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Research Article

**BATCH AND FLOW INJECTION SPECTROPHOTOMETRIC
DETERMINATION OF NITRITE AND NITRATE IN
WASTEWATER SAMPLES OF ERBIL CITY****Hijran Sanaan Jabbar^{1*} and Hazha Omar Othman²**^{1,2} Department of Chemistry, College of Science, University of Salahaddin, Erbil, Iraq¹ Email: Hijran.jabbar@su.edu.krd; Mob: +9647504682450**Abstract:**

Two simple and direct batch spectrophotometric methods for the determination of nitrite and nitrate and two simultaneous spectrophotometric flow injection methods developed for the determination of nitrite and nitrate in wastewater samples. The methods based on the direct reaction between nitrite with barbituric acid (BBA) and/or thiobarbituric acid (TBA) and the absorbance's are measured at 536 and 589 nm respectively. The reaction is selective for nitrite and provides a basis for a new spectrophotometric determination. In Batch method nitrate was reduced to nitrite using Jones reductor and analyzed as nitrite, while simultaneous flow system based on on-line nitrate reduction using Jones reductor column. The optimized chemical and physical conditions gave linear calibration range between 1.3-53 and 0.3-40 µg/ml using BBA and TBA in batch method, and gave linear range between 6.5-43 and 3.0-40 µg/ml using BBA and TBA in flow injection method. The methods developed were applied to the determination of nitrite and nitrate in waste water samples of Erbil city.

Keywords: Spectrophotometric; Batch; Flow injection analysis; Nitrite, Nitrate; Simultaneous determination; Wastewater

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INTRODUCTION

The determination of nitrite and nitrate plays an important role in the analysis of surface and wastewaters. The WHO set the maximum contamination levels in drinking water to 0.1 and 10mg nitrogen per liter (mg-N/L) of nitrite and nitrate, respectively. Similar limits are set-up in many Europe countries by national authorities [1]. Determination of nitrite generally is importance because it produces carcinogenic nitrosamines in the human body through its reaction with amines or amides [2]. Traces of nitrite and nitrate in drinking water may lead to methemoglobinemia in infants and with long term exposure is a possible cancer risk [3].

Various methods have been used for nitrite determination such as spectrophotometry [4-7], flow injection spectrophotometry [8-10], derivative spectrophotometry [11], chemiluminescence [12-14], fluorimetry [15], ion chromatography [16, 17], Raman spectrometry [18, 19] and electrochemical methods [20, 21].

The most frequently used approach to the spectrophotometric detection of nitrite is the Griess Assay which involves a diazo-coupling procedure [22]. For more than a century, the Griess Assay played an incomparable role in the determination of nitrite. However, it has been recognized that this method suffers from poor sensitivity and numerous interferences from other ions such as Cu^{2+} , Fe^{3+} , S^{2-} and I^- [23]. A spectrophotometric method developed for the determination of nitrite in water samples based on the nitrite reaction with barbituric acid in acidic solution to give the nitroso derivative, violuric acid and measured at analytical wavelength of 310 nm [5].

The present paper reports two batch and two flow injection methods designed on the principle of redox speciation for the determination of nitrite and nitrate. These methods involve the use of a single solution of the BBA and/or TBA without using acids and bases.

EXPERIMENTAL

Apparatus

A JENWAY 6405 UV/Vis. Single-beam spectrophotometer with 1 cm quartz cell was used for the spectral and absorbance measurements.

Chemicals

All chemicals used were of analytical grade and double-distilled water was used for the preparation

and dilution of reagents and samples. Nitrite and nitrate stock solutions were prepared using pre-dried solid NaNO_2 (Fluka) and KNO_3 (Fluka).

Recommended procedure

Batch method

To a series of 25 ml volumetric flask 1.7 mL of 0.015 mol/L BBA (or 3.0 ml, 0.015 mol/L TBA) were added, then 0.3 – 60 $\mu\text{g/mL}$ nitrite was added. The solution set for 3.0 minutes at 30°C using BBA (or at 25°C for TBA). Then, the solution mixed, diluted to the mark with distilled water and the absorbance was measured at 536 and 589 nm against reagent blank which was prepared in the same way for BBA and TBA respectively.

For nitrate determination, a 5.0 ml portion of solution containing 0.3 – 100 $\mu\text{g/ml}$ nitrate transferred into the modified Jones reductor as described in the AOAC official methods of analysis [24] where the flow rate is adjusted to 3.0 ml/min. The reductor is then washed with 5 ml of water. The nitrite solution obtained from the reductor is treated as described in the above procedure for spectrophotometric determination of nitrite.

Flow injection spectrophotometric determination

The flow injection manifold used for determination of nitrite and nitrate in this method is shown in Figure 1. It consists of a variable speed multi-channel peristaltic pump (Desagapl - Heidelberg, England peristaltic pump) to propel carrier stream (distilled water) and BBA or TBA solution with flow rate of 1.0 ml/min. A 100 μl of nitrite/nitrate solution (or sample) was injected to the carrier stream using Rheodyne-USA injection valve supplied with variable loops. The injected sample stream divided to two lines; the first line react with the reagent to produce a signal of nitrite, while the second line passed through a home-made Jones reductor column with 25 cm length and 2.5 mm internal diameter to reduce nitrate to nitrite and then merged with the reagent stream producing a signal of total nitrite and nitrite. The detection and absorbance measuring was carried out at 536 and 589 nm for BBA and TBA respectively using a single beam spectrophotometer with a flow cell (10 mm path length, 100 μl). The detector output was connected to one line Yokogawa Model 3021 – Japan recorder on which peak height were recorded.

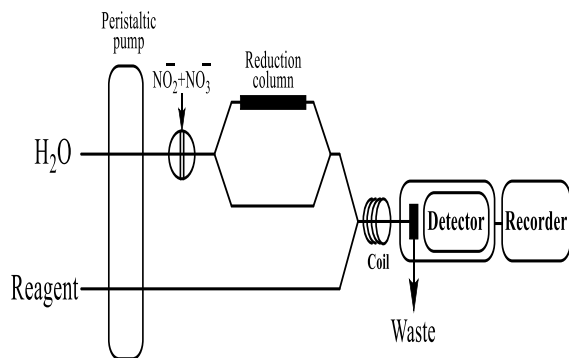


Fig. 1: Schematic diagram of flow injection spectrophotometric manifold used for the determination of nitrite and nitrate.

RESULTS AND DISCUSSION:

Absorption Spectra

The proposed spectrophotometric methods based on the reaction between nitrite and BBA (or TBA). As shown in Figure 2, the absorption spectrum of nitrite – BBA shows a maximum absorption at 536 and for nitrite – TBA shows a maximum absorption at 589 nm.

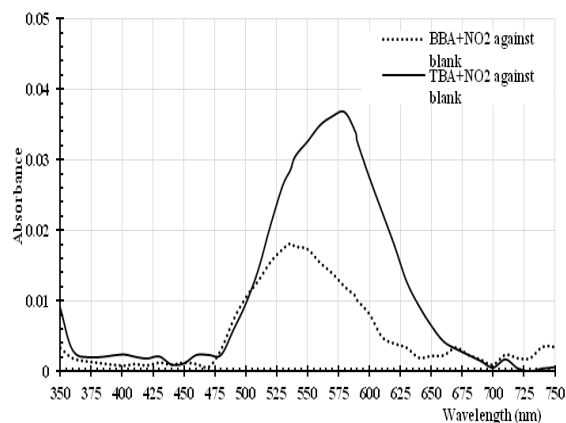


Fig. 2: Absorption spectra of BBA-NO₂ and TBA-NO₂ against blank using 20 µg/ml nitrite.

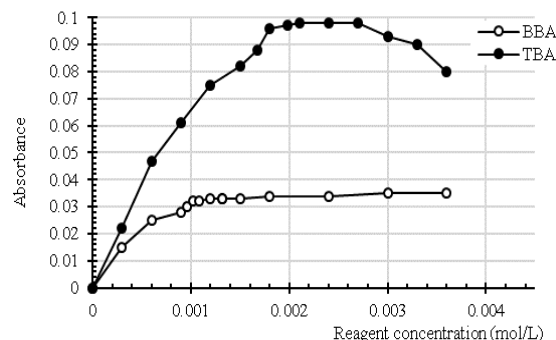
Optimization of Reaction Variables

Since the developed methods depends on the formation of colored product between nitrite and BBA or TBA so, optimization studies were carried out to find the optimum conditions for the reaction taking nitrite (15 µg/mL) as a representative example for these studies. Preliminary investigations showed that using acid, base or buffer solutions decreases the absorbance of colored product.

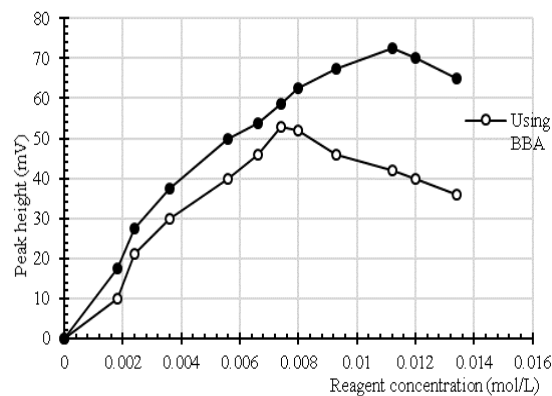
Optimization of the chemical parameters

Barbituric acid and thiobarbituric acid were used in

the reaction for formation of colored product. The influence of BBA (and TBA) concentration on the peak height was examined. The results showed that the peak height increased as the concentration of the reagent increased. When the concentration of the reagent was above 0.00102 mol/L for BBA and 0.00180 mol/L for TBA in batch method (Figure 3a), and above 0.0074 mol/L BBA and 0.0112 mol/L TBA using flow system (Figure 3b) the peak height decreased. Thus, these concentrations were used as optimum.



(a)



(b)

Fig. 3: Effect of reagent concentration on the absorbance: (a) batch method (b) flow method.

Optimization of the physical parameters of batch method

The effect of reaction time using different temperatures on the color intensity of the product was studied using optimum reagent concentration. An increase in absorbance observed with increasing reaction time from 1 min to 3 min at 30 °C using BBA and 25 °C using TBA. While increasing of absorbance limited after 3 min as shown in Figure 4. Therefore, it is recommended that the reaction should be carried out at 30 °C when BBA used as reagent and at 25 °C for TBA.

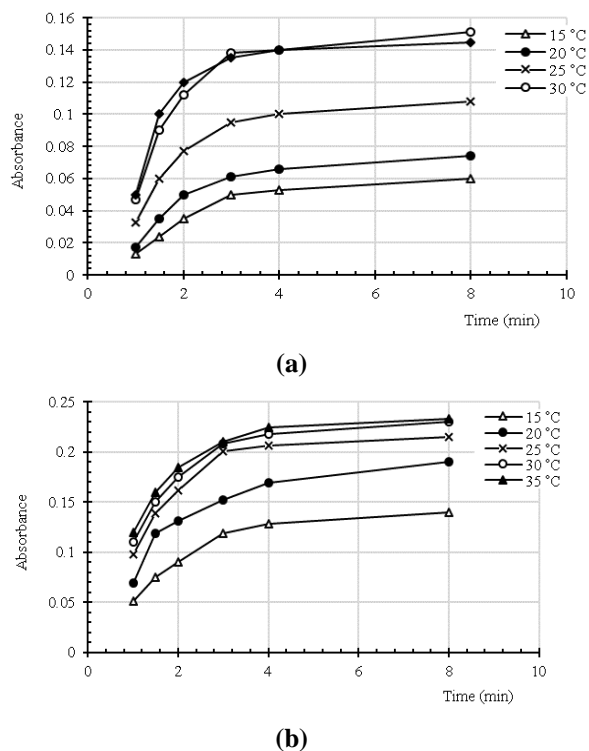


Fig. 4: Effect of reaction time and temperature on the colored product using: (a) BBA (b) TBA as reagent.

Optimization of the physical parameters of flow system

The influences of flow rate on peak heights for the colored reaction are shown in Figure 5. It is known that very low flow rates can originate small and enlarged peaks due to a high dispersion of the sample. On the other hand, at very high flow rates the reaction may not progress enough to give an adequate signal-to-noise ratio [25]. An optimum flow rate was 1.0 ml/min using BBA solution and/or for TBA solution.

A reaction coil was used in the flow system for mixing and reaction between nitrite and reagent as shown in flow schematic diagram. It was found that

Table 1: Optimum chemical and physical conditions for the proposed methods.

Parameter	Batch		Flow injection	
	BBA	TBA	BBA	TBA
Reagent concentration (mol/L)	0.00102	0.0018	0.0074	0.0112
Mixing time (min)	3.0	3.0	–	–
Temperature (°C)	30	25	25	25
λ_{max} (nm)	536	589	536	589
Flow rate (ml/min)	–	–	1.0	1.0
Coil length (cm)	–	–	40	30

40 cm reaction coil length, for using BBA as reagent, enough to producing maximum peak height in studied range (10 – 60 cm), while 30 cm reaction coil gave best result using TBA as shown in Figure 6. Therefore, these coil reactions length were used in subsequent studies.

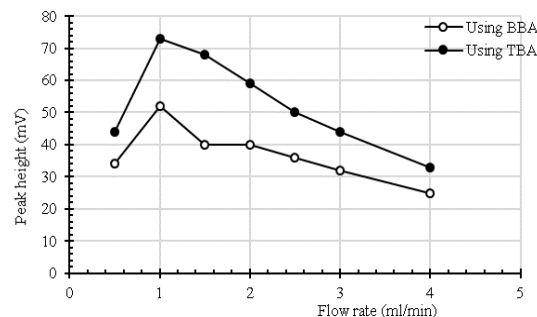


Fig. 5: Effect of flow rate on the reaction.

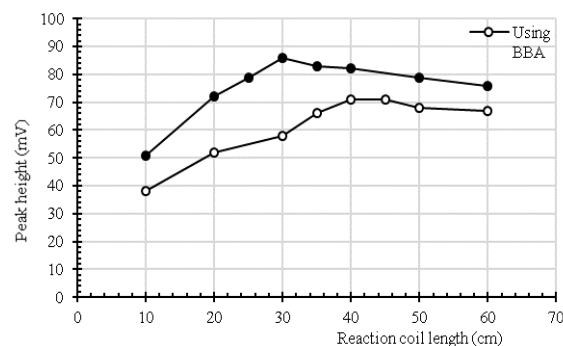


Fig. 6: Effect of reaction coil length on the colored product.

Stability of the colored product

Stability time was obtained by following the absorbance readings of the developed reaction product for 24 hours at room temperature ($25 \pm 1^\circ\text{C}$). It was found that the produced color was stable for more than 8 hours.

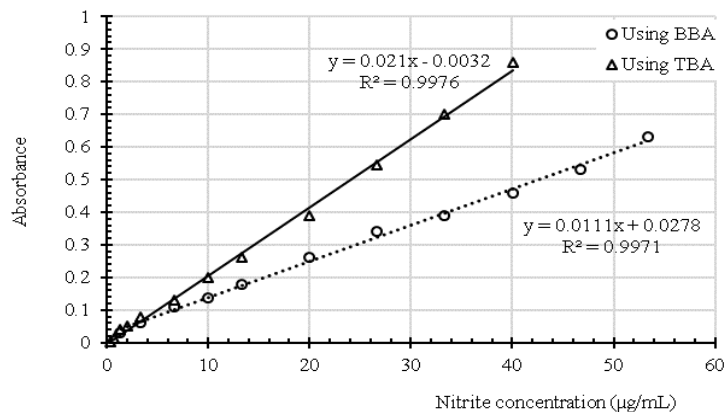
The optimum chemical and physical conditions for the determination of nitrite using proposed methods are shown in Table 1.

Validation of the methods

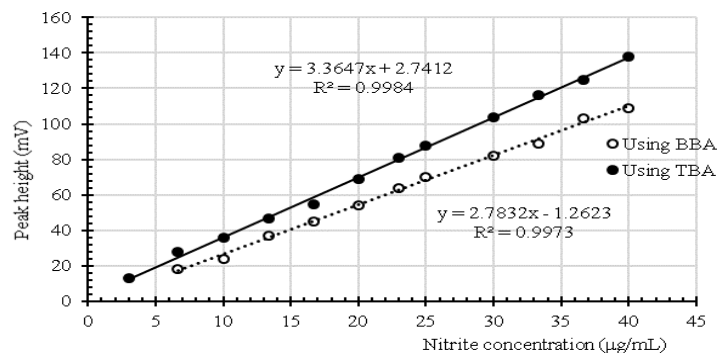
Calibration graph

Linear relationship was obtained between nitrite concentration and absorbance applying the developed methods (Table 2). Good linearity of the calibration

curves were clearly evident by good correlation coefficients which ranged from 0.9985 to 0.9992 and coefficients of determination ranged from 0.9971 to 0.9984.



(a)



(b)

Fig. 7: Calibration graph for determination of nitrite: (a) batch method (b) flow injection method.

Table 2: The statistical parameters of calibration results for the determination of nitrite.

Parameters	Value			
	Batch		Flow injection	
	BBA	TBA	BBA	TBA
Analyte	Nitrite	Nitrite	Nitrite	Nitrite
Linear range (µg/ml)	1.3 – 53	0.3 – 40	6.5 – 43	3.0 – 40
Detection limit (µg/ml)	0.5	0.05	3.0	1.0
Slope	0.0111	0.0210	2.7832	3.3647
Intercept	0.0278	0.0032	1.2623	2.7412
Correlation coefficient (R)	0.9985	0.9988	0.9986	0.9992
Coefficient of determination (R ²)	0.9971	0.9976	0.9973	0.9984

Accuracy and precision

The accuracy of the methods were determined by investigating the recovery of nitrite for each methods at three concentration levels covering the calibration range (five replicates of each concentration). The results shown in Table 3 depict good accuracy and error percentage ranged from -0.37 to -2.73%.

As shown in Table 3, the small values of standard deviation (SD) and relative standard deviations (RSD%) point to high precision of the proposed methods.

Table 3: Accuracy and precision measurements of the proposed methods.

Method		Nitrite ($\mu\text{g/ml}$)		Accuracy (E %)	RSD%
		Added	Found ($\bar{X} \pm \text{SE}; \text{SD}$)		
Batch	Using BBA	15	14.66 \pm 0.120 ; 0.207	-2.29	1.42
		25	24.32 \pm 0.113 ; 0.195	-2.73	0.80
		40	39.20 \pm 0.099 ; 0.171	-1.99	0.44
	Using TBA	15	14.91 \pm 0.020 ; 0.035	-0.62	0.24
		25	24.76 \pm 0.026 ; 0.045	-0.95	0.18
		40	39.38 \pm 0.029 ; 0.050	-1.55	0.13
Flow injection	Using BBA	10	09.73 \pm 0.027 ; 0.047	-2.73	0.49
		25	24.78 \pm 0.029 ; 0.050	-0.89	0.20
		35	34.81 \pm 0.031 ; 0.053	-0.54	0.15
	Using TBA	10	09.86 \pm 0.015 ; 0.025	-1.43	0.26
		25	24.91 \pm 0.015 ; 0.025	-0.37	0.10
		35	34.83 \pm 0.029 ; 0.050	-0.49	0.14

X=Mean of five replicate (n=5), SE=Standard Error, SD=Standard Deviation, E%=Relative error, RSD%=Relative standard deviation.

Selectivity

The selectivity of the proposed methods was studied by determining the effect of various chemical species on the estimation of nitrite (Table 4). The tolerance limit was defined as the concentration of added ion

(cation or anion) causing less than $\pm 5.0\%$ relative error for the nitrite determination. To avoid the effect of some interfering cations 2 ml of 2% EDTA solution was used before adding of the reagent.

Table 4: Effect of common interferences on the determination of Nitrite.

Method	Interfering ion	Reagent	MAC	Nitrite ($\mu\text{g/ml}$)		Recovery %	RSD %	TCR
				Added	Found ($\bar{X} \pm \text{SE}; \text{SD}$)			
Batch	Sodium	BBA	250	25	24.82 \pm 0.026 ; 0.046	99.28	0.18	10
		TBA	250	25	24.92 \pm 0.023 ; 0.040	99.67	0.16	10
	Potassium	BBA	200	25	24.87 \pm 0.024 ; 0.042	99.47	0.17	8
		TBA	200	25	24.93 \pm 0.021 ; 0.036	99.72	0.14	8
	Ammonium	BBA	150	25	24.53 \pm 0.031 ; 0.053	98.12	0.22	6
		TBA	150	25	24.64 \pm 0.036 ; 0.062	98.56	0.25	6
	Calcium	BBA	225	25	24.91 \pm 0.012 ; 0.020	99.64	0.08	9
		TBA	225	25	24.96 \pm 0.015 ; 0.025	99.83	0.10	9
Continue.....								

	Magnesium	BBA	150	25	$24.27 \pm 0.032 ; 0.056$	97.08	0.23	6	
		TBA	150	25	$24.37 \pm 0.037 ; 0.064$	97.47	0.26	6	
	Chloride	BBA	250	25	$24.73 \pm 0.032 ; 0.055$	98.91	0.22	10	
		TBA	250	25	$24.76 \pm 0.028 ; 0.049$	99.03	0.20	10	
	Carbonate	BBA	200	25	$24.74 \pm 0.035 ; 0.061$	98.96	0.25	8	
		TBA	200	25	$24.78 \pm 0.003 ; 0.006$	99.11	0.02	8	
	Bicarbonate	BBA	175	25	$24.71 \pm 0.026 ; 0.045$	98.83	0.18	7	
		TBA	175	25	$24.74 \pm 0.015 ; 0.026$	98.96	0.11	7	
	Sulphate	BBA	100	25	$24.33 \pm 0.120 ; 0.208$	97.33	0.86	4	
		TBA	100	25	$24.48 \pm 0.042 ; 0.072$	97.92	0.29	4	
	Phosphate	BBA	100	25	$23.81 \pm 0.096 ; 0.167$	95.24	0.70	4	
		TBA	100	25	$24.15 \pm 0.074 ; 0.129$	96.59	0.53	4	
	Flow injection	Sodium	BBA	250	25	$24.80 \pm 0.010 ; 0.017$	99.20	0.07	10
			TBA	250	25	$24.90 \pm 0.009 ; 0.015$	99.59	0.06	10
		Potassium	BBA	200	25	$24.84 \pm 0.019 ; 0.032$	99.37	0.13	8
			TBA	200	25	$24.91 \pm 0.007 ; 0.012$	99.63	0.05	8
		Ammonium	BBA	150	25	$24.51 \pm 0.023 ; 0.040$	98.03	0.16	6
			TBA	150	25	$24.61 \pm 0.026 ; 0.046$	98.44	0.19	6
Calcium		BBA	225	25	$24.88 \pm 0.018 ; 0.031$	99.53	0.12	9	
		TBA	225	25	$24.93 \pm 0.017 ; 0.030$	99.72	0.12	9	
Magnesium		BBA	150	25	$24.23 \pm 0.018 ; 0.031$	96.91	0.13	6	
		TBA	150	25	$24.32 \pm 0.010 ; 0.017$	97.28	0.07	6	
Chloride		BBA	250	25	$24.71 \pm 0.012 ; 0.021$	98.83	0.08	10	
		TBA	250	25	$24.74 \pm 0.023 ; 0.040$	98.95	0.16	10	
Carbonate		BBA	200	25	$24.71 \pm 0.032 ; 0.055$	98.83	0.22	8	
		TBA	200	25	$24.75 \pm 0.025 ; 0.044$	99.00	0.18	8	
Bicarbonate		BBA	175	25	$24.68 \pm 0.015 ; 0.025$	98.73	0.10	7	
		Continue.....							

		TBA	175	25	24.72 ± 0.015 ; 0.026	98.88	0.11	7
	Sulphate	BBA	100	25	24.28 ± 0.091 ; 0.157	97.11	0.65	4
		TBA	100	25	24.45 ± 0.029 ; 0.050	97.81	0.21	4
	Phosphate	BBA	100	25	23.77 ± 0.064 ; 0.110	95.09	0.46	4
		TBA	100	25	24.13 ± 0.067 ; 0.115	96.53	0.48	4

MAC= Maximum Allowable Concentrations, X=Mean of three replicate (n=3), SE=Standard error, SD= Standard deviation, RSD%= Relative standard deviation, TCR= Tolerable Concentration Ratio with no interferences = (Interferent (µg/mL) / Nitrite (µg/mL)).

Sample analysis

The proposed methods were applied to the determination of nitrite and nitrate in waste water samples of Erbil city/Iraq. The water samples were collected from five different places, and were filtered before analysis.

The performances of the proposed methods were compared statistically in terms of student's t-values and the variance ratio F-test. At 95% confidence level, the calculated t value and F value do not exceed the theoretical values for the methods.

Table 5: Standard addition method for the assay nitrite and nitrate in waste water using proposed methods.

Method	Reagent	Wastewater samples	Nitrite found (µg/ml)		E %	RSD %
			Proposed method X ± SE; SD	Standard method X ± SE; SD		
Batch	BBA	Makhmur's way	519.33 ± 2.848 ; 4.933	512.00 ± 2.082 ; 3.606	1.43	0.95
		Peshasazy Bashur	547.33 ± 1.453 ; 2.517	540.67 ± 1.453 ; 2.517	1.23	0.46
		Hawkary	462.00 ± 1.528 ; 2.646	458.00 ± 1.528 ; 2.646	0.87	0.57
		Galawezh	437.67 ± 2.404 ; 4.163	435.67 ± 1.764 ; 3.055	0.46	0.95
		New Zanko Village	392.67 ± 1.856 ; 3.215	391.00 ± 2.517 ; 4.359	0.43	0.82
	TBA	Makhmur's way	516.67 ± 1.202 ; 2.082	512.00 ± 2.082 ; 3.606	0.91	0.40
		Peshasazy Bashur	535.67 ± 2.728 ; 4.726	540.67 ± 1.453 ; 2.517	-0.92	0.88
		Hawkary	457.00 ± 1.732 ; 3.000	458.00 ± 1.528 ; 2.646	-0.22	0.66
		Galawezh	435.33 ± 1.202 ; 2.082	435.67 ± 1.764 ; 3.055	-0.08	0.48
		New Zanko Village	389.67 ± 0.667 ; 1.155	391.00 ± 2.517 ; 4.359	-0.34	0.30
Flow injection	BBA	Makhmur's way	524.00 ± 1.000 ; 1.732	512.00 ± 2.082 ; 3.606	2.34	0.33
		Peshasazy Bashur	550.67 ± 2.333 ; 4.041	540.67 ± 1.453 ; 2.517	1.85	0.73
		Hawkary	464.00 ± 1.528 ; 2.646	458.00 ± 1.528 ; 2.646	1.31	0.57
			Continue.....			

TBA	Galawezh	441.33 ± 1.453 ; 2.517	435.67 ± 1.764 ; 3.055	1.30	0.57
	New Zanko Village	395.00 ± 0.577 ; 1.000	391.00 ± 2.517 ; 4.359	1.02	0.25
	Makhmur's way	514.00 ± 1.528 ; 2.646	512.00 ± 2.082 ; 3.606	0.39	0.51
	Peshasazy Bashur	539.67 ± 2.963 ; 5.132	540.67 ± 1.453 ; 2.517	-0.18	0.95
	Hawkary	459.67 ± 1.453 ; 2.517	458.00 ± 1.528 ; 2.646	0.36	0.55
	Galawezh	437.67 ± 1.202 ; 2.082	435.67 ± 1.764 ; 3.055	0.46	0.48
	New Zanko Village	391.67 ± 1.764 ; 3.055	391.00 ± 2.517 ; 4.359	0.17	0.78

X=Mean of three replicate (n=3), SE=Standard error, SD= Standard deviation, RSD %= Relative standard deviation, E= Error.

CONCLUSIONS

The developed batch spectrophotometric methods and flow injection spectrophotometric methods for the determination of nitrite and nitrate in waste water samples are precise, accurate and sensitive. Statistical analysis proves that the methods could be applied for the analysis of waste water and drinking water.

The proposed method neither involves extraction nor did demand sophisticated instruments and not involve any stringent reaction conditions. The required chemical reagents are readily available and are in additional nontoxic and non-carcinogenic. The proposed methods are simple and reduce the time required to obtain results. The simultaneous method was successfully used to determine the nitrite and nitrate concentrations of wastewater samples from Erbil, Iraq. As suggestion, it can be applied in pharmaceutical analysis for the determination of nitrate or nitrite containing drugs.

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