DiazO-Coupling Reactions for Spectrophotometric Estimation of Nitrite in Some Cured Meat Products

Hemn A. Qader

Department of Pharmaceutical Chemistry, College of Pharmacy, Hawler Medical University, Erbil, Kurdistan Region, Iraq.

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*Corresponding Author:
Hemn A. Qader
hemn.abdul@pha.hmu.edu.krd

A B S T R A C T

This work reported a rapid, simple and sensitive spectrophotometric procedure for determination of nitrite ion by diazo-coupling reactions. 2-Aminobenzoimidazole (dissolved in H2SO4) was charged with nitrite ion, which leads to produce a water-soluble diazonium ion that subsequently reacted with orcinol as coupling agent to yield an azo compound, reaching maximum absorption at 305 nm. Many parameters of the reaction conditions have been studied and optimized to enhance the sensitivity of the proposed method. After plotting the calibration curve, it was found that Beer's law is obeyed over the concentration range of 0.05 – 3.0 µg/mL of nitrite, with 0.03µg/mL and 1.55×10^4 L/mol.cm of detection limit and molar absorptivity, respectively. The accuracy and precision of the method were tested and obtained values were acceptable. The influence of common cations, anions and interferences was studied, then nitrite ion in some cured meat samples determined with the proposed method. The results were compared with the standard method and favorable agreement between the obtained data was observed.

1. INTRODUCTION

The nitrite ion considered as an important intermediate in the cycle of biological nitrogen, and it is a versatile chemical compound that has many applications ranging from food preservatives in food industry, meat curing process, manufacturing of dye, bleaches, fertilizers, corrosion inhibition of water of industrial process and as well as for medicinal purposes (Mousavi and Shirkhanloo, 2009, Yilong et al., 2015).

Nitrite and nitrate salts of sodium and potassium (NaNO2, NaNO3, KNO2, and KNO3) are used in meat curing process. Due to the strong reducing agent with characteristic antibacterial properties of nitrite, hence inhibit some spoilage and food poisoning anaerobic microorganisms, they stabilize red colour of meat, hinder the development of oxidative rancidity and participate to produce a special flavor. Nitrate considered being a reservoir of...
nitrite, because nitrite is formed due to the microbiological reduction of nitrate (Zanardi et al., 2002, Rished and Fakhre, 2008). Thus among these ingredients in curing meat process, using of sodium and potassium salts of nitrite plays a multi-faced role (Rished and Fakhre, 2008).

Nitrite, also it is a toxic and hazardous inorganic contaminant and has a direct impact on the human health, that toxicity due to its reaction with amines or amides in human body and forming different types of nitrosoamines, which are very powerful carcinogens (Yilong et al., 2015, Moghimi et al., 2016). Excessive intake concentration of nitrite could be health hazardous agent, especially for pregnant women and infants, because hemoglobin iron content of the red blood cells is oxidized by nitrite ion and forming the methemoglobin (disease known as methemoglobinemia), which loses its ability to carrying oxygen (Moghimi et al., 2016). Nitrites can also hazardous and cause damage to aquaculture via its toxicity to aquatic animals, including fish and crustaceans (Yilong et al., 2015).

Due to concern potential hazards and health damage caused by nitrite to human health and aquaculture environments, most international organizations have been established sets of relative standards to limit the concentration of nitrite content in potable water, food and aquaculture. The amount of 3.0 mg/L as nitrite ion (or 0.9 mg/L as nitrite-nitrogen) was recommended by the World Health Organization (WHO) as a maximum nitrite concentration in drinking water, while the European Union limit nitrite concentration for drinking water is 0.1 mg L\(^{-1}\) of NO\(_2\)-N. Also according to the WHO maximum allowed nitrite concentration in meat products is 200 ppm (WHO, 2011, Moldovan, 2012). While maximum amounts of nitrite to be added in different meat products at the beginning of the process and imposes a maximum residue of 50 μg/mL nitrites and 250 μg/mL nitrates in cured meat products that have not been thermally treated suggested by the current legislation (EU Directive 95/2)(Zanardi et al., 2002).

Due to its potential toxicity and impact on biological systems, industry, environment, agricultural an interest has been aroused for developing large numbers of new analytical procedures for estimation trace amounts of nitrite ion in different sample types regarding environmental protection and public health (Mousavi and Shirkanloo, 2009, Yilong et al., 2015). Thus many methods have been proposed and reported in the literature for quantitation of nitrite and nitrate including: spectrophotometric methods (Sastry et al., 2002, Afkhami et al., 2004, Aydn et al., 2005, Rahshid, 2006, Al-Okab and Syed, 2007, Mohammed, 2008, Veena and Narayana, 2009, Narayana and Sunil, 2009, Hassan and Ali, 2010, Nagaraja et al., 2010, Pourreza et al., 2012, Bilal and Bashir, 2013, Porche, 2014, Galán-Vidal et al., 2014, Zhang et al., 2014, Zhang et al., 2016, Bahadoran et al., 2016, Nagaraja et al., 2016, Altunay et al., 2017, Zahedi et al., 2017, Fraihat, 2017), kinetic methods (Ghasemi et al., 2004, Shariati-Rad et al., 2017), FIA (Higuchi and Motomizu, 1999, Ensafi et al., 2004, Pourhossein et al., 2005, Pasquali et al., 2007, Yaqoob et al., 2013), electrochemical methods such as; potentiometry (Hassan et al., 2003, Vlascici et al., 2006, Crespo et al., 2015, Deng et al., 2016), voltammetry (Yildiz et al., 2014, Baciu et al., 2015, Üzer et al., 2016, Terbouche et al., 2016), and polarography(Yilmaz and Somer, 2016, Somer et al., 2016), chromatographic methods (Chou et al., 2003, Wu et al., 2013, Burhan et al., 2016, Lopez-Moreno et al., 2016), and electrophoresis (Della Betta et al., 2014, Kalaycıoğlu and Erim, 2016).

Spectrophotometric methods among them are the most common and widely used techniques for estimation of nitrite and most of
them are based on diazo-coupling reactions leads to azo dye formation, although these procedures have some drawbacks of pH dependence, diazotization temperature, coupling time and using of large sample volume of carcinogenic reagents, but these are characterized by excellent limit of detection or high sensitivity, convenient and relatively inexpensive (Moghimi et al., 2016, Afkhami et al., 2004). Therefore, in the current work, spectrophotometric procedure is described for estimation of nitrite with the aid of diazo-coupling reactions. Thus, a water soluble diazonium ion is produced upon the reaction of nitrite ion with 2-aminobenzoimidazole as the diazotizing agent, which directly react with orcinol as coupling agent to give an azo dye (Scheme 1), that have λmax at 305 nm. The method has been applied successfully to the nitrite estimation in some cured meat products those are available at Erbil markets.

2. EXPERIMENTAL

2.1. Apparatus

The spectral records were performed on a CECIL CE 3021 single beam UV/Vis scanning spectrophotometer. While measurements of absorbance were carried on JENWAY 6305 UV-Vis spectrophotometer with 10-mm path length quartz cell.

2.2. Reagents and chemicals

All of the used chemicals were of analytical reagent grade.

2.2.1. Stock nitrite solution (100 μg/mL) (Fluka): 0.1499 g of sodium nitrite compound was dissolved and diluted to the mark with distilled water in a 1000 mL volumetric flask. A pellet of NaOH was added to prevent liberation of nitrous acid and for inhibiting the bacterial growth, 1.0 mL of chloroform was added (Ensafi et al., 2004). Each working standard solutions were freshly prepared by further diluting the stock solution with distilled water.

2.2.2. 2-Aminobenzoimidazole solution (0.2%) (Fluka): 0.2 g of the compound was dissolved and prepared in 0.1 M H2SO4 to 100 mL in a volumetric flask.

2.2.3. Orcinol solution (1.0%) (BDH): 1.0 g of orcinol was dissolved in distilled water and diluting to 100 mL in a volumetric flask with the same solvent.

2.2.4. Carrez Reagent I Solution (15% w/v) (SCP): 15.00 g of potassium hexacyanoferrate (II), K4[Fe(CN)6].3H2O was dissolved and diluted to 100 mL with distilled water (ALI, 2007).

2.2.5. Carrez Reagent II Solution (30.0% w/v) (SCP):30.0 g of zinc sulphate, ZnSO4.7H2O was dissolved and diluted to 100 mL with distilled water and the Carrez reagent II (30.0%) solution was obtained (ALI, 2007).

2.3. Recommended procedure

A serious of known volume of aqueous nitrite ion solutions containing (0.05 – 3.0) μg/mL were charged with 0.6 mL of 0.2% 2-aminobenzoimidazol solution (dissolved and prepared in 0.1 M H2SO4), 3.0 mL of orcinol solution (1.0%). The reaction mixture solutions in 25 mL volumetric flasks completed to the
mark with distilled water. The reagent blank was prepared as the same manner but without of nitrite ion. The absorbance of the azo compound was measured at 305 nm.

2.4. Sample preparation

Accurately 5.0 g of well-homogenized meat samples were taken into a beaker, and about 50 mL of boiling distilled water was added and mixed well then boiled on a water-bath for about 15 minutes, to extract of nitrite from the meat samples (hot water extraction). After the solutions were cooled to 15-25 °C, successively 3.0 mL from each of Carrez I and Carrez II reagent solutions were added to the sample mixture, to de-proteinisation (precipitation of proteins) and clarification. The pH was adjusted to 8±0.2 with 0.1 M of sodium hydroxide solution and mixed. The contents of the beakers were transferred quantitatively to 100 mL volumetric flasks and were make up to the mark with distilled water, and mixed well. After filtration and centrifugation, the first few milliliters of the samples were discarded and the clear filtrate used to the test (ALI, 2007), then after applying the recommended procedure spectrophotometric measurement was carried out.

3. RESULTS AND DISCUSSIONS

3.1. Absorption spectra

After treating of nitrite ion according to the recommended procedure, spectral recording of the azo compound showed that maximum absorption can be obtained at 305 nm. While, the reagent blank has no absorbance in this region, as it is shown in the Figure 1.

Figure 1: Absorption spectra of reagent blank against distilled water (1) and azo compound against reagent blank (2) treated according to the recommended procedure

3.1.1. Optimization of reaction conditions

Since 2-aminobenzoimidazole is dissolved in acid, therefore, effect of the type and acid concentration for dissolving it were studied. Using of 0.1 M sulfuric acid was found to give better results than 0.1 M of each one of hydrochloric and acetic acids (Figures 2 and 3). The effects of volume of 0.2% acidified 2-aminobenzoimidazole and 1.0% orcinol solutions as diazotizing-coupling reagents were examined, as shown in Figures 4 and 5, using of 0.6 mL of acidified 2-aminobenzoimidazole and 3.0 mL of 1.0% orcinol solution gave the maximum absorbance, therefore, were selected as optimum conditions. In general, colour of azo compounds developed in alkaline solution, therefore, the effect of different alkali solutions such as NaOH, Na₂CO₃ and NaHCO₃ were examined, the results showed that using of alkaline solution leads to form a transient yellow colour which it is very unstable and absorbance cannot be measured manually in the batch method, therefore, using of alkaline solution is avoided. The order of the reactants addition should be followed, as mentioned in the recommended procedure. The effect of using ice (low temperature) on the formation of the diazonium and the azo compound was studied, the results showed that there was no
significant difference in the azo compound absorbance with or without using of ice, therefore, the experiments have been carried out at room temperature. Stability of the azo compound was tested under the optimized conditions, the results indicated that the azo compound developed instantaneously and remains stable for t more than 2.0 hour.

3.2. Calibration curve and its statistical data

Under the chosen optimum parameters, a calibration curve was constructed (Figure 6). The azo compound seems to obey Beer’s law within the concentration range of 1.25-75.0 μg of nitrite per 25 mL (i.e. 0.05-3.0 μg/mL). Table 1 summarized the statistical data of the calibration curve of proposed spectrophotometric method for estimation of nitrite.
Table 1: Statistical data of the calibration curve for determination of nitrite spectrophotometrically

| Value                  | Parameter                        |
|-----------------------|----------------------------------|
| \( \lambda_{\text{max}} \) (nm) | 305                              |
| Linear range (μg/mL)  | 0.05 – 3.0                       |
| Regression equation   | \( A = 0.3403 \ [\text{NO}_2^-] - 0.0002 \) |
| Slop (L/mg.cm)        | 0.3403                           |
| Intercept             | 0.0002                           |
| Molar absorptivity (L/mol.cm) | 1.55×10⁴                      |
| Correlation coefficient | 0.9999                         |
| Detection Limit (μg/mL) | 0.03                            |
| Sandell’s sensitivity (μg/cm²) | 0.0029                        |

3.3. Accuracy and precision

Depending upon the values of the relative error percentage (Error %) and percentage of the relative standard deviation (RSD %), accuracy and precision of the proposed method for determination of nitrite ion were studied, respectively, for five replicate measurements. Table 2 summarizes the results.

Table 2: Accuracy and precision and of the proposed spectrophotometric method

| Nitrite ion concentration (μg/mL) | aRSD % | bError % |
|----------------------------------|--------|----------|
| 0.05                             | 1.98   | + 3.185  |
| 1.5                              | 0.585  | - 1.95   |
| 3.0                              | 1.493  | + 1.5    |

a and b are averages of 5 replicate measurements (n=5)

3.4. Interferences study

The method selectivity of the proposed method was examined by studying the effects of common cations, anions and interferences on the determination of 1.5 μg/mL nitrite ions. The interferent is a species that caused a relative error percentage in the sample absorbance greater than ±5.0%. Results are summarized in Table 3. The effect of all studied interfering cations was removed completely by using 1.5 mL of 5.0% EDTA solution as a masking agent (ALI, 2007).

4. APPLICATION OF THE METHOD

The recommend method was applied to the estimation of nitrite ion in some cured meat products. In the present method, 5.0 mL of meat extract was taken and the proposed procedure was applied. The obtained results are compared with those obtained for standard NEDA method (Sun et al., 2003) and the results seem in good agreement. Table 4 shows the results.

5. CONCLUSIONS

The proposed method was applied successfully for estimation of nitrite ion in some cured meat products. The proposed method offers some advantages such as; simplicity, easily soluble compound, i.e.2-aminobenzoimidazole and orcinol compare with some used reagents, more sensitive (higher molar absorptivity value ) than some of the reported methods (Rished and Fakhre, 2008, Moghimi et al., 2016, Sastry et al., 2002, Veena and Narayana, 2009, Pourreza et al., 2012, Zhang et al., 2014, Nagaraja et al., 2016, Zahedi et al., 2017), reproducibility, and wide applicable range compare with other published methods (Rished and Fakhre, 2008, Moghimi et al., 2016, Afkhami et al., 2004, Rashid,
Table 3: Tolerance limits of interfering species in the spectrophotometric estimation of 37.5 μg/mL of nitrite ion in a final volume of 25.0 mL.

| Interfering species     | Amount added to nitrite (μg) | Error %    |
|-------------------------|-----------------------------|------------|
| Aluminum (III)          | 5000                        | +3.926     |
| Chromium (III)          | 2500                        | -1.389     |
| Zinc (II)               | 2500                        | +1.308     |
| Cadmium (II)            | 2500                        | +2.09      |
| Cobalt (II)             | 2000                        | +3.141     |
| Magnesium (II)          | 2000                        | -1.04      |
| Ammonium (I)            | 750                         | -1.574     |
| Iron (III)              | 250                         | +4.712     |
| Nickel (II)             | 25.0                        | -3.926     |
| Manganese (II)          | 12.5                        | +3.664     |
| Copper (II)             | 12.5                        | -3.149     |
| Iodate                  | 5000                        | +1.842     |
| Bicarbonate             | 2500                        | -3.42      |
| Acetate                 | 2500                        | +1.315     |
| Bromide                 | 2500                        | -2.63      |
| Chlorate                | 2500                        | -3.10      |
| Chloride                | 1750                        | +3.68      |
| Cyanide                 | 1250                        | +2.63      |
| Oxalate                 | 1125                        | +2.762     |
| Fluoride                | 1000                        | +1.933     |
| Carbonate               | 750                         | +2.59      |
| Phosphate               | 375                         | -1.57      |
| Periodate               | 125                         | -4.443     |
| Bromate                 | 125                         | -1.10      |
| Iodide                  | 75                          | +4.143     |
| S₂O₃                    | 25                          | -4.97      |
| Thiocyanate             | 25                          | -1.837     |
| S₂O₅                    | 25                          | -5.0       |
| Sulphite                | 17.5                        | -2.887     |
| Citric acid             | 875                         | +4.26      |
| Ascorbic acid           | 500                         | +4.01      |

Table 4: Nitrite determination in some cured meat samples

| Meat samples               | Origin          | Nitrite found (μg) |
|----------------------------|-----------------|-------------------|
|                            |                 | Proposed method   | Standard NEDA method |
| Great Chicken Luncheon     | Holland         | 83.37             | 84.56               |
| Altayeb Chicken Luncheon   | Saudi Arabia    | 106.91            | 106.84              |
| Del Monte Chicken Luncheon | Jordan          | 69.94             | 72.04               |
| California Garden Chicken Luncheon | United Arab Emirates | 71.29         | 71.91               |
| Baidar Chicken Luncheon    | Saudi Arabia    | 85.50             | 83.97               |
| Altunsa Chicken Luncheon   | Turkey          | 68.00             | 69.86               |
| Farm Beef                  | Brazil          | 55.03             | 53.07               |
| Product          | Country  | Nitrite  | Nitrate  |
|------------------|----------|----------|----------|
| Bordon Corned Beef | Brazil   | 128.09   | 126.68   |
| Boca Beef        | Brazil   | 80.94    | 81.88    |
| Shilaneh Tuna Fish | Iran     | 58.13    | 56.27    |
| Kasih Tuna Fish  | Thailand | 49.61    | 48.93    |
| Americana Tuna Fish | Thailand | 83.27    | 86.08    |
| Altunsza Tuna Fish | Turkey   | 53.59    | 54.36    |
| Shabab Tuna Fish | Iran     | 61.09    | 58.23    |
| Heinz Tuna Fish  | Thailand | 59.72    | 58.51    |
| Al-Sayad Tuna Fish | Jordan  | 68.19    | 66.19    |

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