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التقدير الطيفي للنتريت في نموذج اللحم امنه عدنان الفارس جامعة الموصل / كلية العلوم / قسم الكيمياء amenaadnan@uomosul.edu.iq

الملخص

تم اقتراح طريقة طيفية بسيط ودقيقة وحساسة لتقدير النتريت في عينات اللحوم. كان التفاعل الرئيسي للتحديد المقترح هو أيون النتريت في أزوتة سلفاسيتاميد في وسط حامضي لإنتاج ملح ومستقرة الذي يقترن فورًا بكاشف الريسورسينول لينتج صبغة أزويه ملونة قابلة للذوبان بالماء ومستقرة تتمتع بأقصى امتصاص عند ٩٤٤ نانومتر. الطريقة الحالية مناسبة لتقدير أيون النتريت من ٢.٥ إلى ٢٥ ميكروغرام/٢٥ مل. الامتصاصية المولارية وحساسية ساندل للصبغة الآزوية المتكونة هي ٢.٣٢٢ × ١٠٠ لتر .مول⁻ . سم⁻ و ٢٩٢٠٠٠ ميكروغرام. سم^{-٢} على التوالي. تم تطبيق الطريقة لتقدير النتريت في عينات لحوم مختلفة وكانت النتائج التي تم الحصول عليها مرضية (مع عناق الأخطاء التحليلية). أشارت نتائج طريقة الإضافة القياسية المواد المضافة إلى علب اللحوم عينات اللحوم المختلفة إلى أن الطريقة الحالية من تداخلات المواد المضافة إلى علب اللحوم المعبأة والمواد الحافظة.

الكلمات المفتاحية: نتربت، ازوتة، سلفااسيتاميد، نموذج اللحم.

Spectrophotometric Determination of Nitrite in Meat Sample Amenah A. Alfares University of Mosul /College of Science/ Department of Chemistry amenaadnan@uomosul.edu.iq

Abstract:

A simple, accurate, and sensitive spectrophotometric determination of nitrite in meat samples has been suggested. The main reaction for the suggested determination was occupied of nitrite ions in the diazotization of





sulphacetamide in an acidic medium to produce the diazonium salt which immediately coupled with resorcinol reagent to produce a soluble and stable colored azo dye has maximum absorption at 494 nm. The present method is suitable for the estimation of nitrite ion from 2.5 to 75 μ g/25ml. The molar absorptivity and Sandell sensitivity of the formed azo dye are 2.392 x 10⁴ 1. mol.⁻¹ cm⁻¹ and 0.00192 μ g.cm⁻², respectively. The method has been applied to estimate nitrite in various meat samples and the results obtained are satisfactory(with the range of analytical errors). The results of the applied standard addition method in the estimation of nitrite in various meat samples indicated that the present method is free from interferences of the additives added to packaged meat cans and preservatives.

Keywords: Nitrite, sulphacetamide, diazotization, meat sample.

Introduction

Sodium nitrate and sodium nitrite are nitrogen oxide compounds, they are natural components present in nature(water and vegetables) that are usually , they have many uses, and the most important use is to add them to canned meat, which can be well-kept without deterioration at a cold temperature and not the degree of freezing. The importance lies in the fact that sodium nitrite gives a red color to meat and is acceptable to shoppers (sodium nitrite is a cosmetic material) and also prevents the formation of microorganisms that work on spelling the meat and cause of food poisoning for those who consume it. The limitation of the quantity of sodium nitrite in meat is not more than 200 ppm. Sodium nitrate and sodium nitrite the two compounds have a carcinogenic effect, so that the Food and Agriculture Organization & World Health Organization recommended for the quantity of nitrate was 3.7 mg / kg body weight/ per day(Nerdy, 2018, 2983; Larsson, 2011, 28; González, 2015, 80).

If the factory wants to make the color of the meat to be red color, it must add quantities in some cases that exceed 10-20 minutes, than if the purpose of adding is only to preserve it.





From the literature review there are various spectrophotometric methods used in the estimation of nitrite in various samples. Most of the previous methods are based on using the Griess reaction, which included using quantity of nitrite in the formation of the colored azo dye via using nitrite in diazotization of aromatic amine(in acidic medium) and then the corresponding diazonium sault coupling with phenol or its derivative or an aromatic amine. Many of the methods in literature have good sensitivity and selectivity but some of them require control of pH and fixed temperature. The type of above reaction included two reagents, amine to be diazotized (in presence of acid) and coupling reagent to produce the colored azo dye: paminophenylmercaptoacetic acid with N-(I-naphthy1)ethylenediamine di hydrochloride in acidic medium to form an colored (bluish violet) azo dye has maximum absorption at 565 nm(Tarafder& Rathore, 1988, 1073-1076). p-nitroaniline in the presence of diphenylamine in micellar media(Afkhami & Bahram, 2004, 1009-1011), benzidine to produce the corresponding bisdiazonium ion, the colored azo dye resulted from coupling with resorcinol(Nagaraj & Chandrashekara, 2016, 101-105), p-bromoaniline to converted to diazonium ion then coupled with salbutamol in ammonia solution to form a yellow stable azo dye (Hamoudi & Fakhre, 353-542), and 2-aminobenzoimidazole to produce corresponding diazonium salt, then subsequently coupled with orcinol (Qader, 2013, 153-156). Direct reaction of nitrite with barbituric acid in acidic solution to produce the nitroso derivative of barbituric acid (violuric acid), that has maximum absorption at wavelength of 310 nm(Aydın & Taşcıoğlu, 2005, 1181-1186).Captopril can be used in determination of nitrite(Porche, 2014).UV absorption spectra based on multiple linear regression is employed(Jiao & Yan, 2013, 2273-2277).

Various techniques have been used in estimation nitrite these techniques: electrochemical technique(Yang, 2021, 54; Wang, 2021, 221), voltammetric(Lu, S., Hummel, 2020,167), HPLC-DAD(Tatarczak-Michalewska, 2019, 1754), capillary ion chromatography(D'Amore, 2019, 12), and GC/MS(Luckovitch, 2020, 55).





During the literature survey there is no any research used sulphacetamide as amino compound and phloroglucinol as coupling agents in any spectrophotometric determination of nitrite, therefore the reaction of sulphacetamide diazonium salt resulted from reaction of a known amount of nitrite and then coupling with phloroglucinol are used in this paper for estimation of nitrite ion in various samples

Material and methods

Apparatus:

The absorption spectra and absorbance measurements are performed using Jasco V-630 digital double beam UV-Vis spectrophotometer equipped(Japan) with 1.0-cm matched Silica cells. Bp3001 professional bench top pH meter devices were used for pH measurements.

Material: All chemicals that used in this work were purchased from Fluka and B.D.H companies.

Reagents:

All experiments were performed with analytical- reagent grade.

Solutions:

Sodium nitrite solution $(25\mu g.ml^{-1})$ **:** This solution was prepared by dissolving 0.0025g of sodium nitrite(68.99 g.mol⁻¹) in 100 ml distilled water in a volumetric flask.

Sulphacetamide solution (500µg/ml): the solution was prepared by dissolving 0.0500 g sulphacetamide in 80 ml distilled water and completed to the mark in 100 ml volumetric flask with distilled water.

Resorcinol (0.1%): Accurately 0.1000 g of resorcinol (110 g.mol⁻¹) was dissolved in 100 ml distilled water in a volumetric flask.

Sulphuric acid (0.5 M) and (2 M NaOH) solutions: were also prepared by prepare dilution of concentrated sulphuric acid(98 g.mol⁻¹) and dilution ampule of standard sodium hydroxide (Fluka, 100 ml, 10 M) to 500 ml distilled water.

Meat sample solution





The meat solution was prepared by mixing two containers of meat (bordon, Brazilian). Crushing 5 g of a sample and mixing it with 10 ml of hot distilled water (80 0 C), good mixing for the purpose of obtaining high homogeneity. Then the constituents were completely transferred to a larger size beaker, and the digestion process was carried out using a steam bath for two hours. A 1 ml of saturated mercuric chloride (1 g dissolved in 10 ml of distilled water) is added to prevent bacterial growth then the mixture is cooled to the laboratory temperature and filtered to complete the volume to 100 ml in a volumetric flask(Younis, 1998, 120-150).

Analytical procedure for calibration curve:

An aliquot 0.1-3ml of standard solution of sodium nitrite (25 μ g.ml⁻¹) was transferred into a series of 25 ml calibrated flasks, to each flask 1.5 ml of sulphacetamide (500 μ g.ml⁻¹) and 0.7 ml sulphuric acid. The flasks were stand for 2 minutes. Then1.5 ml of 0.1 % resorcinol was added, 5 ml of sodium hydroxide solution (2M) was added, The volumes were completed to mark with distilled water, (Figure 1) shows the calibration curve which indicates that Beer's law was obeyed over the concentration range (2.5-75) μ g.25ml⁻¹. The molar absorptivity was 2.392 x 10⁴ l.mol⁻¹.cm⁻¹.



Figure 1 Calibration curve of Nitrite (NO_2^-) determination using the proposed method

Results and discussion:





Principle of the method:

The first step of reaction was preparation of diazonium salt from the reaction of NO^{2-} with sulphacetamide in presence of sulphuric acid(Scheme 1).



Sulphacetamide **Scheme 1:** Preparation of diazonium salt.

The second step induced the coupling of diazotized sulphacetamide with resorcinol in presence of sodium hydroxide to produce a colored azo dye(Scheme2).



Scheme 2: Coupling of diazotized sulphacetamide with resorcinol.

The primary experiment:

1.00 ml of sodium nitrite solution (50 μ g.ml⁻¹) with 0.5 ml of 0.5 M sulphuric acid was taken in 25 ml volumetric flask and mixed with 1.0 ml of





(500 μ g.ml⁻¹) sulphacetamide, 1.5 ml of resorcinol and 4 ml of 2M sodium hydroxide were added and diluted to the mark with distilled water. The absorption spectrum shows that the colored azo dye has a maximum absorbance at 494 nm, so that this wavelength was used in the measured of absorbance in the next experiment.

Optimum reaction conditions:

The effect of various parameters on the absorption intensity of the formed azo dye(in room temperature = 22 ± 2 ⁰C) were optimized.

Effect of Diazotization Acid:

The effect of various acid solutions (0.5 M) such as HCl, CH₃COOH, HNO₃ and H₂SO₄ have been investigated as acid in diazotization of sulphacetamide (SAA) in order to produce intense colored azo dye. The experimental investigations showed that H₂SO₄ was the most suitable acidic medium for obtaining maximum absorbance and it was used in all subsequent experiments. The effect of different volumes (0.2-1.0 ml) of 0.5 M H₂SO₄ has been examined and the absorbance of formed azo dye was measured at 494 nm (Table 1).

ml of (0.5M)	Absorbance				
sulphuric acid	0.25	0.5	1.0	1.5	\mathbf{R}^2
0.2	0.1206	0.2228	0.4624	0.7270	0.9981
0.5	0.0959	0.2199	0.4825	0.7072	0.9988
0.7	0.1013	0.2310	0.4575	0.7145	0.9995
1.0	0.0922	0.2160	0.4508	0.6925	0.9993

Table 1 The effect of sulphuric acid amount on absorbance.

The results in (Table1) indicated that 0.7 ml of the sulphuric acid is the more suitable to give high absorbance and also the highest value of determination coefficient.

Effect of sulphacetamide amount

The effect of SAA amount has been investigated by using various amount of SAA (1-3 ml of 500 μ g.ml⁻¹), the results shown in Table 2.

 Table 2 The effect of SAA amount on absorbance.



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ml of	Absorban	ce/ µg.ml ⁻¹ of nit	\mathbf{D}^2	
500 μg/ml	0.5	1.0	1.5	ĸ
1.0	0.1403	0.225	0.3592	0.9832
1.5	0.2801	0.4516	0.7187	0.9844
2.0	0.2363	0.4347	0.6997	0.9932

The results in Table 2 indicate that 1.5 ml of SAA (500 μ g/ml) gave the highest absorbance of formed azo dye, so that it recommended to use in next experiments.

The effect of resorcinol amount

The effect of the coupling reagent (resorcinol) on the intensity of azo dye has been studied by adding various amounts (1-2 ml of 0.1%) to various amount of NaNO₂ (0.5-2 μ g.ml⁻¹) and adding the other solutions with optimum values (Table 3).

ml of						
Resorcinol (0.1%) solnution	0.5	1.0	1.2	1.5	2	\mathbf{R}^2
1	0.2374	0.4365	0.5811	0.7098	0.9527	0.9953
1.5	0.2405	0.4721	0.5992	0.7320	0.9577	0.9979
2 44	0.2338	0.4569	0.5577	0.7435	0.9318	0.9941

Table 3 The effect of resorcinol amount on absorbance

The result in (Table 3) indicated that 1.5 ml of resorcinol was the optimal, according to high absorbance of formed azo dye and the highest value of R^2 (0.9979).

Effect of Base:

The produced azo dye was carried out in basic medium. Therefore, the effects of various alkaline solution (2M) were investigated such as NaOH, Na_2CO_3 , KOH, $NaHCO_3$ and NH_4OH . The experimental investigations showed that the formation of azo dye required a strong basic solution of NaOH or KOH, while Na_2CO_3 and $NaHCO_3$ exhibited weak color contrast which is apparently due to pH variation. The most suitable basic solution to



give maximum absorbance is NaOH solution (Table 4). Also the effect of sodium hydroxide on absorbance has been studied and the results illustrated in Table 5.

ml of	Absorbance/	Absorbance/ml of nitrite added				
(2M) NaOH	0.5	1	1.5			
1	0.2138	0.3694	0.5275			
1.5	0.2190	0.4237	0.6660			
2	0.2380	0.4650	0.7216			
2.5	0.2490	0.4962	0.7522			
3	0.2606	0.5343	0.7806			
4	0.2615	0.5193	0.8114			
5	0.2649	0.5375	0.8190			
6	0.2486	0.5029	0.7972			

Table 4 The effect of different types of bases on absorbance.

Table 5 The effect of the amount of sodium hydroxide on absorbance.

Base (2M)	Absorbance/ml added of bases	рН
NaOH	0.4431	13.22
КОН	0.4239	12.39
Na ₂ CO ₃	0.0641	9.30
NaHCO ₃	ميبة وطرابق اللدير 0.0557 ⁴	8.06

The results in (Table 5) indicated that 5 ml of 2M sodium hydroxide was the optimal volume, it gave high absorbance.

Effect of solvent:

Various solvents were used in the dilution of solution. The results illustrated in Figure 2 and Table 6.





Figure 2 The spectra of formed azo dye in various solvents.

580

485

	Solvent	λ _{max}	Absorbance
Α	Distilled Water	494	0.5357
В	Acetic acid	394	0.3790
С	Ethanol	433	0.4189
D	Acetone	498	0.4715
Ε	Propanol >1 >	- <u>499</u>	0.4268
F	Formic acid	400	0.3358

 Table 6 The effect of solvents on absorbance.

The results in Fig.2 and Table 6 indicated that the water was the optimum solvent, it gave high absorbance, therefore distilled water was used in dilution.

Effect of time on color development: To test the effect of time on the absorbance of colored azo dye at 494 nm, two different amounts of sodium nitrite(25 and 50 μ g/25 ml) under the optimal, experimental conditions have been taken, and the absorbance was measured at different intervals of time up to 60 minutes (Table 7).

Table 7 The effect of time on absorbance.



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	ugof	Absorbance/minute standing time	

µg of		Absorbance/minute standing time						
nitrite/25	0	5	10	20	30	40	٥.	٦.
ml	U	5	10	20	50	ΨU		
25	0.535	0.535	0.535	0.535	0.535	0.535	070	070
50	0.937	0.936	0.935	0.935	0.935	0.936	• 970	• 970

Final absorption spectra:

Sodium nitrite was treated according the recommended procedure; the absorption spectrum shows a maximum absorption at 494 nm. The reagent blank solution has no absorption at this wavelength (Figure 3).



Figure 3 Absorption spectra of 25μ g.ml⁻¹ sodium nitrite treated according to the recommended procedure and measured Vs. blank (A), (B) azo dye measured Vs. distilled water, and C blank Vs distilled water

Nature of the dye:

The composition of the intense orange dye that result from the reaction of diazotized-SAA with RES in basic medium has been established using the continuous variation and mole ratio methods, the results indicate that the dye has a combination of 1:1 ratio of diazotized SAA to RES (Figure 4 and Figure 5).







Figures 4 and 5 indicated that the azo dye formed in ratio 1:1 SAA:RES, the diazonium salt prefers to bonded with the reagent RES at the para site for one of the hydroxyl groups and ortho for the other group, and this is proven by the literature (Zakaria, 2018, 38-43; Othman, 2017, 60-66), therefore the suggested structure of colored azo dye as shown in Figure.6.





Figure 6 The suggested structure of colored azo dye. **Application of the method**

Various amount of meat sample solution were taken and the steps of proposed method were applied and the results shown in Table 8.

ml of meat solution	Absorbance	Concentration µg/25 ml	mg NO ²⁻ /g sample	Average mg NO ²⁻ /g sample
1	0.1305	5.76	0.00461	
3	0.4230	19.83	0.00528	0.00499
5	0.6611	31.27	0.00500	A CARACTER STATE OF A CARACTER STATE

Table 8 The results of application

The results above in Table 8 indicated that the amount of nitrite ion in meat sample is very low, and it is with the accepted amount for humane used of meat.

To prove that the method was free from interference of addition added in manufacture of meat, the standard addition method was applied in estimation 1ml of meat sample solution. The results are illustrate in Figure 7.



Figure 7 The application of standard addition method in application of the method

The percentage of mg NO_2^- /g meat constructed from the relationship of linearity according application of standard addition method equal to 0.00458



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mg NO^{2-}/g sample, and it is very close to the value calculated via calibration curve.

Conclusion

A method to assay nitrite in meat samples using a sensitive spectrophotometric method has been proposed. Nitrite ion occupancy during the diazotization of sulphacetamide to produced the diazonium- salt, which instantly combined with resorcinol reagent to generate a soluble and persistent colored azo dye measured at 494 nm. The application section to determine the nitrite content of various meat samples. and the outcomes, despite the spectrum of analytical errors, are good.

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