Fabrication of Two Micro Flow Injection Cells and Investigation of the Effect of Flow Cell Volume on Dispersion and Repeatability: A Novel Study

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A new study of the effect of a previously unstudied factor on dispersion, and repeatability of the injected sample. The design idea is based on manufacturing two new microcells with different volumes used in flow injection (FI) analysis systems and determining the measurements of the external structure, internal paths, and the measuring chamber with high accuracy. The efficiency of the FI system was tested by measuring the dead volume, and it was found to be zero. All measurements (n=5) were implemented by using the concentration 10.00 mg/L of Toluidine Blue dye solution at the wavelength 625.0 nm, flow cells with the volumes (35.0, 80.0, and 450.0) µL, sample volumes (39.25 and 78.50) µL, and flow rates (2.6, 4.5, and 6.6) mL/min. The results of the study indicate a relationship between flow cell volume and dispersion values. This relationship becomes apparent when other factors, such as sample volume and flow rate, are changed.

Keywords: homemade micro flow cells, dispersion, repeatability, dead volume, toluidine blue

Introduction

The literature has recorded an impressive success for flow injection analysis and its very wide applications. It is easy to notice the extent of the success of FI in simplifying chemical measurements, reduction the consumption of chemicals and solutions, and analyzes due to many reasons compared to the other techniques used in analytical chemistry. It is worth noting that improvements and developments can be made to flow injection systems, especially when the parts of the system are manufactured from available and low-cost materials, such as the injection valve, flow cell, and detector [1-6].

The traditional flow injection analysis refers to a system which allows the injection of small volumes of both sample and reagent solutions into a flowing carrier stream. The advantages of traditional flow injection system made it ideal for the analysis of small amounts of samples [7]. Over time, the urgent need for miniaturization has been increased for many reasons including: minimizing the sample and reagent consumption, improving the portability, reducing the amount of waste produced, increasing the simplicity of fabrication, analyzing the complex samples, and minimizing the impact of chemicals and materials on the environment. The use of microchannels and micro devices enabled miniaturization and development of micro flow injection analysis (µFIA). The two essential differences between traditional FIA and µFIA are the precise mixing of the sample and reagent at reduced microscale and enhanced control of the flow rates [8-10].

Dispersion is the degree of dilution of sample/reagent segment in the manifold of flow injection system. Dispersion is the most important physical phenomenon in all injection systems, which occur as a result of the convergence of two different liquids in concentrations. The advantages of dispersion process in flow injection technique are that they are reproducible and controllable through the possibility of exploiting and changing the flow parameters and geometrical dimensions of the flow channels. The analysis is characterized by high frequency repeatability due to the timing of the injection from the valve to the detection area and the matching of the injected sample volume by using of injection loops of specific volumes. As a result, the dispersion process is controlled and therefore the analysis is successful and the best results can be obtained [11-13].

The factors affecting the dispersion process can be divided into two main types, the first related to the flow system and how it is designed to suit the chemical reaction under study, and the second type relates to the nature of the prepared sample and the chemical reaction. The factors affecting the cross-sectional dispersion process involved the following: Flow rate, sample concentration, length and diameter of reaction

coil, length and diameter of tubing etc. [14,15].

This study describes two main purposes of manufacturing both micro flow injection cells. The first purpose was to minimize the consumption of chemicals and solutions. The very low consumption of the reaction components leads to high precision, accuracy, and consequently efficient flow system. The second purpose, which represents the focus of the current research, was to study the effect of flow cell volume on the dispersion.

Experimental Part

Materials

In the current work, the chemicals were of analytical grade and all the solutions used without neither purification nor standardization.

Sample Solution

A 250 (mg/L) stock solution was prepared by dissolving 0.25 g of toluidine blue dye in 1000 mL of distilled water. For the purpose of conducting the absorbance measurements, (Shimadzu UV-1700, Japan) was utilized. The wavelength of toluidine blue dye was 625 nm as a maximum absorption. The rest standard solutions was prepared by the successive dilution of the stock solution [16].

Installing Flow Injection System

Basically, the installing process of FIA system adopted on the connection of the following components: four-channel peristaltic pump from Ismateic (Germany), home-made flow injection valve, a single-beam APEL (PD-303, Japan) UV spectrophotometry was utilized for the purpose of obtaining UV-VIS spectra for spectrophtometric measurements as a detection device of the FIA system, and the Kompensograph recorder C1032 Siemens (Germany). The flow cell with volume 450 *μL* was used from Helmma (UK). Two manufactured micro flow cells were also used with volumes 35 *μL and 80 μL.*

Fabrication and Operation of FIA-Valve

The homemade flow injection valve was made of the hard plastic and acrylic pieces as raw materials for utilizing to manufacture the platform of the valve. Then the valve was provided by two three sub-way to control the inputting and outputting of the solutions. Two loops of different volumes made of Teflon with i.d 1.0 mm were used for loading of solutions. The loop was fixed through designated and specified vents on the valve and its closing and opening was controlled through the three sub-way. Regarding the mechanism of injecting solutions and loading them towards the detector, this process was completely controlled by opening and closing specific ports in the valve via changing the directions of the three sub-way as illustrated in Figure 1.

Fabrication of Micro Flow Injection Cells

The manufacturing process of the new micro flow cells goes through several stages. In the structure manufacturing stage, the detailed plan is drawn on the first plastic chip (a transparent poly acrylic plastic chip) with a thickness of 4 mm from which the cell is made, with dimensions measured with high precision. As for the second plastic chip, its thickness is 1 mm. Each micro flow cell takes the shape of the letter T after the two chips are glued together.

After the dimensions of the two chips were determined and the process of engraving was carried out on the 4 mm thick chip according to the dimensions specified in the previous stages, then the process of gluing the two chips, comes the final stage, which is painting the micro cell completely black, except for the micro cell measuring chamber, in order to prevent any radiation to pass through the cell. In this case only the light of the device lamp passes through the measuring room. The black color has the ability to absorb light and does not have the ability to reflect the light falling on it (Figure 2).

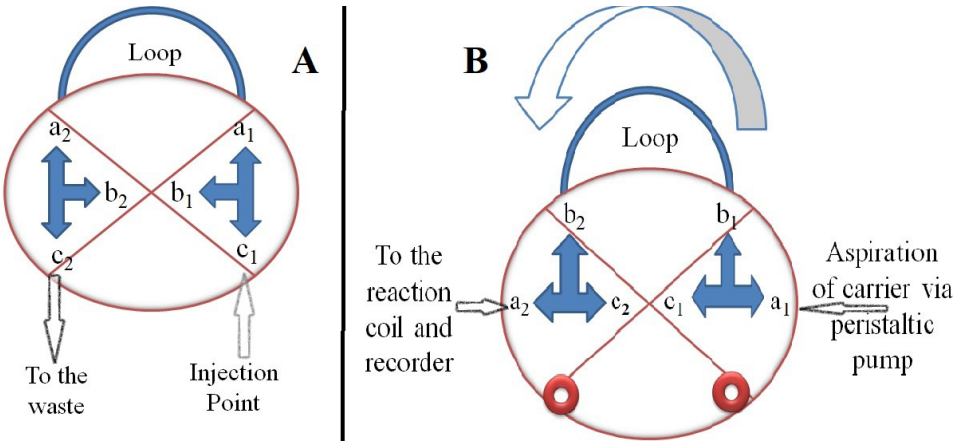

Figure 1. The utilizing and operation of home-made FIA-Valve, A: the mechanism of injection step, where the directions c1, a1, a2, and c2 are opened, and the directions b1 and b2 are locked, B: the mechanism of loading step, where the directions a1, b1, b2, and a2 are opened, and the directions c1 and c2 are locked.

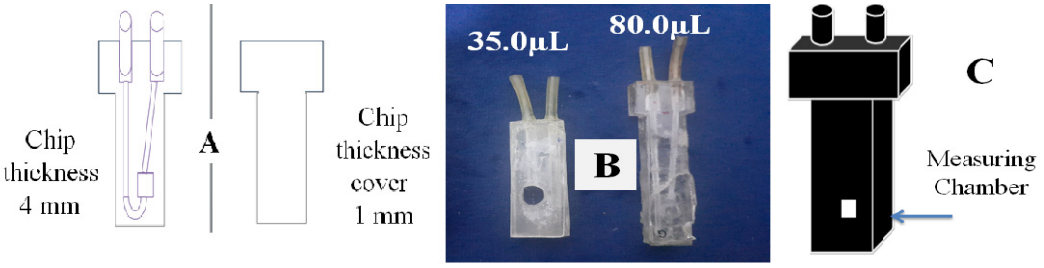

Figure 2. The designing stages of the two microcells, where A; preparation the two chips before gluing process, B; the two microcells after gluing process and inserting inner tubing, C; The process of painting the entire cell black, except for the measuring chamber.

Results and Discussion

Dead Volume

Dead volume is one of the most effective parameters of FI analysis. The Dead volume is a measure of the efficiency of FI system during the quantitative analysis of the sample. The dead volume is also called as residual volume in the flow injection valve. The dead volume results in double and distorted peak or the appearance of response in the baseline in this case the value of dead volume not equal to zero. The zero value of dead volume can be observed if all the peaks have the same baseline [17,18].

The dead volume experiment was performed using the three cells with the volumes 35 µL, 80 µL, and 450 µL separately. The conditions for the three experiments included the use of 10.0 mg/L of sample concentration and the sample volume was 78.50 µL at the wavelength 625 nm. It was observed through the three experiments that the analytical signal starts from the baseline and rises to the peak, after which comes the washing phase, followed by the descent of the analytical signal to the baseline again as shown in Figure 3. Therefore, the dead volume will be zero, and this indicates that there is no residual volume of the sample in the parts of the flow system, starting from the injection valve and ending with the flow cell.

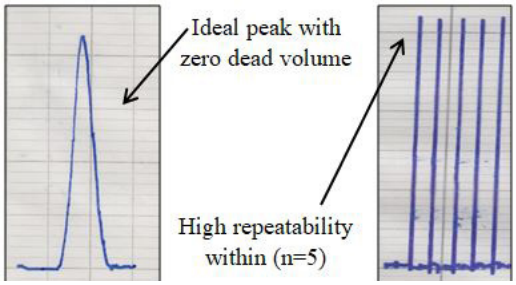

Figure 3. Ideal peak at zero dead volume and repeatability.

Effect of Flow Cell Volume on Dispersion

Table S1 summarizes an extensive study of the effect of flow cell volume on the dispersion coefficient using three flow injection cells with different volumes (35, 80, and 450) µL, flow rates (2.6, 4.5 and 6.6) mL/min, and the sample concentration is 10 mg/L at the wavelength 625 nm.

According to the results in Table S1 and Figure 4 which demonstrate the relationship between the flow cell volume and dispersion. When the flow cell volume and dispersion are variables, the remaining reaction conditions, such as, sample concentration and sample volume are constant. In general, when the sample volume is 78.50 µL In general, an increase in the dispersion was observed in proportion to the increase in flow cell volumes (35.0-450.0) µL at the three flow rates (2.6, 4.5, and 6.6) mL/min. The lowest dispersion value measured was 1.205 when the sample volume was 78.50 µL, the cell volume was 35.0 µL, and the flow rate was 6.6 mL/min. The highest dispersion value recorded was 3.28 when the sample volume was 78.50 µL, the cell volume was 450 µL, and the flow rate was 2.6 mL/min.

Effect of Flow Rate on Dispersion

Through the results of Table S1, an adverse relationship between dispersion and flow rate was observed. There was a decrease in dispersion with the increase of flow rate from 2.6 to 6.6 mL/min. It is worth noting that the inverse relationship between dispersion and flow rate was observed experimentally using flow cells with different volumes (35, 80, and 450) µL, while holding the physical and chemical reaction conditions constant included sample concentration, sample volume, room temperature, tubing i.d., wavelength, detection devise …etc. The literature mentioned the relationship between flow rate and dispersion through the equation (D=KF), where D, dispersion, K, constant, and F, flow rate. This means that there is a direct relationship between dispersion (D) and flow rate (F), noting that the flow rates used are within the range (0.5-5.0) mL/min [19].

Effect of Sample Volume on Dispersion

In light of the results obtained from the Table S1, it is possible to conclude that the dispersion value decreases with increasing of sample volume from 39.25 µL to 78.50 µL. In other words, when using the sample volume 39.25 µL, the amount of dispersion is higher with a constant flow cell volume. However, in the case of using the sample volume 78.50 µL, a decrease in the amount of dispersion was observed when the flow cell volume was constant, and this applies when using the three flow cell volumes (35.0, 80.0, and 450.0) µL. All he measurements were conducted using the sample concentration 10.0 mg/L, and the flow rates (2.6, 4.5, 6.6) mL/min at λ_{max} 625 nm.

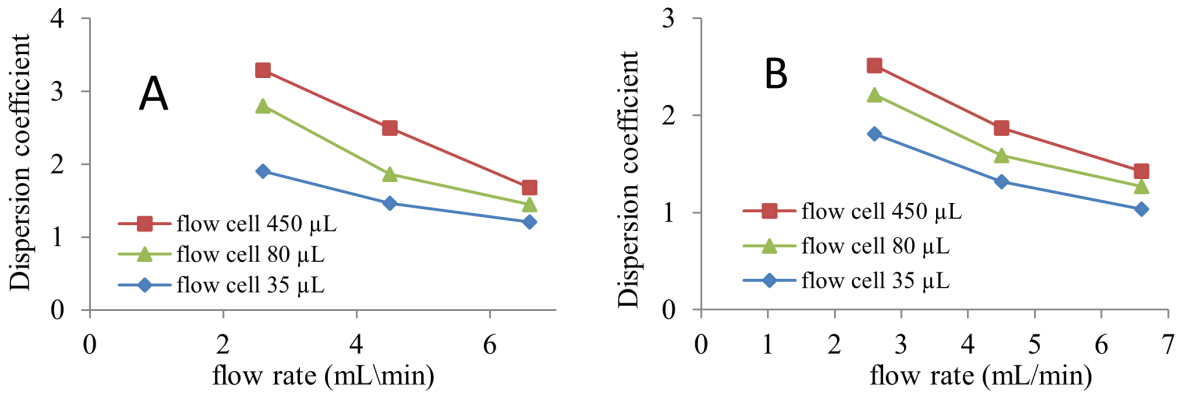

Figure 4. Effect of flow cell volume on dispersion coefficient (D) values. A: sample loop volume 78.5 µL, B: sample loop volume 39.25 µL.

The Sensitivity of Detection

The detection sensitivity in FI analysis depends on the analytical signal which is represented by the form and height of the peaks. Peak form is affected by the parameters peak width and height, which consequently influence the accuracy and precision of detection. Many factors affect detection sensitivity, including: the type of detector, path length of the sample cell, sample volume, flow rate, loop design, and the length of mixing coil. All the factors that previously mentioned could be optimized to improve the detection sensitivity. The FIA systems can be designed to achieve the lowest limit of detection and the highest analytical sensitivity. And therefore, FIA technique develops into one of the most powerful analytical techniques in qualitative and quantitative analysis. In view of the above, the achieving peaks of the current study were relatively symmetrical according to the terms of FIA technique in many respects, such as the width, height, base line, and the distance between one peak and another [20,21].

Repeatability

Repeatability is a term refers to the possibility to generate similar or identical measurements in experiments that conducted under the same optimal conditions. Repeatability is a very important concept in the field of scientific research in general and analytical chemistry and quality control in particular. It is also refers to the repeated measurements by using the solution of the same sample to yield the same value under the same optimal physical and chemical conditions via applying multiple experiments [22,23].

Table S2 demonstrates the closeness and agreement of the results. The experiment was carried out by using five successive injections of 10.0 mg/L of toluidine blue solution (Figure 3 as example) at different flow rates (2.6, 4.5, 6.6) mL/min and the maximum absorption is 625 nm. This experiment was repeated by utilizing the three flow cell volumes (35.0, 80.0, and 450.0) µL. The ranges of the values for relative standard deviation (RSD %) were between (0.628-1.045), (0.513-2.142), and (0.717-2.597), when the three flow cell volumes (35.0, 80.0, and 450.0) µL is used respectively. It was observed that the lowest values of the RSD% were when using the flow cell volume of 35.0 µL. In general, the results in the Table 2 showed high repeatability, which in turn enhanced the reliability and validity of the results.

The Relationship between flow cell volume and Standard Deviation SD

The relationship between flow cell volume and standard deviation was studied at different flow rates (2.6, 4.5, and 6.6) mL/min at the concentration 10.0 mg/L and sample volume 78.50 µL. When comparing the results using flow cell volumes (35.0, 80.0, and 450.0) µL, it was noted that the ranges of the values for the standard deviation were (0.0447-0.0547), (0.0547-0.0707), and (0.0894-0.1517) respectively. The standard deviation values demonstrated that there is a direct relationship between the flow cell volume and the standard deviation. And thus, the larger the flow cell volume, the larger the standard deviation values. Through this study, it was concluded that using the flow cell volume 35.0 µL leads to lower standard deviation values. In other words, the precision of the analytical results increases by using the flow cell volume 35.0 µL as shown in Figure 5.

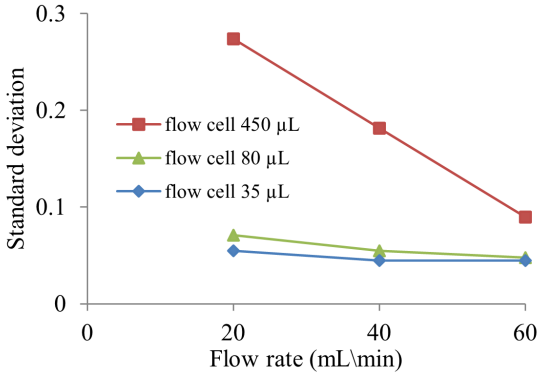

Figure 5. Effect of flow cell volume on standard deviation values at different flow rates.

Conclusions

The very low consumption of the reaction components leads to high precision and accuracy and consequently efficient flow system with low cost. The new micro flow cells were inert towards most of chemical reactions with very small volume and the easiness of the washing processes. The plastic materials used in the manufacture of the injection valve and flow cells are recyclable materials. Most of the parts used in the manufacturing process were plastic pieces that were thrown away as waste, and therefore it can be said that an additional step towards green chemistry has been achieved due to the minimizing the impact of materials on the environment. Dispersion values are affected by changing the flow cell volume and this effect increases when changing the sample volume and using different flow rates.

Recommendations

The recommendations can be made regarding implementation of applications using environmental, pharmaceutical, and biological samples. The effect of other factors, such as temperature, on the sensitivity of the analytical signal can be studied. Using other plastic polymeric materials to manufacture flow cells and studying the extent of their impact on the sensitivity of the analytical signal.

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