Lab – Built semi-automated Stop–flow System for Spectrophotometric Determination of Nitrite in Different water Samples

K H Al-Sowdani¹ and Mohammad Th. K. Al-Balaawi¹

¹Chemistry Department, Education College for Pure Sciences, University of Basra, IRAQ.
²Marine Chemistry Department, Marine Science center, University of Basra, IRAQ.

E-mail: alsowdanifia@yahoo.com

Abstract. A lab built semi-automated stopped-flow system was employed for spectrophotometric determination of nitrite in different water samples. The constructed system consists two microcontrollers (Arduinos) supplied with a home-made software programs. The first one was UNO type used to manipulates a home-made injection-pump stepper-motor type and to control the reaction-time in the flow cell. The other one was Mega type which was used as data-logger to record the results as stopped-flow peak by using Microsoft Excel 2010 program. The linearity was in the range (0.05 – 0.5) µg/ml with regression coefficient of 0.9960. The detection limit was 0.025 µg/ml and the samples can be analyzed at rate exceeding 45 samples/h. The concentration of nitrite in the different water samples were in the range (0.062 – 0.326) µg/ml.

Keywords: Stopped-flow, Adriano, Uno, Mega, injection pump, nitrite.

1. Introduction

There are many techniques were used for spectrophotometric determination nitrite¹-³. Flow injection analysis is the most frequently used techniques⁴. A stopped–flow, which is a modern technique driven from flow injection analysis⁵. This mode of flow analysis is based on stopping the carrier flow and let the reaction to take place in the measuring flow cell. The flow is stopped by a shut down the propelling pump or by means of the valve. Stopped-flow system has many advantages as simple, easy to handle, flexible in application, inexpensive and allowed better measurements sensitivity⁶.

Currently, a wide variety of advanced technologies have been applied in the field of analytical chemistry⁷. Open sources platforms such as Arduino micro-controllers have gained considerable attention from chemists, due to their low cost, integrated development interfaces and do not require expert knowledge³,⁷, and ⁸.

So, thought of combing the advantages of stopped-flow technique as sensitive, selective and low consumption of reagent and sample with Arduino micro-controllers will offer a suitable semi-automated Stop–flow System for Spectrophotometric Determination of Nitrite in Different water Samples
two Arduinos were supplied with suitable software. First one was Uno type to efficient, reproducible control to the home-made injection pump stepper motor type and to stop the chemical reaction spontaneously in the flow cell was the reaction take place with high precision. The second one was Mega types which used as a data logger to manipulate and recording the results of the stopped-flow system as a peak height using Excel program 2010.

It was decided to construct and build up a semi-automated stopped-flow system which can readily be assembled from inexpensive components and evaluated the system for Nitrite determination.

To the best of our knowledge such as that lab-built semi-automated stopped-flow system equipped with Arduino micro control platform and suitable software have not previously been applied for phosphate or other substance in Basra University.

2. Material And Methods

2.1. Reagents and samples:

During analytical application with lab- build stopping flow system deionized distilled water was used throughout and all reagents employed were analytical grade unless otherwise stated.

A stock nitrite solution of 100 µg/ml was prepared by dissolving 0.1500 g of sodium nitrite in 1L of water.

The working standard solution was prepared by appropriate dilution of the stock solution with water. 1.0% w/v sulphanilamide solution (BDH) solution was prepared by dissolving 2.5 g in 130 ml of water. 0.3% w/v n-(1-naphthyl)Ethylenediamine dihydrochloride (BDH) was prepared by dissolving 1.5 g of n-(1-naphthyl)Ethylenediamine dihydrochloride in 500 ml of water.

2.2. Instrumentation

Fig. 1, A and B show the lab-build stopped flow system which consists.

Figure (1, a): Shows the lab build
2.3. Procedure

Tecator application note 10 methods were modified to be used in this work for nitrite determination by the lab-build stopped –Flow System. Fig. (2) Shows the flow chart of the program running in the system. After adjusting the wavelength of the spectrophotometer on 454 nm.

The constructed system consists of two microcontrollers (Arduino) supplied with a homemade software program. The first one was UNO type used to manipulates a home-made injection-pump stepper-motor type and to control the reaction-time in the flow cell. The other one was Mega type which was used as data-logger to record the results as a stopped-flow peak by using Microsoft Excel 2010 program.

![Flow Chart](image-url)
The Arduino type Uno used to control the injection pump, first of all, wash the manifold spontaneously for 10 sec. then refilled the 60 ml. plastic syringe with a reagent. A 180 µL the of nitrite sample injected manually through injection valve (Rhyeoyne, catalog, California) into mixed reagent stream. Then the home-made injection pump type stepper motor stopped when the reaction zone of the sample and reagents react at the flow measuring cell for 10 Sec. The other Arduino type Mega to recording the signals as peak height with aid Microsoft Excel 2010 program. After that injection pump restarts to wish the flow system and restart for other samples.

3. Results an Discussion:

Fig. (3) Shows the manifold of the system used for phosphate determination, which clearly indicated the most crucial and novel part of this system is to use the role of two microcontrollers. The first Arduino type UNO was programmed by Arduino software V1.8.3, which used to control the movement and provided by the power supply (12V). The home-made injection-pump type stepper motor (Nema17). In order to determine the phosphate by using the microcontroller to control the starting and fill up the injection pump. The micro control unit equipped with variable resistance 50 ohms (Fig.2) which supply the system with 1024 analog signal value which can make each signal as the order to let the injection stepper motor work in suitable style. In this lab-built only (10-100) range analog signal was fixed in order to order to a stepper motor to start and (101-200) range for fill the injection-pump with a reagent. The injection-pump consist mainly from the stepper-motor Fig. with a relatively high moment and the most significant properties is the motor which can be adjusted precisely the volume of injected reagents by the number of steps.

The moment selection for this stepper-motor spends on the number of syringes used in this system. It is possible to use two 60 ml volume plastic syringes or only one syringe filled with a mixture of the two reagents which is used in this system. The sample is injected by the injection valve and propelled to the measuring flow cell, by the carrier stream in the spectrophotometer (U.V 303 Apel) and the absorbance measured at 880 nm. This spectrophotometer contains an out-pw to the analog signal which proportional to the absorption value of the colored complex in the measuring cell. This analog signal converts to Digital signal through a microcontroller l Adriano type Mega which work as a data logger to the mat and recording the data by lab tab equipped with Microsoft Excel 201010.

![Diagram](image_url)  
**Figure (3):** Manifold of the stop-Flow System
The Optimum conditions for nitrate Determination by the proposed system:

The lab-build semi-automated stopped flow system as showing in Fig. (1) was used to optimize the variable that affecting the peak height by carrying out a series of experiments to establish the optimum analytical condition that in influence the peak height. Table (1) lists all optimum results which were used in subsequent work.

Table (1): the Optimum conditions for determination of phosphate

| Parameter                        | Value            |
|----------------------------------|------------------|
| Flow rate                        | 7.5m l/min       |
| Sample volume                    | 180 µl           |
| Cell volume                      | 450 µl           |
| Mixing coil length               | 20 cm            |
| Stopping time                    | 10 Sec           |
| Sulphanilamide                   | 0.015 M          |
| N-(1-Naphthyl)Ethylenediamine dihydrochloride | 1x10^{-3} M |
| wavelength                       | 454 nm           |
| pH                               | 3.5              |

Calibration curve:

Under the established conditions listed in Table(1) a calibration curve for nitrate was obtained Fig. (4). It is linear over the range 0.125-2.0 µg/ml. The linear curve has a regression coefficient, the detection limit. and R.S.D% .09962, 0.05 µg/ml and 3.8 respectively

Accuracy:

In order to establish the validity and the accuracy of Lab-built system for the determination of Phosphate in water one standard and two representatives samples were examined by using standard additions method\(^5\) listed in table 2. The same batch of samples was analyzed by classical manual method \(^11\). The recoveries and RSD% were calculated ( Table 2). The value of RSD%for reproducibility obtained was 1.37% which clearly indicated that lab-built stopped-flow system has a high reproducibly which can be used for phosphate determination in the different water sample (Fig.5).

Table (2): Determination of Phosphate in Standard and representative samples using standard additions method.

| Claimed conc (µg/ml.) of Phosphate | Determination by Lab-Build Stopped-Flow method | Determination by Classical method |
|-----------------------------------|-----------------------------------------------|----------------------------------|
|                                   | Found (µg/ml.) | Recovery | R.S.D%* | Found (µg/ml.) | Recovery | R.S.D% |
| 50 ppm (Riccachemical)           | 51.55          | 96.9     | 0.82    | 50.05          | 95.2%    | 0.93   |
| 0.8 ppm                          | 0.81           | 101.25   | 0.94    | 0.79           | 98.4%    | 1.07   |
| 1.2 ppm                          | 1.18           | 98       | 0.78    | 1.19           | 97.7%    | 0.87   |

* For Triple successive stopped-flow peaks.
Applications:

The proposed system was used successfully for the determination of phosphate in different water samples. Table (3) lists the results obtained by using the addition standard method to avoid all the possible interference. The results were in the range (0.74-1.35 µg/ml) which mean that all water samples were not polluted.

Table (3): Sites of Water samples

| Sample                              | Concentration Of Phosphate (µg/ml) | RSD% |
|-------------------------------------|-----------------------------------|------|
| Tap water –Qibla                    | 0.70                              | 1.069|
| Tap water – Karamat Ali             | 0.74                              | 1.654|
| Bottled water –Seraj water          | NA                                | --   |
| Bottled water-Naba’a Al-Basra       | NA                                | --   |
| Grave river                         | 0.78                              | 1.699|
| Arab Gulf                           | 0.35                              | 1.450|
| Aquarium- Basra science Center      | 1.35                              | 1.707|
| Al-Aziz river-Basra                 | 0.75                              | 1.006|
| Tiger river-Qurna-Basra             | 0.650                             | 1.778|

4. Conclusion:

Assembling a lab-build semi-automated stopped-flow system form few available, simple and low price components. For the first time a microcontroller(Adrian type Uno and Mega) supplied with a suitable software were used in our laboratory as a controller to the proposed system especially the stepper-moto3r and data logger to recording the data, respectively. The lab-build system offers simple, sensitive, reproducible means for phosphate determination.

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