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Determination of Nitrite in Meat by Azo Dye Formation

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ABSTRACT
A nitrite has been determined by developing a simple and sensitive spectrophotometric method which is involve the reaction between nitrite and ρ-bromoaniline to form the corresponding diazonium ion which is afterward coupled with salbutamol in the presence of ammonia solution to form a yellow water-soluble and stable azo dye showing maximum absorption at 442 nm. A 0.1 - 3 µg NO_2^-/10 mL is follow of Beer's law, i.e., 0.1 - 3.0 ppm with Sandell's sensitivity index of 4.24×10^{-4} µg.cm^{-2} and ε, the molar absorptivity of 1.08606×10^5 L.mol^{-1}. cm^{-1}. The limit of detection and the limit of quantitation is calculated for determination of nitrite which is 0.0199 µg.ml^{-1} and 0.0274 µg.ml^{-1} severally. The applicability of this method has been tested by as saying nitrite in curing meat samples.

Keywords: Azo dye, ρ-bromoaniline, Curing meat, Nitrite, Spectrophotometric determination.

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INTRODUCTION

Nitrogen for both animals and plants is consider as a vital nutrient which is found in the environment in the form of nitric oxide (NO), nitrite (NO_2^-) and nitrate (NO_3^-)[1]. The nonstop use of nitrite in bleaches ,food , fertilizers as well as for medicinal purposes, unify with disclosure of their potential toxicity has raised a lot of concerns [2 ,3]. The biological oxidation of ammonium formed nitrite which can be converted subsequently to nitrate [4].

In mammalian systems nitrate is converted to nitrite by bacterial and mammalian enzyme action, then nitrite reacts with amines, amides, and amino acids to form N-nitroso compound [5].

The red color in cured meat and hence come from the combination of nitrite with myoglobin to form nitrosohaemoglobin, which is sometimes used as meat preservatives [6]. As a bacteriostatic, nitrite stop the growth of bacteria specifically Clostridium botulinum which causes botulism, also it inhibits the growth of spores when it used as a sporostatic [7]. The minimum quantity of added nitrite needed to secure a safe shelf life of curing meat is 25 ppm while the maximum allowed amount of nitrite in curing meat is 200 ppm [7,8]. Therefore, the need for assaying of nitrite was undisputed.

For the determination of NO_2^-, many techniques have been proposed cover chromatographic method [9,10], electrochemical method [11,12] , colorimetric method [7], solid-phase spectrophotometric method [13,14], capillary electrophoresis method [12,15], UV Absorption Spectra [16] and spectrophotometric methods [17- 19].

During the past 15 years, different techniques for the determination of nitrite is developed [20]. The most widely used methods for nitrite determination is spectrophotometric methods, due to the excellent limit of detection obtained and facile assay-type protocols [21]. The aim of the study is to develop a sensitive and simple method for the determination of nitrite in curing meat using the reaction of the coupling diazotization. The study is based on a coupling on the reaction of nitrite with ρ-bromoaniline to form the corresponding diazonium ion which is subsequently coupled with salbùtamol in the existence of ammonia solution forming a water-soluble and stable azo dye showing highest absorption at 442 nm.

METHODS AND MATERIAL

APPARATUS

The spectral and absorbance measurements were done by a UV-1800 SHIMADZU scanning UV/Vis spectrophotometer and AE-UV 1609 UV-Vis spectrophotometer sequentially using 1-cm match quartz cells, while (HANNA pH-211) pH meter is used for the pH measurements.

REAGENTS

The chemicals used in this method were of analytical reagent grade.

Stock NO_2^- solution (100 µg/ml): A 0.1499 g of $NaNO_2$ (Fluka) was dissolved in distilled water after that diluted to 100 ml in a volumetric flask. The stock solution then used to prepare working standard solution which it prepared freshly.

ρ-bromoaniline reagent solution, 0.1 % and 0.05% : A 0.1 and 0.05 g of ρ-bromoaniline (Ridel-deHaën) sequentially is thaw in 75 ml of distilling water , shake and warm the solution if necessary then it's made up to volume in 100 ml volumetric flask. The solution is preserved in a refrigerator in a brown bottle and is stable for at least three days.

Salbutamol salphate, 0.05%: A 0.05 g of salbutamol sulphate (from N.D.I, the state company for drug industries and medical appliances- Ninevah) is solve in distilled water and then completed to 100 ml with distilled water in a volumetric flask.

Sodium hydroxide solution, 1M: A 4 g of sodium hydroxide (BDH) is dissolving by distilling water and the volume is completed to 100 ml in a volumetric flask and standardized with HCl.

Ammonium hydroxide solution, 1M: A 18.55 ml of concentrated ammonium hydroxide (13.48 N) (Ridel-deHaën) is prepared by dilution with distilled water to the mark in 250 ml volumetric flask.

Hydrochloric acid, 3 M: A 24.84 ml of concentrated hydrochloric acid (12.076 N) (Ridel-deHaën) is diluted with distilled water to the mark in 100 ml volumetric flask.

Sample preparation

The meat samples were treated by boiling it with hot water with each one of concentrated Carrez reagent I and II , according to the Roche Applied Science procedure, Cat

No.11746081001, 68298Mannheim, Germany then samples were extracted followed by filtration [22].

RECOMMENDED PROCEDURE

In a 10 ml of volumetric flask a known volume of aqueous sample of NO_2^- containing 3 µg of nitrite was charged with 1 ml of hydrochloric acid (1M) then 1 ml of 0.1% ρ-bromoaniline, 0.5 ml of 0.05% salbutamol sulphate and 5 ml of sodium hydroxide (1 M) were added. The composition solution diluted to the mark with ethanol absolute to the mark. In the same way the reagent blank was prepared without nitrite ion. The absorbance measuring is done at 334.5 nm.

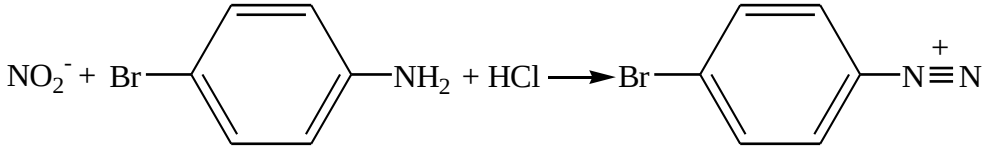
RESULTS AND DISCUSSION

Absorption spectra

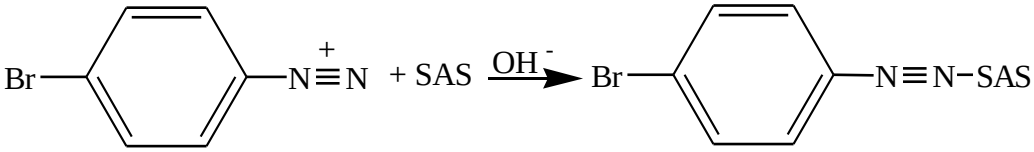
According to the recommended procedure, the nitrite ion was treated. The azocompound is showing maximum absorption at 334.5nm. While, the blank in this region has no absorbance.

The principal of the method

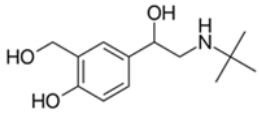
Nitrite is reacted with ρ-bromoaniline in the existence of hydrochloric acid to form the corresponding diazonium ion as showmen in the equation:



Then salbutamol sulphate is coupling with diazonium ion in the presence of basic solution to form a water-soluble and stable azo dye :



SAS (salbutamol sulphat) =



It was observed that the color intensity of the dye formed depends on the amount of nitrite present.

Effect of Acid Types

The effect of different types of 1 M acids (hydrochloric, sulphuric, acetic, formic and nitric acids) has been investigated. From the results in Table (1) hydrochloric acid gives the higher intensity so it recommended for the sequence experiments.

Table 1: Effect of acid types (1M)

Acids (ML)	Absorpance
HCl	0.443
H_2SO_4	0.207
HNO_3	0.195
HCOOH	0.076
H_3COOH	0.302

Effect of HCl Concentration

The effect of the concentration of (1, 2, 3,4 ,5 ,6, and 7 M) of HCl on the colour intensity of the azo dye was calculated. The outcome indicated that 3 M of hydrochloric acid is chosen in the recommended procedure because the azo compound is stable and the blank solution has no noticed absorbance at this wavelength.

The HCl volume effecting on absorbance

The effect of different volume of 3 M of hydrochloric acid on the absorbance of the azocompound have been studied as shown in Table (2)

Table 2: The HCl volume effecting on absorbance

ML of 3 M HCl	Absorbance of sample
0.1	0.062
0.3	0.133
0.5	0.440
1.0	0.700
1.5	0.617
2.0	0.036

From the above results, 1 ml of 3M of hydrochloric acid gives highest absorbance so it is chosen for the following experiments.

Effecting of ρ- bromoaniline reagent on absorbance

The effect of various volumes (0.1,0.3,0.5,0.7,1.0,1.5 and 2.0 ml) of 0.1% of ρ- bromoaniline reagent was investigated. The results give indistinct values, therefor we decided to change the concentration of the reagent from 0.1% to 0.05%, then repeated the experiment with the new concentration 0.05% of ρ- bromoaniline reagent as showed in Table (3).

Table 3: Effect of (0.05%) of ρ-bromoaniline volume in absorbance:

ML of 0.05% PBA	Absorbance of sample
0.1	0.658
0.3	0.857
0.5	0.824
0.7	0.794
1.0	0.716
1.5	0.660
2.0	0.585

The above results indicated that the highest intensity is 0.3 ml of 0.05% of ρ-bromoaniline therefore it's recommended for the next experiments.

Effect of salbutamol salphate volume on absorbance

Effecting of different volumes (0.1,0.3,0.5,0.7, 1.0, 1.5,2.0, 2.5 and 3.0) ml of 0.05% of salbutamol salphate have been studies. The results designated that 1.5 ml of 0.05% of SAS give highest intensity, so it's chosen for the subsequence experiments.

Effect of bases type on azo dye

A 5.0 ml of different types of 1.0 M of bases such as (sodium carbonate, sodium hydrogen carbonate, sodium acetate, ammonia (ammonium hydroxide), potassium hydroxide and sodium hydroxide) were examined. From The results obtains potassium hydroxide give highest intensity while ammonia give yellow color, that is mean the reaction shifted to visible area, therefore λmax for ammonia has been measured which it is 442 nm. Ethanol is exchange by distilled water, so the reaction is become occur in aqueous medium. Ammonia is used for the next experiments.

Effect of the volume of ammonia solution

To achieve the maximum colour density of the azo dye the effect of various volume of (3.0, 4.0, 5.0, 6.0 and 6.5) ml of 1 M of ammonia solution on absorbance were investigated . The result shows that 5 ml of 1M of NH_3 gives highest absorbance so it is chosen for the next experiments. To reduce the volume, ammonia has been prepared in a concentration of 5 M instead of 1 M, therefore 1 ml of 5 M of ammonia is suggested in the next experiments.

Order of addition

For the purpose of studying the effect of changing the sequence of additives on the absorption of the resulting azodye. A number of sequences were selected (24 sequence) different in the addition of solutions .

It is noted from the results obtained that sequencing (NO_2^- + HCl+ PBA+ SAS+ NH_3) which is used in previous experiments, give the highest absorption of the azodye formed compared to the rest of the sequences so it was adopted in subsequent experiments.

Colour Stability of the Azo Dye

The time of the development and stability is effecting of the colored dye therefore it is studied under the above described optimum experimental conditions. The colored dye formation has been completed immediately and remained constant for at least 3 days.

Final Absorption Spectra

(Fig. 1) showing the absorption spectra under the optimum conditions of the azocompound and that of the

corresponding reagent blank. According to the spectrum, the 442.00 nm and 277.20 nm are the maximum absorbance of the yellow colored compound and the reagent blank , respectively . The maximum absorption wavelength is 442 nm which is used for the calibration curve.

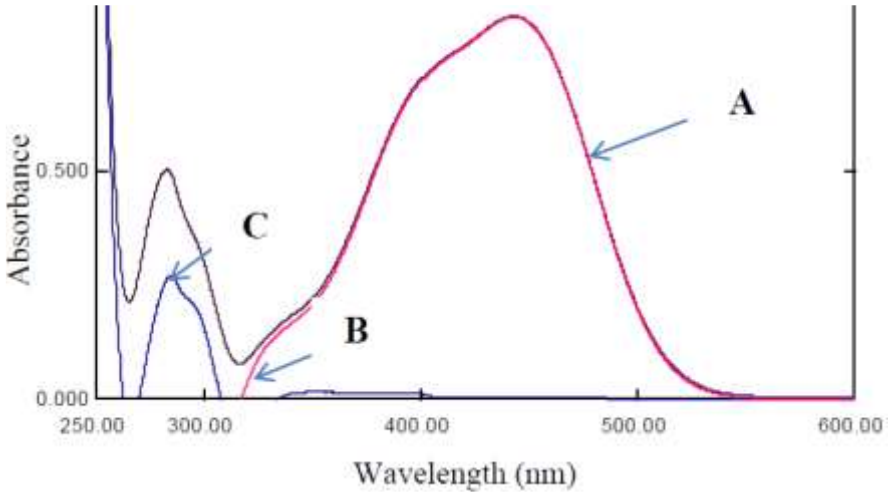


Figure 1: The absorption spectra of 30 µg of NO_2^- /10 ml treated as the present study and measured: (A) against blank, (B) against distilled water and (C) blank against distilled water.

Procedure and calibration graph

Under the optimum reaction conditions, nitrite concentration effecting is studied according to the equation of Beer's law. The study is done by transferring aliquots of nitrite solution into 10 ml volumetric flasks to involve the range 1- 55 µg /mL ; i.e., 0.1-5.5 ppm of nitrite. The 1.0 ml of 3 M HCl, 0.3 ml of 0.05% ρ-bromoaniline and 1.5 ml of 0.05% SAS, 1.0 ml of 5 M of NH_3 and the volume are completing with distilled water to the mark. At 442 n, the absorbance is measured for reaction mixtures against the reagent blank prepared in the identical manner but in the absence of NO_2^-. The calibration graph results obtain is obvious in (Fig. 2).

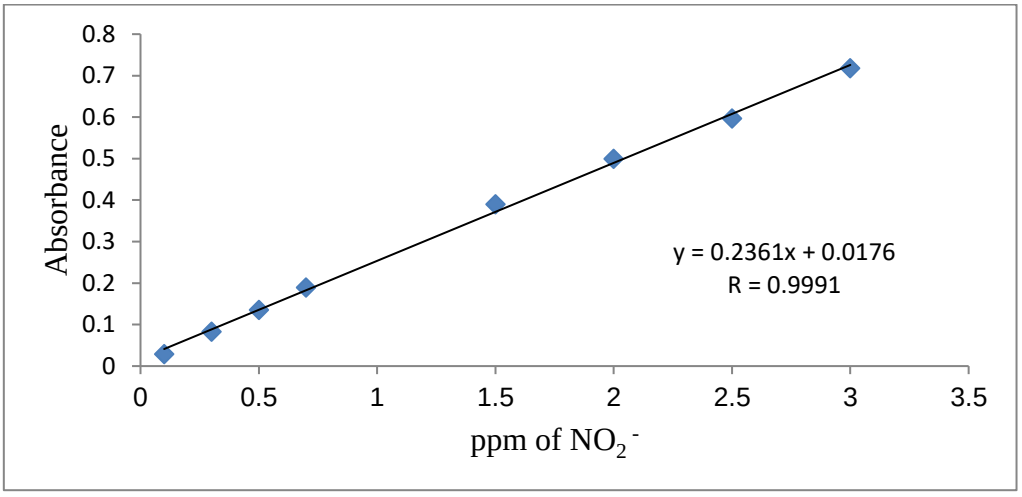


Figure 2: Calibration graph for nitrite determination with diazotized ρ-bromoaniline reagent

A straight line passing through the origin is getting from the plot above. The absorbance is linear for 0.1 – 3 µg NO_2^-/10 mL; i.e., 0.1- 3 ppm . From the equation of calibration graph, the molar absorptivity of the azo dye is calculated and found to be 1.08606×10^5 $L.mol^{-1}.cm^{-1}$ with Sandell's sensitivity index of 4.24×10^{-4} $\mu g.cm^{-2}$. A negative deviation is observed at higher concentration of NO_2^- , r^2 (coefficient of determination) is 0.9991, showing an excellent linearity as shown in Fig.2.

$$LOD = \bar{X}_B + 3\sigma_B$$

The detection limit was calculated to estimate nitrite by applying the following mathematical relationship (23):

$$LOQ = \bar{X}_B + 10\sigma_B$$

Where $\bar{X}_B$ is the mean concentration of the blank (n = 10) and σ_B is the standard deviation of the blank (23).

A 10 of the blank solution was prepared and the absorbance was measured at 442 nm $\bar{X}_B$ compared to the distilled water to be found in the and then to find the standard deviation. The detection limit was 0.0199µg.ml^{-1}. In the same way, quantification limit was calculated and used the following mathematical relationship:

It was found to be 0.0274 µg.ml^{-1}.

The Accuracy and precision of the method

To examination the accuracy σ_B and precision of the proposed study, nitrite is measured under the above-established conditions at three various concentrations. The results exhibit in Table (4) indicated that the methods is satisfactory.

Table 4: The Accuracy and Precision of the method

Nitrite concentration (µg/ml)	Relative Error,%*	Relative standard deviation ,%*
0.3	-0.36	±0.65
1.0	-0.61	±0.62
2.5	+0.28	±0.05

* Average of three determination

Study of Interferences effects

The foreign species interfering effects on the determination of nitrite in the proposed study is examined by determination 20µg of nitrite in existence of each interfering ion. Table (5) display the results obtain.

Table 5: Study of interferences effects

Foreign ion	Form added	Interferences* %		
		Amount added, µg		
		250	500	1000
Cu^{2+}	$CuCl_2.2H_2O$	-2.55	+2.98	-8.94
Zn^{2+}	$ZnSO_4.7H_2O$	+5.53	+2.55	+4.26
Co^{2+}	$Co(NO_3)_2.6H_2O$	+12.77	+8.09	+22.76
Ni^{2+}	$Ni(NO_3)_2.6H_2O$	-0.85	+3.83	+7.66
Na+	NaCl	+8.06	+1.38	+1.03
K^+	KNO_3	-0.62	+3.31	-2.07
Cr^{3+}	$Cr(NO_3)_2.9H_2O$	Light turbid	Light turbid	Light turbid
Ag^+	$AgNO_3$	Turbid	Turbid	Turbid
NH_4^+	NH_4Cl	-7.13	+8.15	+3.87
Pb^{2+}	$Pb(NO_3)_2$	+0.62	+22.53	+31.41
Tl^+	$Tl(NO_3).6H_2O$	-31.20	-9.10	-31.20
Fe^{3+}	$FeCl_3.6H_2O$	0.0	Ppt.	Ppt.
Fe^{2+}	$(NH_4)FeSO_4.6H_2O$	Ppt.	Ppt.	Ppt.
Sr^{2+}	$Sr(NO_3)_2.2H_2O$	-7.74	+13.85	1.43
Cd^{2+}	$3CdSO_4.8H_2O$	12.91	+4.12	-15.11
Mg^{2+}	$MgCl_2.6H_2O$	-3.08	+2.40	-0.34
Al^{3+}	$AlCl_3.6H_2O$	+0.83	-0.83	+17.73
NO_3^-	KNO_3	-2.61	+1.77	+3.23
I^-	KI	-40.66	-55.16	-64.84
IO_3^-	KIO_3	0.0	-4.62	-1.10
$C_2O_4^-$	$Na_2C_2O_4$	+13.85	+8.13	-0.44
ClO_3^-	$KClO_3$	-94.51	-94.51	-97.14
Cl^-	NaCl	+1.09	+4.13	-0.65
IO_4^-	KIO_4	-30.29	-32.95	-24.57
HCO_3^-	$NaHCO_3$	-18.13	-16.48	-22.80
Br^-	KBr	-6.87	+18.48	9.34
$S_2O_3^{2-}$	$Na_2S_2O_3$	-89.84	-96.70	-96.98
Mg^{2+}	$MgCO_3$	+0.62	-3.92	+5.57
Ba^{2+}	$Ba(NO_3)_2$	+4.85	+3.92	+2.06
CH_3COO^-	CH_3COONH_4	-5.77	+4.12	+9.07

Citric acid	Citric acid	+1.54	+0.44	-4.18
BrO_3^-	$KBrO_3$	-77.62	-83.95	-75.00
$S_2O_5^{2-}$	$K_2S_2O_5$	-97.94	-97.71	-98.85
SCN^-	NH_4SCN	+8.84	+0.45	+1.59

*For at least four replications

APPLICATION OF THE METHOD

The recommended study was applied successfully to the determination of NO_2^- in curing meat samples. As shown in Table (6) the results obtaining with this method agreed well with those obtaining by the standard NEDA method [24].

Table 6: The Determination of NO_2^- in cured meat samples

Meat samples	Nitritr found (µg) in 10 ml of final volume	
	N-NEDA method	Proposed method
Bodroon	1.74	1.68
Baidar	1.68	1.56
Sqada	1.35	1.48
Al Taghziah	1.45	1.55
Roasty	1.40	1.54
Ghadeer	1.50	1.60

COMPARISON OF THE METHOD

Table (7) displays the comparison between the analytical variables of this method with those of another method [25].

Table 7: Comparison of the method

Analytical parameter	Present method	Literature method (25)
Medium	Basic	acid
lmax (nm)	442	584
ε, 1. mol-1.cm-1	1.08606×10^5	5.57×10^4
Beer's law range, ppm	0.1- 3	0.1-1.6
Sensitivity index, µg. cm^{-2}		0.82
Time for colour development, Min	0	10
Accuracy	---	---
Precision, RSD% (n=5)		1.8
Cost of reagent	cheap	cheap
Toxicity of reagent	----	----
Colour of dye	yellow	Violet
Application of the method	Application to meat	Application to meat

The results obtained in Table (7) show that the current method is highly sensitive. The method of using the (ρ-Aminoazobenzene) reagent requires a quantity of acetone, while the current method using the reagents (ρ-bromoaniline and salbutamol salphate) does not require an organic solvent for the process of dissolving. A ρ-aminoazobenzene is stable for only three hours while ρ-bromoaniline and salbutamol salphate is stable for at least three days. In order to increase the color stability of p-aminoazobenzene, we need to add a surfactant factor (sodium dodecyl sulphat), while the current method does not need to add any surface surfactant factor to increase the stability of the resulting color where the color of the resulting dye stable for at least three days.

CONCLUSION

A nitrite has been determined by improving a sensitive and simple spectrophotometric method which is depend on the reaction between nitrite and ρ-bromoaniline to form the corresponding diazonium ion which is then coupled with salbùtamol in the presence of ammonia solution to form a water-soluble and stable azo dye .The method has been applied successfully in curing meat samples.

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REFERENCES

1. Moshoeshoe, M. N., & Obuseng, V. (2018). Simultaneous determination of nitrate, nitrite and phosphate in environmental samples by high performance liquid chromatography with UV detection. *South African Journal of Chemistry*, *71*(1), 79-85.
2. Jiao, L. Z., Dong, D. M., Zheng, W. G., Wu, W. B., Feng, H. K., Shen, C. J., & Yan, H. (2013). Determination of nitrite using UV absorption spectra based on multiple linear regression. *Asian Journal of Chemistry*, *25*(4), 2273-2277.
3. Yilong, Z., Dean, Z., & Daoliang, L. (2015). Electrochemical and other methods for detection and determination of dissolved nitrite: A review. *Int. J. Electrochem. Sci*, *10*, 1144-1168.
4. Fraihat, S. M. (2017). Green methods for the determination of nitrite in water samples based on a novel diazo coupling reaction. *Green Processing and Synthesis*, *6*(2), 245-248.
5. Rezaei, M., Fani, A., Moini, A. L., Mirzajani, P., Malekirad, A. A., & Rafiei, M. (2014). Determining nitrate and nitrite content in beverages, fruits, vegetables, and stews marketed in Arak, Iran. *International scholarly research notices*, *2014*.
6. Nemade, K. S., & Attarde, S. (2014). Development and validation of UV spectrophotometric method for simultaneous estimation of nitrate and nitrite in water, soil, drug and vegetable samples. *International Journal of Advanced Chemistry*, *2*(2), 74-79.
7. Khanfar, M. F., Abu Eisheh, N. J., Al-Ghussain, L., & Al-Halhouli, A. A. T. (2019). Lab on a Chip for the Colorimetric Determination of Nitrite in Processed Meat Products in the Jordanian Market. *Micromachines*, *10*(1), 36.
8. Ammar, M.B. and Wadala A.W.(2013). Photometric assay of nitrite in various samples. Raf. J. Sci., 24(1) , 60-83.
9. Zhao, J., Wang, J., Yang, Y., & Lu, Y. (2015). The determination of nitrate and nitrite in human urine and blood by high-performance liquid chromatography and cloud-point extraction. *Journal of chromatographic science*, *53*(7), 1169-1177.
10. Moshoeshoe, M. N., & Obuseng, V. (2018). Simultaneous determination of nitrate, nitrite and phosphate in environmental samples by high performance liquid chromatography with UV detection. *South African Journal of Chemistry*, *71*(1), 79-85.
11. Foresi, N., Correa-Aragunde, N., Amenta, M., Arruebarrena, A., Creus, C., & Lamattina, L. (2016). Detection of Nitric Oxide and Determination of Nitrite Concentrations in Arabidopsis thaliana and Azospirilum brasilense. *Bio-protocol*, *6*, 1-10.
12. van Staden, J. K. F., Nuta, R. G., & Tatu, G. L. (2018). Determination of Nitrite from Water Catchment Areas Using Graphite Based Electrodes. *Journal of The Electrochemical Society*, *165*(13), B565.
13. Gavrilenko, N. A., Saranchina, N. V., Fedan, D. A., & Gavrilenko, M. A. (2017). Solid-phase spectrophotometric iodometric determination of nitrite and selenium (IV) using a polymethacrylate matrix. *Journal of Analytical Chemistry*, *72*(5), 546-550.
14. Nesterenko, E. P., Murphy, B., Murray, E., Moore, B., & Diamond, D. (2016). Solid-phase test reagent for determination of nitrite and nitrate. *Analytical methods*, *8*(35), 6520-6528.
15. Martínková, E., Křžek, T., & Coufal, P. (2014). Determination of nitrites and nitrates in drinking water using capillary electrophoresis. *Chemical papers*, *68*(8), 1008-1014.
16. Jiao, L. Z., Dong, D. M., Zheng, W. G., Wu, W. B., Feng, H. K., Shen, C. J., & Yan, H. (2013). Determination of nitrite using UV absorption spectra based on multiple linear regression. *Asian Journal of Chemistry*, *25*(4), 2273-2277.
17. Porche, M. (2014). *Spectrophotometric Determination of Nitrite by Derivatization with Captopril* (Doctoral dissertation, Miami University).
18. Qader, H. A. (2013). Spectrophotometric determination of nitrite in curing meat samples. *Applied Science Report*, *3*(3), 153-156.
19. Nagaraj, P., Gopalakrishna Bhat, N., & Chandrashekara, K. G. (2016). Spectrophotometric determination of nitrite and nitrate ions by diazo coupling method. *International Journal of Chemical Studies*, *4*(3), 101-105.
20. Wang, Q. H., Yu, L. J., Liu, Y., Lin, L., Lu, R. G., Zhu, J. P., ... & Lu, Z. L. (2017). Methods for the detection and determination of nitrite and nitrate: A review. *Talanta*, *165*, 709-720.
21. Bshabshe, A.A., Al-Shehri, H., Assiri, A., Jamil, A. An unusual presentation of systemic lupus erythematosus as evan syndrome: A case report and review literature (2018) European Journal of Molecular and Clinical Medicine, 5 (1), pp. 37-40.
22. Afkhami, A., Masahi, S., & Bahram, M. (2004). Spectrophotometric determination of nitrite based on its reaction with p-nitroaniline in the presence of diphenylamine in micellar media. *Bulletin of the Korean Chemical Society*, *25*(7), 1009-1011.
23. GmbH RD.(2016). Nitrite/nitrate, colorimetric method, Cat. No. 11 746 081 001, Photometric endpoint determination, Version 6, Roche Applied Science, Germany, 68298, Manheim.
24. Shrivastava, A., & Gupta, V. B. (2011). Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles of young scientists*, *2*(1), 21.
25. American Public Health Association, American Water Works Association, Water Pollution Control Federation, & Water Environment Federation. (1992). *Standard methods for the examination of water and wastewater* (Vol. 2). American Public Health Association. 85-86.

26. Maroa SMA.(2010). Colorimetric determination of nitrite via diazotization coupling reaction application to meat. H.D. Thesis. Mosul - Iraq: Department of Chemistry, College of Science, Mosul University. 24-25.