Journal of Chemical Technology and Metallurgy, 60, 3, 2025, 511-520

TRACE ANALYSIS OF BRILLIANT GREEN DYE BY FLOW INJECTION TECHNIQUE

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Received 24 October 2024
Accepted 27 January 2025
DOI: 10.59957/jctm.v60.i3.2025.17

ABSTRACT

A low-cost and high throughput flow injection analysis (FIA) system design is conducted to determine Brilliant Green (BG) dye. The research includes studying the chemical and physical variables and control of optimal conditions of the FIA system. The merging zone technique uses a homemade flow injection valve to determine BG dye. The optimum conditions are studied, such as the flow rate of the carrier, optimal dye volume, repeatability, dead volume, and dispersion coefficient. The flow rate is 3.0 mL min^{-1}, the optimal volume of dye is 82.425 µL, the repeatability is high for eight measurements (n = 8), and the values of dispersion coefficient are 1.41 and 1.44 for the concentrations of 4 and 7 mg L^{-1}, respectively. The calibration graphs have linear ranges of (0.5 - 10) mg L^{-1} and (0.07 - 16) mg L^{-1}, with detection limits of 0.413 and 0.068 mg L^{-1} for the spectrophotometric and FIA methods, respectively. The results obtained were statistically treated. FIA method successfully applied to standard solutions demonstrates its reliability and potential for further applications.

Keywords**:** *flow injection, home-made valve, Brilliant green, dispersion, dead volume.*

INTRODUCTION

Brilliant green (BG) is a synthetic hazardous dye that can cause skin, gastrointestinal tract irritation, lung, and eye problems [1, 2]. BG is presently used for various applications, including silk, dyeing paper, wool, leather, and metal detection and determination [3]. BG dye is employed in paper ink production, biological sample staining, the textile sector, veterinary medicine, cosmetics, mold inhibitors or fungal propagation, the food industry, and disinfectants [4, 5]. Thus, this dye's presence relates to significant environmental impact and potential health disorders [6, 7]. Therefore, our research, which provides a method to determine harmful BG dye after removing it from the water by different treatment techniques, is crucial in addressing these concerns.

Due to the mentioned relevance of BG dye, various analytical methods were documented in the literature for determining BG and similar dyes, including high-performance liquid chromatography [8], electro-chemiluminescence [9], conductometry [10], Micro-Cloud-Point Extraction-spectrophotometry [11], capillary electrophoresis [12], electrochemistry [13], spectrophotometry [14, 15], and liquid chromatography-mass spectrometry [16]. Most of these techniques require skilled analysts, come with substantial operating and maintenance expenses, and are intricate and time-consuming [17].

In recent decades, flow injection analysis (FIA) employing several detection techniques has been established. A peristaltic pump, which aspirates the carrier stream and reagent solution through the system at a steady flow rate, an injection unit, a reaction coil, a detector, and a recorder of the detector signal make up the FIA system [18]. Most of the developed FIA applications identify inorganic analytes, elemental speciation studies, and measure several total water quality indices [19]. For example, FIA has determined organic residues in seawater, natural surface waters, drinking water, and groundwater [20, 21]. These include drugs, dyes, pesticides, surfactants, and phenolic compounds [22]. FIA determination improves operational parameters, including sampling rate, detection limits, required analyte volume, measurement range, and selectivity [23].

The valve is an important component in flow injection analysis devices since it is where the sample and reagents required for the chemical reaction are injected [24]. Initially, a movable handle valve was utilized, like those used in high-performance liquid chromatography, followed by the multi-position valve [25]. Recently, the valves have been programmed to control a material injection, although all those valves are regarded to be extremely pricey [24]. Homemade plastic valves can accommodate a wide range of chemical solutions because of the possibility of setting several loops into the design [26].

The present study aims to determine BG dye in an aqueous solution by a simple, rapid, low-cost FIA method with more miniaturization. This work is based on manufacturing a single-loop injection valve and designing a flow injection system to estimate the dye spectroscopically at 625 nm.

EXPERIMENTAL

Materials

Analytical grade brilliant green dye (Merck, MW: 482.63) is used in this study. As in the literature, it was found practically that the dye absorbs light spectrally at a wavelength of 625 nm [27]. The stock solution of BG was prepared by dissolving 1 g of BG dye in the solid phase and completing the volume of the solution to 1 L with distilled water to obtain 1000 mg L^{-1}. The desired concentrations are prepared using a gradient dilution process. This dye's structural formula and maximum wavelength are shown in Fig. 1. Sodium hydroxide (BDH), and hydrochloric acid (Chem-Lab) were used to pH adjust.

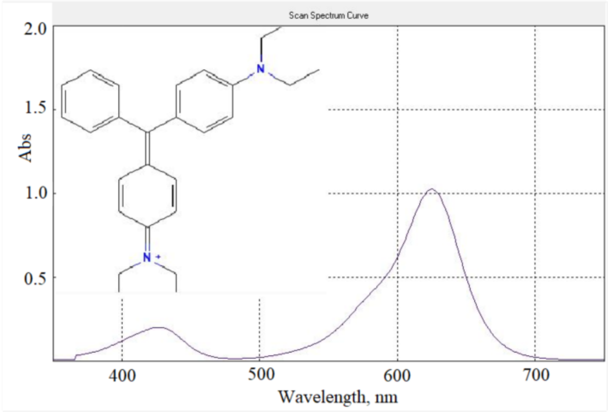

Fig. 1. Structural formula and maximum wavelength of BG dye at 100 mg L^{-1}.

Instrumentation

A one-loop handmade valve linked to the detector and a peristaltic pump (Ismatec, Germany) is included with the FIA system. For both the spectrophotometric and FIA method, a UV-Vis spectrophotometer (Shimadzu UV-1700, Japan) with a 1 cm optical path cell and (PD-303UV APEL, Japan) with a cell volume of 450 µL (Helmma, NY) were used. Kompensograph (C 1032 Siemens, Germany) was connected to a spectrophotometer to record the signal as a peak. Analytical balance (Denver Instrument, Germany), loops of Teflon with an id of 1 mm, and connectors of silicon rubber were used. The pH was adjusted using a pH meter (Philips Pw 9421, Germany).

Work stages of the flow injection valve

First, distilled water is passed into the FIA system. Then, the injection of BG dye into the homemade valve is conducted, followed by pumping the dye with helping of a carrier of distilled water, as shown in Fig. 2. In stage A, the sub-valves (1, 2) are in the direction in which water is pumped into all system parts (plank solution stage). Stage B: the pump is stopped, and the sub valves (1, 2) are moved in the direction that the syringe injects the dye to the sample loop; the excess is excreted through the valve. Stage C: the sub-valves (1, 2) are returned to the first position, and the H_2O carrier loads the dye. The carrier solution of H_2O was adjusted to a pH equal to 7 by using 0.1 M sodium hydroxide or 0.1 M hydrochloric acid.

Fig. 3 demonstrates the process of mechanical process for the manufactured valve. In the first step, distilled water was adjusted at pH = 7 as the carrier was impelled into all the flow injection system parts by a peristaltic pump with a flow rate of 3 mL min^{-1}. The aqueous solution of H_2O stands for the blank. A 0.1 M sodium hydroxide or 0.1 M hydrochloric acid was used to adjust the pH of the carrier solution. In the second step, the pumping of the H_2O carrier stream stopped, and then the solution of 82.4 µL of BG dye was injected into the loop. The dye showed maximum absorption at the maximum wavelength of 625 nm. The change in the height response of the Kompensograph was relative to the brilliant dye concentration /reference carrier H_2O.

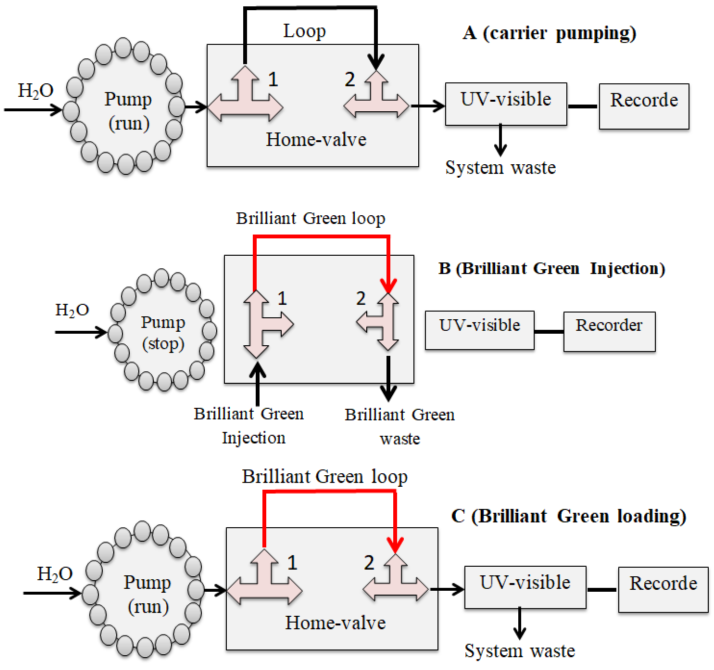

Fig. 2. Work stages of flow injection valve: (A) Pumping distilled water stage through the FIA system, (B) injection of BG Dye stage to loop, and (C) loading stage of the BG sample towards the detector and recording the analytical signal.

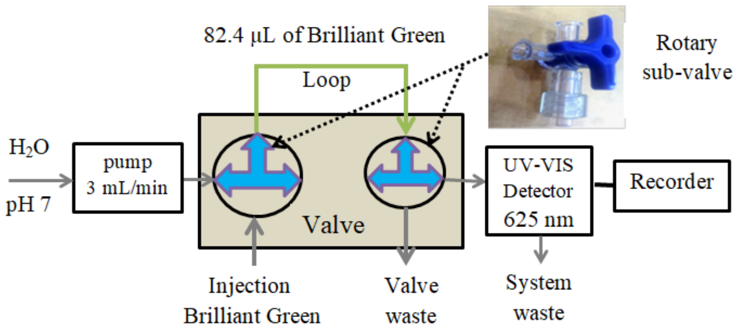

Fig. 3. Graph of the homemade valve´s machine-driven operation.

RESULTS AND DISCUSSION

Effect of flow rate

Flow rate effect on measurement sensitivity is one of the influences that affect the dilution process of the sample zone [28]. The flow rate of the reaction is controlled by changing the speed of the pump propelling the carrier solution [29]. While a slow flow rate is necessary for slow reactions as it increases sensitivity, a high flow rate is characteristic of fast reactions that do not require a long reaction time [30]. A peristaltic pump was used to study the impact of carrier flow rate on peak height in the FIA, ranging from 1000 to 6000 μL per min. The effect of flow rate was studied at wavelength 625 nm for dye concentrations of 4 and 7 mg L^{-1} using a volume of 78.50 μL. It was detected that the response increases gradually when the flow rate increases from 0.70 to 3 mL min^{-1} for both concentrations, reaching the flow rate of 3 mL min^{-1}, where the response is at its maximum. At flow rates upper than 3 mL min^{-1}, a decrease in the response is observed for both concentrations. This can be attributed to the effect of high dispersion. Therefore, the optimal flow rate of 3 mL min^{-1} was adopted in the measurement, as shown in Fig. 4.

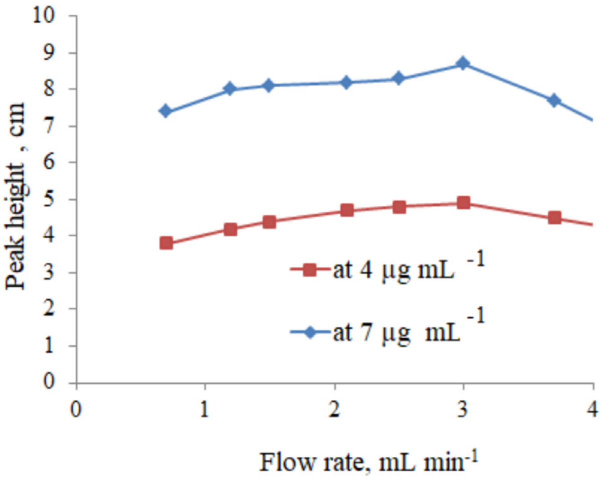

Fig. 4. Flow rate effect on peak height at 4 and 7 mg L^{-1} of BG dye.

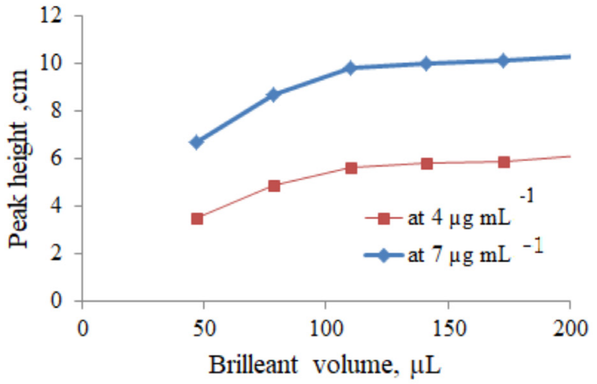

Fig. 5. BG volume effect on peak height at 4 and 7 mg L^{-1}.

Effect of Brilliant Green volume

Sample volume significantly influences the dispersion zone, which was studied using different length loops [28]. Generally, increasing sample volume leads to a rise in response, while accessing sample volume results in imperfect dispersion that produces double and broad peaks [31]. Valve loops of sample injection ranging in volume from 100 to 1000 μL were used to optimize the sample volume in the FIA. At 625 nm, 3 mL min^{-1} flow rate, and 4 and 7 mg L^{-1} of BG dye, the effect of volume dye was studied, and the response was recorded for each volume. The volumes of BG dye under study were 47.1, 78.5, 109.9, 141.3, 172.7, and 204.2 μL. The outcome is shown in Fig. 5. When the BG dye volume was increased, the peak height increased. After the volume of 109.9 μL, the response remains constant for both concentrations of 4 and 7 mg L^{-1}. 109.9 μL of BG dye was selected as the optimum volume.

Repeatability

Repeatability means recurring measurements for the same concentration several times at least six measurements (n = 6) [32]. By measuring the repeatability, it is possible to know the method efficiency and the precision of the manufactured unit under optimal conditions. The impact of the re-injection sample is established at a flow rate of 3 mL min^{-1} and dye volume of 109.9 mL, where the results listed in Table 1 show the repeatability values and response measurement at the concentration 5 mg L^{-1} seven times (n = 8), where the measurements show good repeatability at R.S.D less than 2 %.

Dispersion coefficient

The dispersion coefficient is the proportion between the concentration before and after dilution, denoted by the D [33]. The dispersion coefficient was

Table 1. Repeatability of measurements at 1 and 10 mg L^{-1} of BG.

No. of Reading (n)	Response, cm	
	Conc., 1 mg L^{-1}	Conc., 10 mg L^{-1}
1	1.1	10.4
2	1.15	10.4
3	1.1	10.3
4	1.1	10.4
5	1.1	10.4
6	1.1	10.4
8	1.1	10.4
SD	0.017	0.0451
RSD	1.580	0.4355

Table 2. Determine and calculate the dispersion coefficient.

Conc., mg L^{-1}	Response before dilution H^0, cm	Response after dilution H_{max}, cm	Dispersion coefficient $D = H^0 / H_{max}$
4	9.2	6.5	1.42
7	12.6	8.7	1.45

estimated based on the response height or peak since it is determined by dividing the value of H^o by H_{max} [34]. H^o is the response height before dispersion, while H_{max} is the response height after dispersion. At the optimal conditions for the system designed to estimate the BG dye, which was represented by a flow rate of 5 mL min^{-1} and a dye volume of 109.9 µL and at two different concentrations of 4 and 7 mg L^{-1}, dispersion coefficient value was found, and the results are detailed in Table 2. The values obtained for the dispersion factors 1.42 and 1.45 show that the values are within the limited range of dispersion, which benefits the system due to the high analytical sensitivity.

Sampling rate

The number of samples that can be analysed per h was studied under the optimal conditions previously specified, as the flow rate was 3 mL min^{-1}, and the sample volume of BG dye was five microliters. The time for sample analysis speed was calculated per h using a stopwatch. The time required from the injection sample until the response appeared was 10 sec, while the time required for the response to begin to appear until it returned to the baseline was 21 s. Therefore, the time from the appearance of the peak baseline until the peak reaches the highest level and declines to the zero point is 31 s, which is the time for analysing a single sample. Thus, the system can analyse 113 samples per h, one of the advantages of FIA technology, which is characterized by analysing many samples per h. Fig. 6a displays how the sampling rate was calculated.

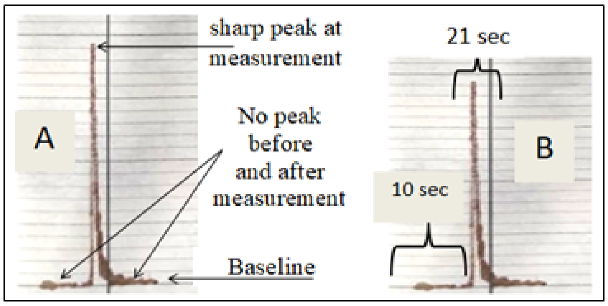

Fig. 6. (a) The dead volume of the homemade valve, (b): sampling rate.

Dead volume

The dead volume is the residual volume in the valve parts throughout the injection of the sample or reagents [35]. It leads to the appearance of a double, distorted, false peak or signal at the baseline, in which case the manufactured valve is imperfect. Nevertheless, the valve is ideal if the dead volume equals zero [36]. The measured responses are in the form of peak heights in which the single peak starts from the zero baselines when analysing and then returns to the zero baselines after the completion of the analysis. No other peaks appear except with re-injection. This case is ideal, and the valve has a zero dead volume. Fig. 6b shows the sharp peak shape in the homemade valve with zero dead volume, where no false peak appears on the baseline before and after the actual injection of the BG dye sample. In previous studies, various designs of homemade valves were manufactured to operate with a dead volume equal to zero [33].

Calibration graph

BG dye calibration graphs of the flow injection method and spectrophotometers were structured by implementing measurements at the optimum conditions. A concatenation of BG dye concentrations was prepared to estimate the calibration curve, as shown in Fig. 7. For the two techniques, the analytical data of the calibration

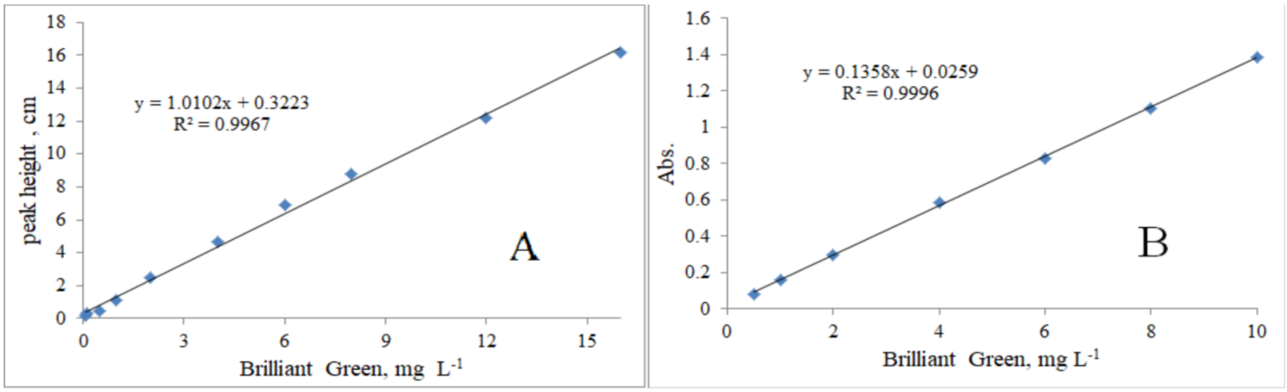

Fig. 7. Calibration curve: (A) FIA method, (B) spectrophotometric method.

graphs, which include Beer's law linearity, an average standard deviation, RSD, detection limit, quantification limit, molar absorptivity, and Sandell's sensitivities are shown in Table 3.

The results are included in Table 3. It must be noted from the above results that the deviation from the Beer-Lambert Law is at a concentration of 10.00 mg L^{-1} for the spectrophotometric method, while this deviation is at a concentration of 16.00 mg L^{-1} for the flow injection method.

Statistical analysis [37]

The statistical analysis includes student 't' tests and analysis of variance (ANOVA). Statistical analyses are performed according to the formulas using Table Curve 2d v5.01 Systat Software. Table 4 shows that the relationship between the concentration of BG dye and the response is linear because that is greater than TTAB in both flow injection and spectrophotometric methods. In the flow injection method (t_{cal} 148.15 >> t_{tab} 2.36) and r = 0.9911, while in the spectrophotometric technique (t_{cal} 27.45 >> t_{tab} 2.45) and r = 0.9960.

From the results of Table 5, it is illustrated that the critical values F_{tab} ($F^1{}_8 = 5.52$, and $F^1{}_7 = 5.99$) are less than the calculated values of F ($F_{stat} = 389.537$ and 757.494) in FIA and spectrophotometric techniques, respectively. At a 95 % confidence interval, the significant and fundamental difference between the variance resulting from regression and the variance resulting from error.

Applications

The newly designed flow injection system has been successfully applied to determine BG dye in the synthesized standard solutions of the dye. Table 6 summarizes the results of flow injection method applications for determining the mentioned dye with 4.00 and 7.00 mg L^{-1} of prepared standard solutions. The results are satisfactory because the reproducibility is high RSD % < 1 % with high recovery, too.

Table 3. The analytical data of calibration curve for determining BGD.

Analytical data of calibration curves		
Parameter	FIA	Spectrophoto-metric
Range, mg L^{-1}	0.07 - 16	0.5 - 10
Molar absorptivity, L mol^{-1} cm^{-1}	4.67×10^5	6.28×10^4
Sandal sensitivity, µg cm^{-2}	99×10^{-5}	736×10^{-5}
Detection limit, mg L^{-1}	0.068	0.413
Quantification limit, mg L^{-1}	0.205	1.23
Intercept	0.3223	0.0259
Slop	1.0103	0.1358
r^2	0.9967	0.9996

Table 4. Synopsis of linear regression equation results of the formula Y = bX + a for the determination of BG dye.

FIA Technique					
Linear range, mg L^{-1}	No. of measurements (n)	Equation of straight line $\hat{Y} = (a \pm s_a t) + (b \pm s_b)$ [Brilliant Green 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 - 12	10	$\hat{Y}$ = 0.35 ± 1.30 + 0.98 ± 19.74 [Brilliant Green dye]	0.9911	148.15	2.36
Spectrophotometric Technique					
Linear range, mg L^{-1}	No. of measurements (n)	Equation of straight line $\hat{Y} = (a \pm s_a t) + (b \pm s_b)$ [Brilliant Green 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 - 10	7	$\hat{Y}$ = - 0.06 ± (- 2.12) + 0.21 ± 27.52 [Brilliant Green dye]	0.9960	27.45	2.45
$\hat{Y}$ = Estimated response					

Table 5. The outcomes of ANOVA test for equation of straight-line Y = bX + a.

FIA Method					
Variance source	Squares sum (SSq)	Freedom degree (Df)	Mean squares (MSq)	F-statistic $F = \frac{S_1^2}{S_0^2}$	F-tabulated
Due to regression	$\sum(\hat{Y}_i - \bar{Y})^2 = 135.11203$	$\nu_1 = 1$	$S_1^2 = 135.11203$	389.537	5.32
Due to error (about regression)	$\sum(Y_i - \hat{Y}_i)^2 = 2.4279684$	ν_2 (n-2) = 7	$S_0^2 = 0.34685263$		
Total	137.54	ν_{Total} (n-1) = 8			
Spectrophotometric Method					
Variance source	Squares sum (SSq)	Freedom Degree (Df)	Mean squares (MSq)	F-statistic $F = \frac{S_1^2}{S_0^2}$	F-tabulated
Due to regression	$\sum(\hat{Y}_i - \bar{Y})^2 = 2.6841422$	$\nu_1 = 1$	$S_1^2 = 2.6841422$	757.494	5.99
Due to error (about regression)	$\Sigma(Y_i - \hat{Y}_i)^2 = 0.021260693$	ν_2 (n - 2) = 6	$S_0^2 = 0.0035434488$		
Total	2.7054029	ν_{Total} (n - 1) = 7			

CONCLUSIONSx

Determination of BG dye by using the homemade FIA unit is characterized by high sensitivity in determining the low concentrations, rapidity in analysis, and extensive range concentration of FIA calibration graphs comparatively with a spectrophotometric method. It is possible to determine BG dye by spectrophotometric method. Still, it requires more time, the consumption of chemicals in large quantities, and the low concentrations cannot be measured. Depending on the results obtained from the current study, the FIA method is more efficient, accurate, and fast for determining BG dye compared to the spectrophotometric method. The simplicity of

Table 6. Determination of BG dye in standard solution by FIA technique.

Sample	Taken conc., mg L^{-1}	Found conc., mg L^{-1}	SD	RSD, %	Er, %	Recovery = 100 ∓ Er, %
BG Dye	4.00	4.01	0.0020	0.0034	0.25	99.75
	7.00	7.02	0.0030	0.0038	0.35	99.65

injection valve manufacturing and the high flexibility of flow injection system design allow more complex designs to be easily developed for application to more complex samples. Recycling plastic materials and utilizing them in the manufacture of flow injection valves is a positive step towards protecting the environment and reducing the economic cost of manufacturing to the lowest possible level.

Acknowledgement

We thank Dr. Dakhil N. Taha, the professor of analytical chemistry at Al-Mustaqbal University College in Babylon City, who offered knowledge and experience that significantly aided the research.

Authors' contribution*: Dakhil Nassir Taha was responsible for supervision and leadership in the planning and implementation of research activities, including external guidance and direction to the core team. Ahmed Saleh Farhood carried out the experimental part, designed the flow injection system, and fabricated the flow injection valve. Amal Saadoon Majeed wrote the manuscript, processed the obtained experimental data, and accomplished the statistical analysis. Rahmah Hashim Abdullah involved in performing the measurements and designed the figures. Nahlah Salman Saddam developed the theoretical part and work out some of technical details. All the authors contributed discussing the results and commenting on the manuscript.*

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