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To cite this article: Yousif Sh. J. Al-Jorani *et al* 2021 *J. Phys.: Conf. Ser.* **1818** 012050

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240th ECS Meeting ORLANDO, FL

Orange County Convention Center Oct 10-14, 2021



Abstract submission due: April 9

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Lab-Made Semi-Automated Microfluidic Flow Injection Spectrophotometric System for Determination of Nitrite in Natural Water

Yousif sh. J. Al-Jorani¹ and K. H. Al-Sowdani² and Salah M. Saleh³

¹Applied marine sciences department, marine sciences faculty, Basrah University, IRAQ

²Chemistry department, college of education and pure science, Basrah University, IRAQ

³Marine chemistry, marine science center, Basrah University, IRAQ

Email: yousif.shafi@yahoo.com

Abstract: A lab-made semi-automated microfluidic flow injection spectrophotometric system for the determination of nitrite in natural water was constructed and designed. It consists of a double line microfluidic chip with dimensions (40 μ l \times 5cm), each one 20 μ l volume aims to allow very small volume from reagent and sample to be used. The microfluidic system also, consists of two Arduino microcontroller programs. The first one was UNO used to dominance mini peristaltic pump and the other one was a mega type which records the results as peaks using Microsoft Excel 2010 program. The linearity was ranged (0.025-0.15) μ g/ml with regression coefficient of six points 0.9997, r.s.d% for ten replicates of 0.1 μ g/ml. The detection limit was 1.75 ng/ml and the samples throughput was 150 samples per hour and one sample required 55.7 μ l of chemical reagents therefore, 150 samples consumed 8.355 ml of chemical reagents which clearly indicated that the consumed reagent and waste were very low. So, the method of determination of nitrite in this system is Eco-friendly. The concentrations of nitrite in the natural water were in the range (0.025-0.4) μ g/ml.

Keywords: Microfluidic, Arduino controller, spectrophotometer, nitrite, natural water

1. Introduction

One of the most important modern trends in analytical chemistry is miniaturizations [1]. This fact can be clearly indicated by the huge number of publications in the website in the last two decades [2]. The Micro-total Analysis and Lab-on-chip systems are examples for using miniaturizations in analytical chemistry [3,4]. The significant interest in miniaturizations in many fields such as environmental studies. These are driven by the need to reduce the instrument cost and space, decrease the consumption of the chemical reagents and waste, increasing the sample throughput and easy to be a fully automated system [5, 6]. The microfluidic technology or science is dealing with small volumes of fluids up to (10⁻⁹-10⁻¹⁸ L) with micro channels in the range of tens to hundreds of micrometers. It is widely used these days in analytical processes due to inherent many advantages of miniaturization. These systems have many attractive features that included short analysis time, low cost, high accuracy, sensitivity, and easy to be constructed and automated. [7,8]. Nitrogen is present in



many forms at the nitrogen cycle in the environment in which nitrite and nitrate are considered as important intermediates. They are present in the surface water land wastewaters [9] which nitrate are indicators of organic pollution from industrial and agricultural waste. Nitrite is more toxic than nitrate to an aquatic environment, but it is less stable and concentrated in the aquatic environment [10]. Nitrate is the dominant form of inorganic nitrogen in lakes and rivers and it is produced through the final oxidation of nitrogen compounds [11]. Many flow injection analytical methods were reported for determination of nitrite in natural water included spectrophotometric [12, 13], ion chromatography [14, 15] colorimetric [16].

Recently, lab-made micro flow injection analysis (μ FIA) semi-automated system was constructing and build-up for phosphate determination [17]. So, it was decided to use this proposed system for nitrite determination in natural water. This microfluidic system thought will offer rapid, low cost, high sample throughput, and Eco-friendly way for nitrite determination.

2. Experimental

2.1 Chemicals

All chemicals were analytical grade, and the solutions were prepared in deionized water. Nitrite standard solution (100 μ g/ml): 0.15g of Sodium nitrite NaNO_2 was dissolved in 1L of deionized water. The working and standards solutions over the range 0.025-0.150 μ g/ml were prepared by serial dilution of stock solution. Sulphanilamid Solution (1% w/v): 2.5g of Sulphanilamid was dissolved in 130 ml concentrated hydrochloric acid and made up to 250 ml with deionized water. N-(1-Nphthyl) ethylene diamine dihydrochloride (NED) solution (0.3% w/v): 1.5g of N-(1-Nphthyl) ethylene diamine dihydrochloride was dissolved in 500 ml deionized water.

2.2 Sampling

Twelve water samples were collected in spring 2020 along the Shatt Al-Arab and other regions, southern of Iraq using 1L clean plastic containers resisting chemicals. The water samples were taken from 30cm subsurface and a few drops of chloroform were added, then filtered by a 0.45mm filter paper (GF/C Whatman) before storing in the freezing [18].

2.3 Microfluidic flow injection manifold and method

Fig. 1 (a) shows a homemade microfluidic chip which was designed in this study. It has double line with dimensions (40 μ l \times 5cm), each one 20 μ l volume aims to allow very small volume from reagent and sample to be used. The microfluidic chip contains in one side two holes, the first one (Inlet) which was connected directly to the injection valve through a 0.2mm diameter Teflon tube for transporting the nitrite sample and Sulphanilamid solution. The other ones (Inlet) for the second reagent N-(1-Nphthyl) ethylene diamine dihydrochloride. They were mixed and react in single channel to form a colored complex which transporting through the flow cell of spectrophotometer to give the analytical signal. The chip was made grooved on plastic plate due to it is low cost and easily to draw the wanted chip and adhesive of the two plastic layers. The 0.2 mm Teflon tube which was used to the whole manifold was fixed to the chip using an epoxy and adhesive.

Fig.1 (b) shows the microfluidic flow injection manifold which used for nitrite determination in the natural water samples. As well as microfluidic chip it consists a homemade micro peristaltic pump with different speeds ranging from (0.4-5.0 ml / min) which was controlled by the ARDUINO type UNO micro controller to move up the chemical reagents to the detector, The micro peristaltic pump consists of several parts: Arduino UNO controller which is programmed by Arduino v1.8.3 software, variable resistance, stepper motor and stepper motor driver which It regulates the movement of the stepper motor and is supplied with an electrical energy of 12 volts through an external power source, as shown in the (table 1) and (Fig. 2). A spectrophotometric (Spectro SC, Labomed, Inc.USA) which supplied with a 7 μ l flow cell (QS Hellma) and the injected sample by Injection Valve (Catati

RHEODYNE California, USA) .Arduino type Mega controller was used as a Datalogger to manipulate and recording the results as peak height corresponding the concentration by interfacing to PC supplied with Microsoft Excel 2010 program.

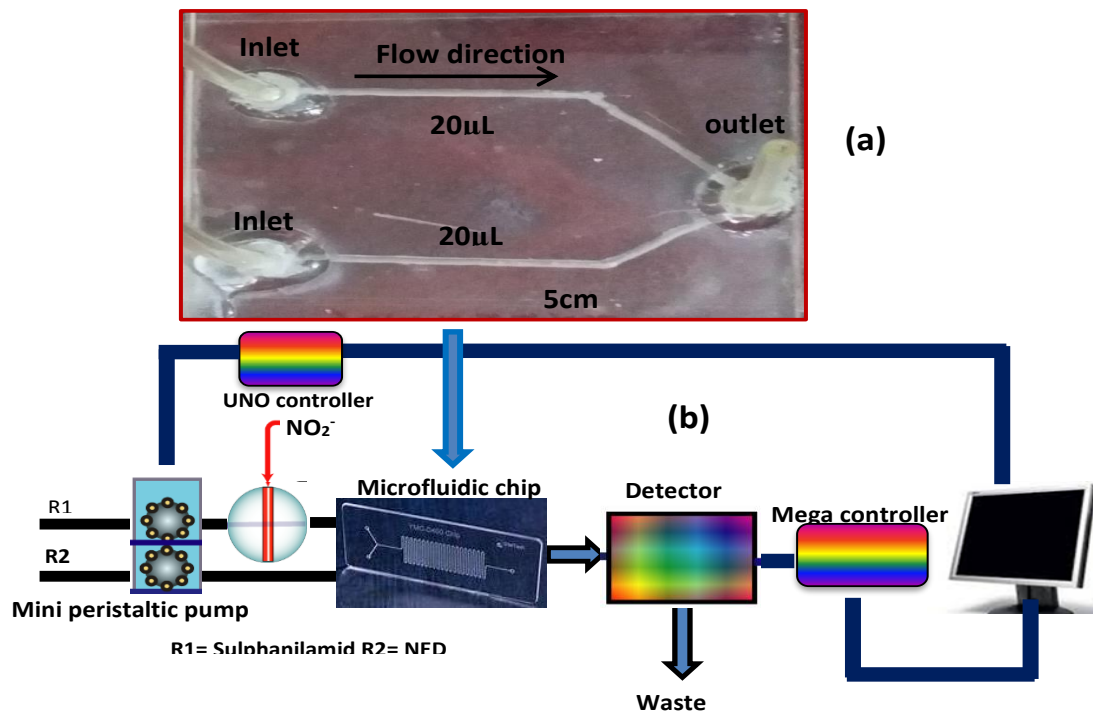


Figure 1. a-Microfluidic chip b- Microfluidic manifold

Table (1) components of the micro unit controlling the peristaltic pump

Component	work	type	Origin
ARDUINO	Micro controller	UNO	Italy
Stepper motor	Injection pump	Nema17	China
Driver Motor	Micro controller	L298N	China
Buzzer	Alarm	HYDZ	China

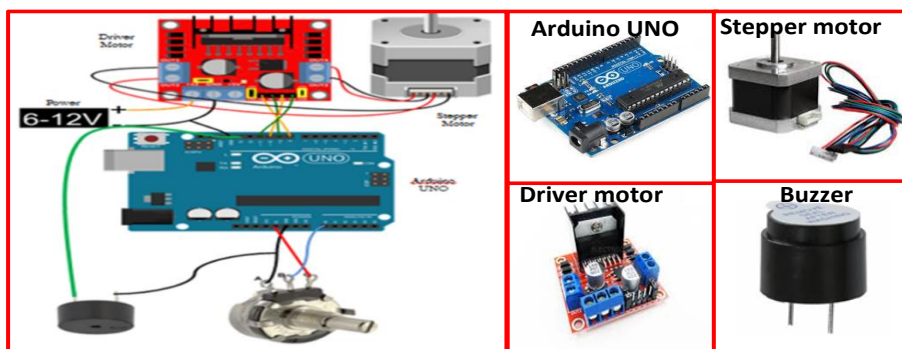


Figure 2. components of the micro unit controlling the peristaltic pump

3. Results and discussion

3.1 Spectral characteristics

A scanning spectrophotometer was used to recording the spectrum of dye complex of nitrite with Sulphanilamid in the acidic medium and coupled with NED (N- (1-naphthyl) ethylene di amine dihydrochloride). The pink color complex show a maximum absorbance at 540nm which was fixed through this work as show in Fig (3) and scheme (1) which was cited from Bhakta et.al [19].

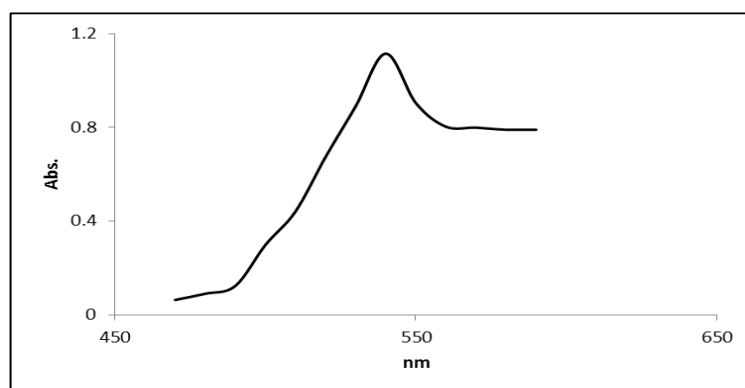
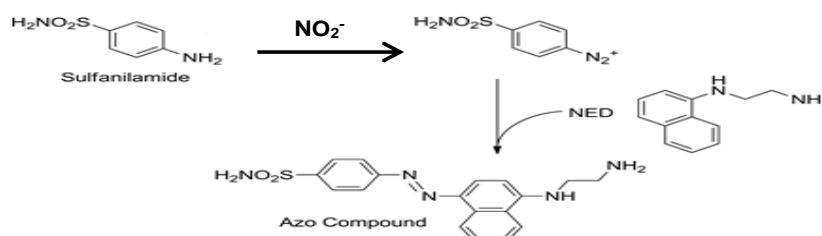


Figure 3. maximum absorbance of pink color complex



Scheme 1. the formation of azo dye pink complex

3.2 Optimum conditions

To achieve high sensitivity and selectivity for nitrite determination in the natural water by microfluidic flow injection system, physical and chemical parameters affecting the performance microfluidic system were investigated.

Flow rate is an important physical factor in the home made microfluidic system. The low flow rate allows the coloured complex to remain more time in the flow cell thus, providing more time for the formation of the colored complex. Fig (4) showed that the peak height of 0.1 $\mu\text{g/ml}$ nitrite in the range (1.4-3.8 ml/min) decreases with increasing flow rate. This can be attributed to the fast of formation of the complex as a result of good mixing for the micro volume of sample and reagents So, 1.0 ml / min was chosen as the best flow rate for subsequent application [20, 21].

The peak height increases gradually with the increase of the sample volume, this leads to an increasing in the absorbance and consequently an increasing in the sensitivity of the method. The injected sample volume ranged (15-35 μL) Therefore, 25 μL of sample volume was chosen as the best volume for subsequent studies to increase the sample throughput and obtain a suitable sensitivity [22, 23] as shown in Fig. (5)

The effect of Sulphanilamid and N- (1-naphthyl) ethylene diamine dihydrochlorid (NED) concentrations on the sensitivity were investigated ranged (0.2-0.8% w/v) and (0.05-0.25% w/v) respectively. Fig. 6 and 7 show these effects on the peak height of 0.1 $\mu\text{g/ml}$ nitrite. The results are clearly indicated that the peak height increase with increasing the concentrations. This was thought to be due to increasing of the formed complex with the increasing concentrations [22,23] Thus, a 0.4 % w/v and 0.15 % w/v were chosen as the optimum concentrations in the corresponding experiment.

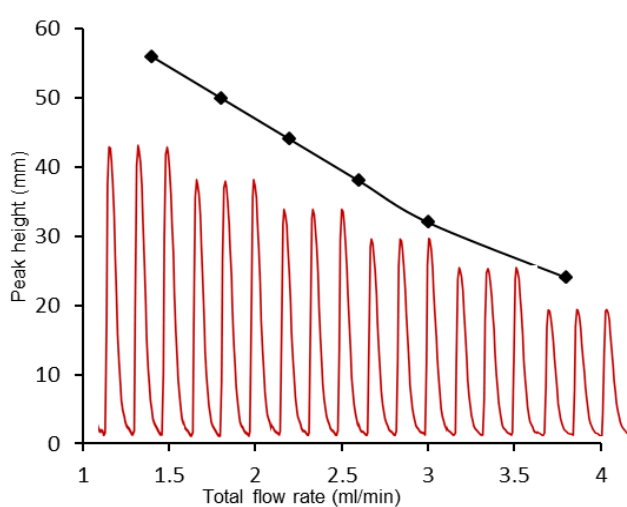


Figure 4. Effect of total flow rate.

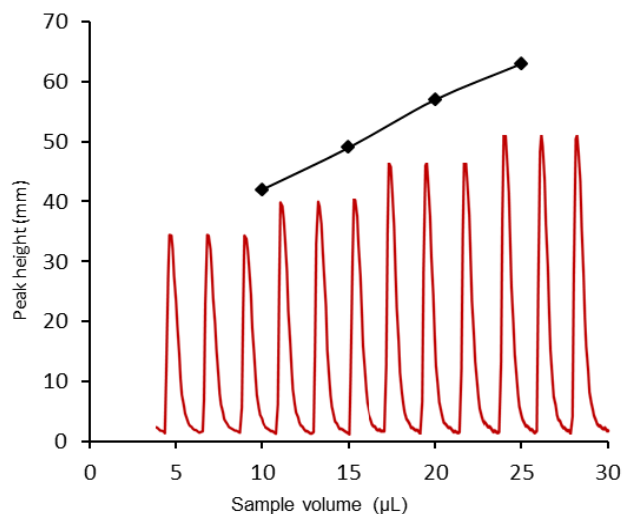


Figure 5. Effect of sample volume.

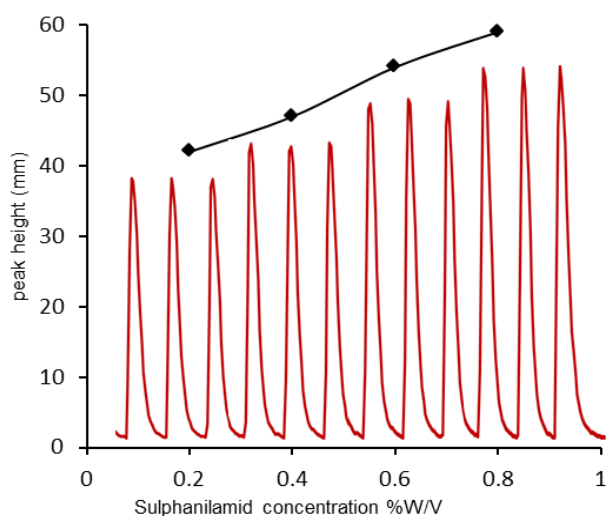


Figure 6. Effect of Sulphanilamid concentration

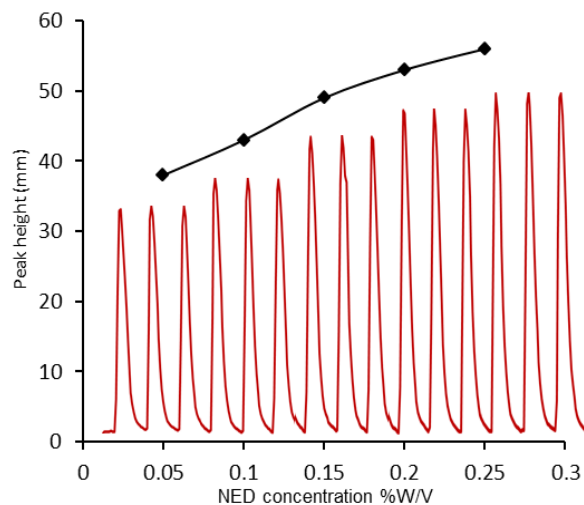


Figure 7. Effect of NED concentration.

3.3 Standard calibration graph

Under the established conditions listed in table (2). Linearity was found the range 0.025-0.15 $\mu\text{g/ml}$ as shown in Fig (8) and Table (3). The regression coefficient of six points was 0.9997, r.s.d% for ten replicates of 0.1 $\mu\text{g/ml}$ nitrite was 0.08% and detection limit was 1.75ng/ml. The equation $y=522.86x+0.0714$ gives the relationship between the peak height and the concentration where y and x are the peak height and the concentration of NO_2^- respectively. The sample throughput was 150 samples per hour and one sample required 55.7 μl of chemical reagents therefore, 150 samples consumed 8.355 ml of chemical reagents which clearly indicated that the consumed reagent and waste were very low, So, the method of determination of nitrite in this system is Eco-friendly. The dispersion coefficient of the manifold was 1.3 as shown in Fig (9).

Table (2) optimum conditions for NO_2^- determination

Parameters	Values
Chip volume	40 μL
Total flow rate	1.0 ml/min
Sample volume	25 μL
Sulphanilamid Conc.	0.4 % w/v
NED Conc.	0.15 % w/v
Tube diameter	0.2mm

Table (3) Standard Calibration graph of nitrite standards

nitrite concentration ($\mu\text{g/ml}$)	Peak height (mm)	(r.s.d %)
0.025	13	0.300
0.050	27	0.200
0.075	39	0.450
0.1	52	0.700
0.125	65	0.300
0.150	79	0.600

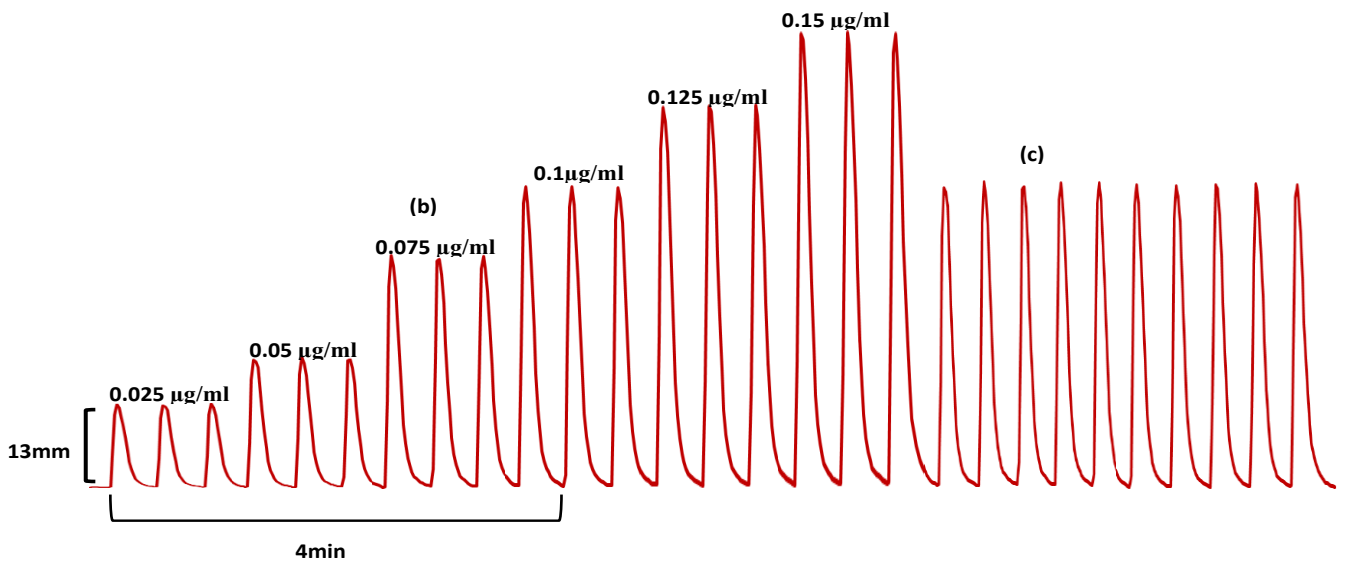
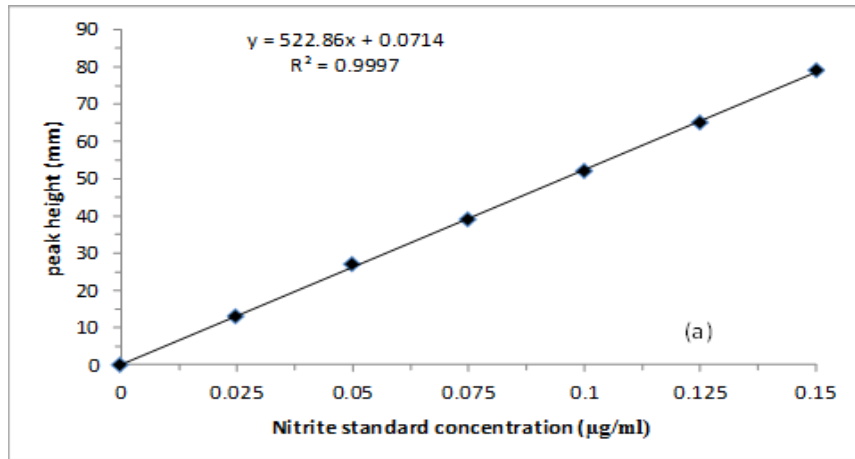


Figure 8. (a). Standard calibration graph for nitrite determination (b) Peaks obtained by injected nitrite Standards (c) 10 replicates of 0.1 µg/ml standard nitrite.

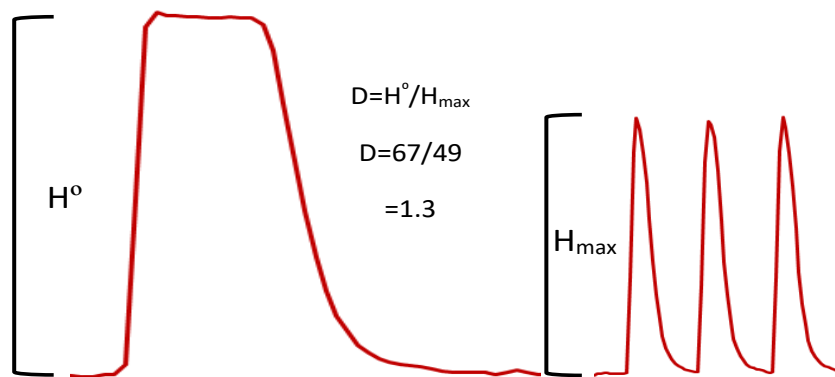


Figure 9. The dispersion coefficient in microfluidic system

4. Interferences study

In order to evaluate the analytical applications of the microfluidic system for NO_2^- determination, the effect of 1, 10, 100, 1000 folds of some Cations and anions on the estimation of 0.1 $\mu\text{g/ml}$ nitrites in natural water were studied. Table (4) list the results which clearly indicated that the anions examined did not interfere with the determination of nitrite. The investigated cations interfered with the determination of nitrite due to their complexation with the organic reagents [24]. The effect of cations can be removed by filtration and adding a 0.5N NaOH or masking agents EDTA and tartrate solution [25, 26].

Table (4) Interferences study of nitrite determination

Foreign ion	Add as	Peak height (mm) at folds			
		1	10	100	1000
Fe^{+2}	FeCl_2	46	44	39	35
Al^{+3}	AlCl_3	52	50	46	39
Cu^{+2}	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	49	39	35	29
Ni^{+2}	$\text{Ni}(\text{NO}_3)_2$	47	35	30	24
Fe^{+3}	FeCl_3	53	44	38	31
Ca^{+2}	CaCl_2	50	52	47	36
PO_4^{-3}	KH_2PO_4	53	50	50	48
Cl^-	NH_4Cl	52	53	54	54
SO_4^{-2}	$\text{ZnSO}_4 \cdot 5\text{H}_2\text{O}$	50	53	51	50
CO_3^{-2}	CaCO_3	52	52	50	50
F^-	Na_2SiF_6	52	52	51	50

5. Analytical Application

To verify the capability of the proposed system to analyze real samples, nitrite was determined in water samples. The nitrite concentrations were determined by the calibration curve investigated by the injection of nitrite standard solutions (Table 5) and evaluated the accuracy by comparing the results with a classical method that showed high agreements between the two methods. The proposed method was compared with some previously reported spectrophotometric methods based on the diazotization coupling reaction for estimation of nitrite in water as shown in (Table 6).

Table (5) Nitrite concentration in water samples

No.	Sample	Nitrite concentrations($\mu\text{g/ml}$)	
		Microfluidic FIA \pm r.s.d%	classical \pm r.s.d%
1	Al-Qurna /Euphrates river	0.025 \pm 0.5	0.03 \pm 0.2
2	Al-Qurna/Tigris river	0.035 \pm 0.4	0.035 \pm 0.3
3	Al-sharsh/ Shatt al-Arab river	0.03 \pm 0.4	0.025 \pm 0.4
4	Al-deer/ Shatt al-Arab river	0.03 \pm 0.7	0.03 \pm 0.2
5	Saad bridge/ Shatt al-Arab river	0.05 \pm 0.7	0.05 \pm 0.75
6	Qurmat ail river	0.1 \pm 0.5	0.1 \pm 0.65
7	Ashar/ Shatt al-Arab river	1.25 \pm 0.4	1.25 \pm 0.7
8	Abu al-Khasib/ Shatt al-Arab river	0.14 \pm 0.8	0.13 \pm 0.5
9	Fao/ Shatt al-Arab river	0.14 \pm 0.3	0.14 \pm 0.2
10	Messan/Tigris river	0.5 \pm 0.4	0.5 \pm 0.2
11	Al-geraf river/Nasiriya	0.075 \pm 0.5	0.07 \pm 0.6
12	Khour al-zubair	0.075 \pm 0.6	0.075 \pm 0.4

Table (6) Comparison of the proposed system with previously reported spectrophotometric method

Diazotization reagent	Coupling reagent	Sample	Detection limit $\mu\text{g/ml}$	Linear range $\mu\text{g/ml}$	Ref.
Sulfanilamide	NED	Soil	0.072	0.164-5.255	[27]
Sulfanilamide	NED	Fresh water	0.008	0.1-1.0	[28]
sulfanilic acid	methyl anthranilate	water, soil	0.93	0.2-8.0	[29]
MMCBAT	N,N-dimethyl aniline	Tap and lake water	0.012	0.05-2.0	[30]
Sulfanilamide	TTA	Water	0.0003	0.001-1.2	[31]
P-Nitroaniline	4-amino-3-hydroxynaphthalene-1-sulphonic acid	Soil and water sample	0.001	0.1-2.0	[32]
Aminoacetophenone	2,3-hydroxynaphthalene	Water and wastewater	-	0.1-5.0	[33]
p-Nitroaniline	MPAT	water	-	0.4-2	[34]
Sulfanilamide	NED	water	0.025	0.062 – 0.326	[12]
Sulfanilamide	NED	Natural water	0.00175	0.025-0.15	Present work

DL: detection limit, NED= N-(1-naphthyl)-ethylenediamine hydrochloride, MMCBAT= 4-(1-methyl-1-mesitylcylobutane-3-yl)-2-aminothiazole, TTA= thenoyltrifluoroacetone, MPAT= 5-methyl 4[phenyl methylene] amino-2, 4 dihydro-1, 2, 3 triazol 3-thione.

6. Conclusion

The homemade microfluidic system which was designed at the first time in the chemistry department Laboratories, Basrah University was simple, fast, inexpensive, accurate and reproducible, applied successfully for nitrite determination in natural water, In addition to that, the proposed system reduced the consumption of samples and chemical reagents, which led to reducing the chemical wastes, so the system is Eco- friendly.

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