# Spectrophotometric Determination of Nitrite in Curing Meat Samples Using Diazo-Coupling Reaction

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## Abstract:
A simple and sensitive spectrophotometric method for the determination of nitrite was described. Nitrite reacts with an acidified sulfanilamide to form a diazonium ion, which is subsequently coupled with orcinol in alkaline medium to form an intensely yellow-colored, stable and water soluble azo dye having maximum absorption at 427nm.The linear absorbance plot with concentration indicates that Beer's law obeyed over the range of 0.005-1.80μg/ml of nitrite, with a detection limit of 0.003μg/ml. The molar absorptivity and Sandell index were $4.36X10^4$ l/mol.cm and 0.00105 μg/cm$^2$, respectively. The optimum conditions affecting the colour intensity and interferences of the foreign ions were studied. The results of the proposed method for determination of nitrite in curing meat samples agreed with those obtained by the NED standard method.

**Key words:** Nitrite, orcinol, sulfanilamide, curing meat

## Introduction:
Sodium and potassium salts of nitrite and nitrate are important additives in the cured meat industry; they give the meat a salty and better taste. The main reasons for the addition of nitrite to meat products are anti-microbial action, color fixation, preservation effect and a significant indirect beneficial effect on flavor [1,2].

High nitrite concentration, however, could lead to infant methaemoglobinaemia, this excess of nitrite is due to bacterial reduction of nitrate or excess nitrite in the curing brine [3,4].It is, therefore, very important to monitor the nitrite content of cured meat in order to avoid negative results such as nitrite burn or a shortened storage life[5,6].At least 125mg/Kg of nitrite is added to the meat products(expressed as sodium nitrite)[7].

The degradation of nitrite is very fast, after two days more than 80% of the nitrite is already degraded. The degradation rate depends also on the addition of ascorbic acid and glucono-δ-lactone.Ascorbate creates reducing conditions in meat which speed up the rate of conversion of nitrite to nitric oxide which is important for stabilizing the meat color. Ascorbate also inhibits formation of nitrosamines in cured products [1]. The limit value for nitrite in meat is 100mg/Kg [7].

Numerous methods have been proposed for the determination of nitrite including electroanalytical[8-11], chromatographic[12,13], chemiluminescence[14,15], and spectrophotometric[16-21] methods. The widely used method is the modified version of Shinn method which is based on the Griess-Ilosvay reaction [5].The present method describes spectrophotometric method for the determination of nitrite based on the reaction of nitrite with sulfanilamide (prepared in 0.1N hydrochloric acid) to form a diazonium salt, then coupling the diazotized sulfanilamide with orcinol in alkaline medium forming an intensely azo-dye. An application part includes determination of nitrite in cured meat samples.

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## Experimental:
### Reagents:
All chemicals used were of analytical grade.

Stock nitrite solution (100 μg/ml)**:** Sodium nitrite (0.150 g) was dissolved in distilled water, 1.0 ml of chloroform (to inhibit bacterial growth) and a pellet of sodium hydroxide (to prevent the liberation of nitrous acid) were added [22], and the solution was diluted with distilled water to 1.0L. Working standard solutions were freshly prepared by diluting the stock solution with distilled water.

Sulfanilamide solution (1.0%):1.00g of the compound was dissolved in 100ml of 1.0N hydrochloric acid

Orcinol solution (1.0%):1.00g of the compound was dissolved and diluted to 100ml with distilled water.

Potassium hydroxide solution (2.0M): 11.2220g of the compound was dissolved and diluted to 100ml with distilled water.

### Apparatus:
The absorption spectra were carried out on a CECIL CE 3021 UV/Vis double beam spectrophotometer, while absorbance measurements were carried out on JENWAY 6305 UV-Vis spectrophotometer, using matched 1-cm glass cells.

### Sample preparation:
Meat samples were treated according to the procdure recommended by the International Standard Organization (ISO 2019 and ISO 3001, 1975) in which aliquot of the sample (containing 0.005-1.80μg/ml of nitrite) were taken, 2.0ml of each of carrez reagents, 5.0ml of borax solution were added and transferred to 250ml volumetric flask, After allowing to stand for 30min. make up to the mark with distilled water, mix and filter [1,2].

### General procedure:
A known volume of aqueous sample containing 0.125-45.0μg of nitrite was charged with 1.2ml of acidified 1.0% Sulfanilamide solution, standing for 2-8 min., 1.0ml of 1.0% orcinol solution and 0.5ml of 2.0M potassium hydroxide solution were added, and diluted with distilled water in 25ml standard volumetric flasks. The reagent blank was prepared in the same way but in the absence of nitrite, and the absorbances are measured at 427 nm.

## Results and discussion:

### Absorption spectra:

When nitrite ion was treated according to the previous procedure, the absorption spectra shown in Fig.1 were obtained. The maximum absorption was 427nm, it was used in the subsequent measurements to obtain the optimum conditions for the formation of the azo dye.

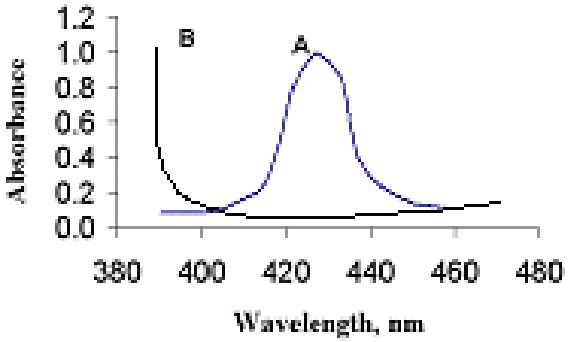


Fig. (1): Absorption spectra of : A. 1.0 µ g/ml of nitrite treated according to the recommended procedure against blank B. Blank against distilled water

### Effect of sulfanilamide concentration:

The effect of different volumes of the composite reagent (sulfanilamide + hydrochloric acid) on the maximum formation of the azo dye was studied. Results showed that 1.0ml of 1.0% sulfanilamide (prepared in 1.0N hydrochloric acid) is enough to form the diazonium ion and it was used in the subsequent experiments.

### Effect of orcinol concentration:

Effect of different volumes of 1.0% orcinol solution was examined. Results indicate that 1.0ml of the reagent solution gives maximum absorption.

### Effect of the alkaline solution:

The presence of alkali solution is essential for the development of an intense yellow color of the azo dye. Effect of different 2.0M alkali solutions (potassium hydroxide, sodium hydroxide and sodium carbonate) was investigated. Results showed that 2.0M potassium hydroxide gives the maximum absorption.

Effect of different volumes of potassium hydroxide solution (2.0M) was also examined. Results indicate that 0.5ml of the solution has a maximum absorption.

### Stability of the azo dye:

The yellow color of the formed azo dye develops instantaneously and remains stable for 6-hrs., then a gradual deterioration happens. The absorbance measurements are carried out in the subsequent experiments after dilution to the mark immediately.

### Calibration curve:

Using the optimum conditions obtained previously, straight line of calibration curve was obtained, which shows that the colored system followed Beer's law over the concentration range of 0.005-1.8µg/ml of nitrite as shown in Fig. (2), with a detection limit of 0.003µg/ml.The correlation coefficient square is 0.9994, the molar absorptivity and Sandell index were $4.36 \times 10^4$ l/mol.cm and 0.00105 µg/cm$^2$, respectively.

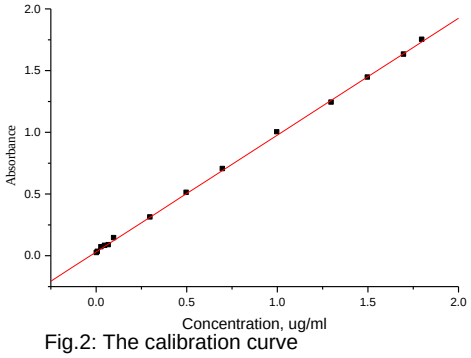


Fig.2: The calibration curve

### Precision and accuracy:

The precision and accuracy of the nitrite ion were studied depending upon the value of the relative standard deviation percentage (RSD %), and relative error percentage (Error %) for five replicates. Results are shown in Table (1)

**Table (1):** Precision and accuracy

| Concentration of nitrite (µg/ml) | RSD % | Error % |
|---|---|---|
| 0.01 | 1.800 | +1.97 |
| 1.00 | 0.070 | -0.99 |
| 1.70 | 0.040 | +0.37 |

## Interferences:

The interferences of several ions which are added as additives to the meat samples on the determination of a solution containing 1.0µg/ml of nitrite were examined by adding various amounts of these foreign ions up to the amounts where the relative errors reach a value of about≤ ± 5% of the expected absorbance. Results obtained are given in Table (2). The results indicated that the ions have no interfering effects except ascorbic acid which was masked with 0.2ml of 0.5% iodine solution. It was noted from the results that small amounts of iodine causes negative error because it is not sufficient to oxidize the ascorbic acid, on the other hand, excess amounts of iodine causes positive error because the formed iodide reacts easily with nitrite and converts it to nitric oxide according to the equation[2] :

$NO_2 + 3I^- + 2H^+ \rightarrow NO + H_2O + I_3^-$

**Table (2):** Effect of interfering ions on the determination of 25µg of nitrite ion in a final volume of 25ml.

| Ions | Acceptable amount of foreign ions to be added in determination of 1.0µg/ml nitrite (µg) | Error % |
|---|---|---|
| Chloride | 5000 | +4.95 |
| Iodide | 3000 | -2.27 |
| Bromide | 2500 | -1.68 |
| Nitrate | 2750 | +2.07 |
| Acetate | 2000 | +4.45 |
| Ascorbic acid | 125 | -4.95 |
| Sulfate | 1250 | -3.36 |
| Phosphate | 1250 | +3.90 |
| Lead(II) | 375 | -4.55 |
| Tin(II) | 250 | +3.66 |
| Citric acid | 150 | +4.12 |

**Application of the method:**

The proposed method was applied to the determination of nitrite in cured meat samples .The curing meat extract was prepared according to the standard method of International Standard Organization (ISO 2019 and ISO 3001, 1975), 5.0ml of the extract was taken, then 0.2ml of 0.5% iodine solution was added (iodine oxidizes ascorbic acid to dehydroascorbic acid) and the recommended procedure was applied. Results as shown in Table (3) indicate that there are no significant difference between values obtained by spectrophotometric method and the standard NED method [23]

**Table (3):** Analysis of nitrite in some curing meat samples

| Sample | Nitrite(μg/ml) | |
|---|---|---|
| | **Present method** | **NED method ref.** |
| Farm | 51.00 | 50.5 |
| Bordon | 122 | 123 |
| Neirs | 126 | 126 |
| Cow brand | 131 | 131 |
| Al-Taghzea | 129 | 128 |

**Comparison with other methods:**

The main features of the present method are the use of non-toxic and easily soluble compounds, i.e. sulfanilamide and orcinol in comparison with other reagents. In comparison with other methods, the present method found to be more sensitive and has a wide range of determination limit as shown in Table (4).

**Table (4):** Comparison of the present method with other spectrophotometric methods

| **Diazotized + coupling reagent** | **$\lambda_{max}$ (nm)** | **Linear range (μg/ml)** | **ε(l/mol.cm)x $10^4$** | **Ref.** |
|---|---|---|---|---|
| p-nitroaniline+ acetylacetone | 490 | 0.05-1.4 | 3.2 | 19 |
| Dapsone + phloroglucinol | 425 | 0.008-1.0 | 4.28 | 20 |
| 3-amino-5-methyl isoxazole + resorcinol | 354 | 0.02-4.0 | 2.19 | 22 |
| Sulfanilamide + orcinol | ٤٢٧ | 0.005-1.8 | 4.36 | Present work |

## Conclusion:

The present method for determination of nitrite has a good sensitivity and wide applicability. It was applied for determination of nitrite in cured meat samples.

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# التقدير المطيافي الضوئي للنتريت مستخدماً تفاعل الازدواج الازو- في نماذج اللحوم المعالجة

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**الملخص:**

تم وصف طريقة مطيافية بسيطة وحساسة لتقدير النتريت، حيث يتفاعل النتريت مع السلفانيل أميد المحمض لتكوين أيون الدايزونيوم والذي يزدوج مع الاورسينول في محيط قاعدي لتكوين صبغة ازو الصفراء والذائبة في الماء ذات أعلى أمتصاصية عند 427nm. تم تطبيق قانون بير ضمن المدى 0.005-1.8µg/ml من النتريت وبحد كشف 0.003µg/ml .الامتصاصية المولارية ومعامل ساندل كانت $4.36X10^4$ l/mol.cm و $0.00105$ µg/cm$^2$ على الترتيب. تم دراسة الظروف المثلى المؤثرة على شدة اللون وتداخل الايونات الغريبة. النتائج المحصلة من الطريقة الحالية لتقدير النتريت في نماذج اللحوم المعالجة كانت موافقة مع الطريقة القياسية NED.