Lab build Fully Automated microfluidic flow injection System

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Abstract. A fully automated microfluidic Flow Injection spectrophotometric system was designed in our laboratory with methylene blue used as an example. The device consists of a double-line microfluidic chip with dimensions (30 μl x 4 cm), each 15 μl volume intended to allow a very small volume of reagent and sample to be used. The microfluidic system also consists of two types of Arduino software. The first was the UNO of a type used to control the two mini peristaltic pumps to drive the water and methylene blue as carrier stream and sample respectively. The Mega type, which was equipped with homemade software, signal-to-peak, was the second Arduino which used as a data logger to record the results in the form of peaks using Microsoft Excel 2016. The peak's height was corresponding to concentration. The linearity was in the range (0.025-0.125 μg / ml) with a five-point regression coefficient (0.9981), RSD% for ten replicates is 0.025 μg / mL. The detection limit was (0.001 μg / ml) and the sample throughput was 300 samples per hour and each sample required 25 μl of chemical reagents. Therefore, 240 samples need only (5.0 ml) from the chemical reagents, which clearly indicates that the consumed reagent and the waste were very low. Therefore, the method for determining methylene blue with a fully automated system is very environmentally friendly, save time, consumption regent, Improve quality, decrease cost-consumption reducing the consume of sample and inherited the reproducibility and accurate from flow injection analysis.

1. Introduction
Automation is the use of technology to perform processes without human aid[1]. In the situation of analytical chemistry, automation may include operations such as measurement of signals and computation of the results, which corresponding to the concentration of the calculated sample[2].

Nowadays, these processes can be easily operated and interfaced with analytical instruments by using a lot of smart devices, which are available in low prices in local markets[3]. Recently, many papers were published concern with the design of homemade microfluidic flow injection systems from our lab for phosphate, chloride, and nitrite determination[4]. In this study, it was decided to developed these systems from semi-automated to fully automated systems. Two microcontrollers types Arduino were used, one UNO to manipulating the two lab-made mini peristaltic pumps to drive the carrier reagent and to suck the right volume of sample by selects the desired speed. The second one Mega was equipped with a home-made software, single- to-peak, which was used as a data logger to recorded the signal as smooth peak height to concentration with aid of Microsoft Excel 2016[5,6,7,8,9,10].

The aim of this work builds up a fully automated microfluidic flow injection system from available components which can be afforded from local markets. The proposed system though to save
time, regent, Improve quality, decrease cost-reducing the consumption of sample and waste, and inherited the reproducibility and accurate obtained results from flow injection analysis.

2. Experimental

2.1. Chemicals
All solutions were prepared with distilled water, where (0.01 M) was prepared by dissolving (3.199 g) of methylene blue in (1000 ml) of distilled water, then a series of concentrations were prepared through serial dilution[6]. A solution of sulfuric acid at a concentration of (0.05M) was prepared by diluting the concentrated acid by adding (1.366 ml) to (500 ml) of distilled water for use as a carrier stream by drawing it with a peristaltic pump[12].

2.2. The Microfluidic device design for automatic injection.

2.3. Figure1. and Table 1. show the components were used in designing the mini peristaltic pump.

Table 1. Components of the micro unit controlling the mini peristaltic pump.

| Component   | work     | type    | Origin |
|-------------|----------|---------|--------|
| ARDUINO     | Micro controller | UNO     | Italy  |
| Driver Motor| Micro controller | L298N   | China  |
| Buzzer      | Alarm    | HYDZ    | China  |

Figure 1. Components of lab – build mini peristaltic pump

Two microcontrollers types Arduino were used, one UNO to manipulating the two lab-made mini peristaltic pumps to drive the carrier reagent and to suck the right volume of sample by selects the desired speed[7,8]. The second one single-to-peak Mega was equipped with a homemade software, single-to-peak, which was used as a data logger (Fig.2) to record the signal as smooth peak height to concentration with aid of Microsoft Excel 2016[13,16].

Figure 2. Ardouino type work Mega as data logger.
Figure 3. Shows the fully automated microfluidic Flow Injection spectrophotometric system

3. Result and discussions

4.1. Spectral characteristics

Fig 4 shows 0.075 µg/ml methylene blue spectrum which show that maximum wavelength at 665 nm which was used in all measurement of methylene blue during this work.

4.2. Optimum conditions

In order to get high performance the physical and chemical parameters affecting the results of the lab-made full automated microfluidic flow injection system were established[8]. One of the important physical parameters is flow rate, especially in the microfluidic system. Figure 5 shows the effect of flow rate on the peak height of (0.075 µg/ml) methylene blue. It is indicated that peak height decrease with increasing flow rate in the range which can be attributed to the increasing the dispersion in the system [14]. So, (1.5 mL/min) was chosen for further work.

The fully automated system was rely on sucking the right volume by the mini peristaltic pump which controlled by the UNO microcontroller. Table 2 lists the period of time and the volume of methylene blue introduce in the 0.05 M sulphuric acid relied on.

Figure 4. Shows 0.075 µg/ml methylene blue spectrum
Figure 5. Effect of flow rate on the peak height of (0.075 \( \mu \text{g/ml} \)) methylene blue.

Table 2. List the period of time and the volume of methylene blue

| No. | period of time (Sec) | volume of methylene blue (\( \mu \text{L} \)) |
|-----|----------------------|----------------------------------------------|
| 1   | 10                   | 20                                           |
| 2   | 20                   | 40                                           |
| 3   | 30                   | 60                                           |
| 4   | 40                   | 80                                           |
| 5   | 50                   | 100                                          |
| 6   | 60                   | 120                                          |

Fig 6 shows that peak height increase with increasing the injected volume which can be due to decreasing of dispersion in the flow system at the flow rate obtained previously[15]. So, (20 \( \mu \text{L} \)) was used in the next experiments to obtained good sensitivity and high sample throughput.

The effect of tube length on peak height was studied in tubes' in the range (3-15cm). Figure 7 shows the results obtained which inducted that peak height decrease with increasing the tube length. This tubes' length was inserted between the mixing point, of injected methylene blue with carrier stream, and the flow cell in the detector. (9Cm) was chosen for further work.
4.3. Standard calibration curve

Under the established optimum condition listed in table 3.

Table 3. The established optimum conditions for methylene blue determination

| Flow rate(min/ml) | Sample volume(µl) | Tube length(mm) |
|-------------------|-------------------|-----------------|
| 15                | 20                | 12              |
| 30                | 40                | 14              |
| 45                | 60                | 16              |

The calibration curve for determination of methylene blue by lab builds a full automated microfluidic flow injection system. The linearity was found in the range (2.5-12.5 µg/ml) of methylene blue (Table 4 and Fig. 8). The regression coefficient (r 2) of five points was 0.9981 and the least square equation was (y = 3.461 x) and the detection limit was (0.001 µg/ml). The sample throughput was (300 sample/1 hour) and each sample required (0.025 mL) of 0.05 M H2SO4 in this case.
Table 4. optimum conditions for methylene blue determination.

| Parameters       | Values     |
|------------------|------------|
| Chip volume      | 20µL       |
| Total flow rate  | 0.5 ml/min |
| Sample volume    | 25µL       |
| Sulphanilamide Conc. | 0.05M   |
| Tube diameter    | 0.2mm      |

Table 5. standard calibration curve of methylene blue

| methylene blue concentration (µg/ml) | Peak height(mm) | R.S.D % |
|-------------------------------------|-----------------|--------|
| 2.5                                 | 9               | 0.0250 |
| 5                                   | 18              | 0.0150 |
| 7.5                                 | 25              | 0.0400 |
| 10                                  | 34              | 0.0450 |
| 12.5                                | 44              | 0.0700 |

Figure 8. Calibration curve for Methylene blue
Figure 9. The peak height of the corresponding calibration curve.

Figure 10. shows the dispersion coefficient and reproducibility which are (1.9) and (25) respectively.

4. Conclusions
The Lab made a fully automated microfluidic flow injection system that was for the first time designed in our laboratories of the Department of Chemistry at the College of Education for Pure Sciences at Basra University. It was a simple, fast, inexpensive, accurate, and reproducible system. It was successfully applied for the determination of methylene blue (as an example). In addition, the proposed system has minimized the consumption of reagents and waste, so the system is environmentally friendly and it is less hazardous due to fully automated with no contact with chemicals.

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