replicated three times ($n = 3$) and the results are expressed as (mean $\pm t_{0.025} \frac{S.D}{\sqrt{n}}$).

The Table 7 shows that t_{cal} is greater than t_{tab} in both two methods, in μ FIA method ($t_{\text{cal}} 16.31 \gg t_{\text{tab}} 2.31$) and $r = 0.9853$, and in spectrophotometric method ($t_{\text{cal}} 10.44 \gg t_{\text{tab}} 2.18$) and $r=0.9995$. Therefore, there is linear relationship between the response and the concentration of Green Malachite Dye.

The critical values F_{tab} ($F_9^1 = 5.12$ and $F_{13}^1 = 4.67$) are less than the calculated values of F ($F_{\text{stat}} = 266.027$, and 10971.2) in μ flow injection and spectrophotometric methods respectively. The results approach to linear state, because, there is significant difference at 95%

confidence interval between the variance due to regression and the variance due to error, as shown in Table 8.

APPLICATION

The micro flow injection method is applied for prepared solutions of Green Malachite Dye successfully. The obtained results show good recoveries and satisfied standard deviation values (Table 9). The percentage of relative error and recovery are calculated by using the equations (2) and (3) respectively.

$$\text{R.E. \%} = \frac{\text{Actual value} - \text{Measured value}}{\text{Known value}} \times 100 \quad (2)$$

$$\text{Recover \%} = 100 \pm \text{R.R. \%} \quad (3)$$

Table 6. Calibration Graph for Green Malachite Dye by Spectrophotometric Method

Seq. No.	Conc. of Dye (ppm)	Absorbance
1	0.01	0.002
2	0.05	0.004
3	0.10	0.006
4	0.30	0.031
5	0.60	0.044
6	1.00	0.099
7	2.00	0.226
8	3.00	0.336
9	4.00	0.463
10	5.00	0.551
11	6.00	0.704
12	7.00	0.791
13	8.00	0.943
14	9.00	1.051

Table 7. Summary of linear regression equation results of the form $Y=bX+a$ for the determination of Green Malachite Dye

μ FIA Method					
Linear range ppm	No. of measurements (n)	Straight line equation $\hat{Y} = (a \pm s_a t) + (b \pm s_b) [\text{Green Malachite Dye}]$	Correlation coefficient (r)	Calculated t-value = $\frac{ r / \sqrt{n-2}}{\sqrt{1-r^2}}$	Tabulated t-value at 95% confidence interval
0.05-18.00	10	$\hat{Y} = 0.93 \pm 1.79 + 0.93 \pm 16.13 [\text{Green Malachite Dye}]$	0.9853	16.31	2.31
Spectrophotometric Method					
Linear range ppm	No. of measurements (n)	Straight line equation $\hat{Y} = (a \pm s_a t) + (b \pm s_b) [\text{Green Malachite Dye}]$	Correlation coefficient (r)	Calculated t-value = $\frac{ r / \sqrt{n-2}}{\sqrt{1-r^2}}$	Tabulated t-value at 95% confidence interval
0.01-9.00	14	$\hat{Y} = 0.01 \pm (-2.06) + 0.12 \pm 104.74 [\text{Green Malachite Dye}]$	0.9995	10.44	2.18

\hat{Y} = Estimated response (mV)

Table 8. The results of ANOVA test for straight line equation $Y = bX + a$

μFIA Method					
Source of variance	Sum of squares (SSq)	Degree of freedom (Df)	Mean squares (MSq)	F-statistic $F = \frac{S_1^2}{S_0^2}$	F-tabulated
Due to regression	$\sum(\hat{Y}_i - \bar{Y})^2 = 353.26556$	$v_1 = 1$	$S_1^2 = 353.26556$	266.027	5.12
Due to error (about regression)	$\sum(Y_i - \hat{Y}_i)^2 = 10.623445$	$v_2 (n-2) = 8$	$S_0^2 = 1.3279306$		
Total	363.889	$v_{Total} (n-1) = 9$			
Spectrophotometric Method					
Source of variance	Sum of squares (SSq)	Degree of freedom (Df)	Mean squares (MSq)	F-statistic $F = \frac{S_1^2}{S_0^2}$	F-tabulated
Due to regression	$\sum(\hat{Y}_i - \bar{Y})^2 = 1.8383322$	$v_1 = 1$	$S_1^2 = 1.8383322$	10971.2	4.67
Due to error (about regression)	$\sum(Y_i - \hat{Y}_i)^2 = 0.0020107257$	$v_2 (n-2) = 12$	$S_0^2 = 0.00016756048$		
Total	1.8403429	$v_{Total} (n-1) = 13$			

Table 9. Determination of Green Malachite Dye in prepared solutions by using the micro valve

Sample	Taken concentration ppm	Found concentration ppm	$E_r\%$	Recovery%
Green Malachite Dye	5	5.02	-0.4	100.04
	12	11.97	0.25	99.75

CONCLUSION

The μflow injection system is designed from available and low cost materials for the determination of Green Malachite Dye. The home-made micro valve is manufactured at Flow Injection Lab in Chemistry Department-College of Science of Babylon University. The μflow injection system is characterized with simplicity, rapidity, sensitivity, wide range of concentrations, and low consumption of chemicals towards greener analytical chemistry. In addition of high efficiency due to zero dead volume, and clear form of responses.

REFERENCES

- [1] Van der Linden, W.E., 1994, Classification and definition of analytical methods based on flowing media (IUPAC Recommendations 1994), *Pure Appl. Chem.*, 66 (12), 2493–2500.
- [2] Ružička, J., and Hansen, E.H., 1975, Flow injection analyses: Part I. A new concept of fast continuous flow analysis, *Anal. Chim. Acta*, 78 (1), 145–157.
- [3] Ružička, J., and Stewart, J.W.B., 1975, Flow injection analysis: Part II. Ultrafast determination of phosphorus in plant material by continuous flow spectrophotometry, *Anal. Chim. Acta*, 79, 79–91.
- [4] Stewart, J.W.B., Ružička, J., Bergamin, H., and Zagatto, E. A., 1976, Flow injection analysis: Part III. Comparison of continuous flow spectrophotometry and potentiometry for rapid determination of the total nitrogen content in plant digests, *Anal. Chim. Acta*, 81 (2), 371–386.
- [5] Ružička, J., Stewart, J.W.B., and Zagatto, E.A., 1976, Flow injection analysis: Part IV. Stream sample splitting and its application to the continuous spectrophotometric determination of chloride in brackish waters, *Anal. Chim. Acta*, 81 (2), 387–396.
- [6] Stewart, J.W.B., and Ružička, J., 1976, Flow injection analysis: Part V. Simultaneous determination of nitrogen and phosphorus in acid digests of plant material with a single spectrophotometer, *Anal. Chim. Acta*, 82 (1), 137–144.
- [7] Hansen, E.H., and Ružička, J., 1976, Flow injection analysis: Part VI. The determination of phosphate and chloride in blood serum by dialysis and sample dilution, *Anal. Chim. Acta*, 87 (2), 353–363.
- [8] Betteridge, D., and Ružička, J., 1976, The determination of glycosol in water by flow-injection analysis - a novel way of measuring viscosity, *Talanta*, 23 (5), 409–410.
- [9] Trojanowicz, M., 2010, *Advances in Flow Analysis*, 2nd Ed., Willey-VCH verlag GmbH and Co. KGaA, 22–25.
- [10] Kolev, S.D., McKelvie, I.D., and Barceló, D., 2008, *Comprehensive Analytical Chemistry: Advances in*

- Flow Injection Analysis and Related Techniques*, 1st Ed., Elsevier, Australia, 25, 83, 96.
- [11] Taha, D.N., and Obaid, Z.S., 2016, Designing flow injection unit for chromates determining, *Res. J. Pharm. Biol. Chem. Sci.*, 7 (6), 2241–2250.
- [12] Naser, N.A., Kadim, K.H., and Taha, D.N., 2012, Synthesis and characterization of an organic reagent 4-(6-bromo-2-benzothiazolylazo) pyrogallol and its analytical application, *J. Oleo Sci.*, 61 (7), 387–392.
- [13] Abbas, G.J., Mashkour, M.S., and Taha, D.N., 2016, Flow injection spectrophotometric determination of penicillamine in pharmaceutical formulation using 1,2-naphthoquinone-4-sulfonate, *PURE*, 5 (4), 92–105.
- [14] Vurck, G., 1981, *Anal. Chim. Acta*, 14, 288.
- [15] Ayala, A., Leal, L.O., Ferrer, L., and Cerdà, V., 2012, Multiparametric automated system for sulfate, nitrite and nitrate monitoring in drinking water and wastewater based on sequential injection analysis, *Microchem. J.*, 100, 55–60.
- [16] Ružička, J. and Hansen, E.H., 1984, Integrated microconduits for flow injection analysis, *Anal. Chim. Acta*, 161, 1–25.
- [17] Skoog, D.A., West, D.M., Holler, F.J., and Crouch, S.R., 2014, *Fundamentals of Analytical Chemistry*, 9th Ed., Brooks/Cole, Cengage Learning, 168.
- [18] Duffy, D.C., McDonald, J.C., Schueller, O.J.A., and Whitesides, G.M., 1998, Rapid prototyping of microfluidic systems in poly(dimethylsiloxane), *Anal. Chem.*, 70 (23), 4974–4984.
- [19] van den Berg, A., and Lammerink, T.S.J., 1998, Micro total analysis systems: microfluidic aspects, integration concept and applications, *Top. Curr. Chem.*, 194, 21–49.
- [20] Quintella, C.M., Watanabe, Y.N., Lima, A.M.V., Korn, M., Pepe, I., Embiruçu, M., and Musse, A.P.S., 2004, Evaluation of transversal and longitudinal dispersion in a flow injection system by exploiting laser induced fluorescence: influence of flow-cell positioning, *Anal. Chim. Acta*, 523, 293–300.
- [21] Taha, D.N., and Majeed, A.S., 2015, Innovative design of microfluidic chip with two asymmetrical & interfered conduits and its applications for the determination of Bi(III) in some pharmaceutical formulations, *Iraqi patent*, 4392.
- [22] Akyar, I. (Ed.), 2011, *Wide Spectra of Quality Control*, InTech.
- [23] Watson, D.G., 1999, *Pharmaceutical Analysis-A Textbook for Pharmacy Students and Pharmaceutical Chemists*, Churchill Livingstone.
- [24] Trojanowicz, M., 2008, *Advances in Flow Analysis*, WILEY-VCH, 16.
- [25] Miller, J.N., and Miller, J.C., 2010, *Statistics and Chemometrics for Analytical Chemistry*, 6th Ed., Pearson Education Limited.
- [26] Hibbert, D.B., and Gooding, J.J., 2006, *Data Analysis for Chemistry: An Introductory Guide for Students and Laboratory Scientists*, Oxford University Press, Inc.
- [27] Taha, D.N., Samaka, I.S., and Mohammed, L.A., 2013, Adsorptive removal of dye from industrial effluents using natural Iraqi palygorskite clay as low-cost adsorption, *J. Asian Sci. Res.*, 3 (9), 945–955.
- [28] Taha, D.N., Samaka, I.S., Mohammed, L.A., and Naige, A.S., 2014, Adsorption studies of direct red 28 dye onto activated carbon prepared from low-cost material, *Civ. Environ. Res.*, 6 (7), 149–159.
- [29] Taha, D.N., and Samaka, I.S., 2012, Natural Iraqi palygorskite clay as low cost adsorbent for the treatment of dye containing industrial wastewater, *J. Oleo Sci.*, 61 (12), 729–736.