J. Basrah Researches, Vol.22 Part 3, 82-90 (1999)

Spectrophotometric Determination of Nirite By The Diazotization Method

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ABSTRACT

Spectrophotometric method for determination of nitrite is achieved. In this method highly water soluble intense yellowish azo dye 5-(P-carboxy phenyl azo) – 2-thiobarbituric acid is formed, due to the reaction of nitrite ion with an acidified P-aminobenzoic acid to produce diazonium salt, which is coupled with 2 - - thiobarbituric acid. The maximum wavelength for the absorption is 420 nm and the concentration range of nitrirte that is obeyed Beer's law is (0.02 – 1.5) ppm. The molar absorptivity is 5.8×10^4 $l.mol^{-1}$ cm^{-1} with the Sandell function is 0.005 µg / cm^2. The best conditions and interferences ions has been studied.

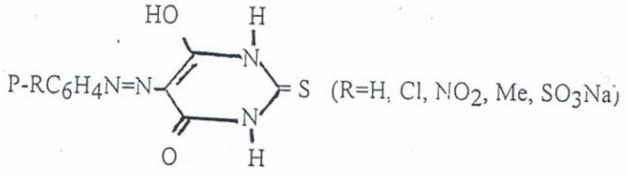
INTRODUCTION

Nitrite ion has been used for many years in foods, fertilizer, detergents and other industries. It is employed in foods for instance, in preparation of cured meat and in the manufacture of certain cheese (Lechowich, 1978), with allowable amounts are 200 mg / kg and 10 mg / kg to preserve them against the growth of some bacteria respectively (FACC, 1978).

Although the nitrite is very important in the preservation of foods, it will seriously effect on both the health and pollution, due to increase its concentration by the microbial oxidation and reduction of ammonia and nitrate respectively, therefore nitrite is played role in the formation of carcinogenous nitrosamines in the products and in the digestive through ingestion of the salts (Sawyer, 1978), so the determination of nitrite at low concentration is very important. The methods of the determination of nitrite have been reviwed by (Kolthoff, 1961); (Sawicki, 1963) and (Streuli, 1970).

Some reported methods for determination of nitrite include, Nakamura (1979) was used 4,5- dihydroxy Coumarine reagent to determine nitrite spectrophotometrically at λ_{max} 410 nm. Bashier (1981) was determined nitrite in the range (1-15) ppm at λ_{max} 499nm by using a reddish – orange azo dye, which is formed from the reaction of nitrite with P-aminobenzoic acid to form a diazonium ion, which is coupled with 8-hydroxy quinoline. Flamerz (1981) was used P-aminosalicycilic acid reagent to determine nitrite in the range (0.1 – 3) ppm at λ_{max} 520 nm. Norwite (1984) was determined nitrite in the range (0.5 – 2) ppm at λ_{max} 530 nm by using indole reagent. Abeed (1992) was measured the absorption of the yellow azo dye at λ_{max} 420 nm by using anthranilic acid reagent to determine (0.1 – 2.8) ppm nitrite.

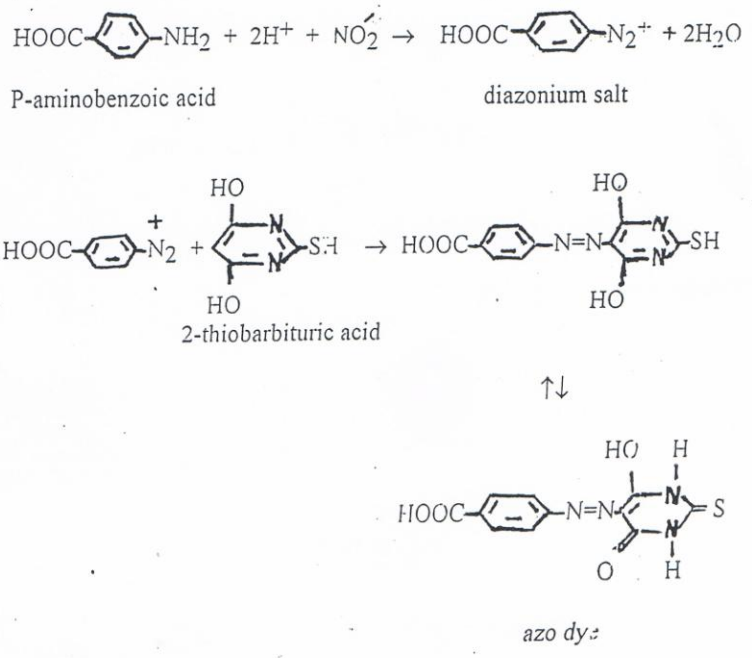
Nesynov (1971) has prepared some azo dyes, which are represented in the following formula:



due to reaction of 2-thiobarbituric acid with different aryldiazonium salts.

In the present work new yellowish azo dye is employed as an analytical method for the determination of nitrite in oqueous solution by using p- aminobenzoic acid is diazotized with nitrite to form the diazonium ion. Consequently the diazonium is coupled with 2-thiobarbituric acid.

This reaction involves the electrophile substitution of diazonium cation at five position of 2 – thiobarbituric acid to form the azo dye 5- (P – carboxy phenyl azo) – 2-thiobarbituric acid (Nesynov, 1972) and it can be represented as follows:



EXPERIMENTAL

Reagents and Chemicals

Double distilled water and analytical reagents were used for preparation solutions.

Stock solution 1000 ppm NO_2 : 0.1499g of $NaNO_2$ was dissolved in distilled water containing 1 ml chloroform to inhibtion bacterial growth, the volume was diluted to 100 ml with distilled water.

P-aminobenzoic acid 1% : 0.1g of the amine was dissolved in 70 ml of distilled water *containing 1.5 ml conc.* HCl and the volume was diluted to 100 ml with distilled water.

2-Thiobarbituric acid 0.1% : 0.1g was dissolved in distilled water to 100 ml volume.

Instrumentation

The CE 292 digital ultraviolet spectrophotometer series 2 from cecil instruments, England was used to measuring the absorbance in vissible region.

Procedure

For the determination of nitrite ion, a series of 10 ml volumetric flasks were prepared containing solution (0.02 – 1.5) ppm of NO_2. Add 2 ml of 0.1% P-aminobenzoic acid reagent solution, 2 ml of 0.1 % 2-thiobarburic acid and final volume 10 ml with distilled water. The absorbance of yellowish azo dye were measured at 420 nm against a blank contaning the same ingredients except nitrite ion.

RESULTS AND DISCUSSION

Spectra

The spectra of the yellowish azo dye which is formed by treating 10 µg of nitrite according to the procedure was recorded within wave length range 350 – 500 nm. It was found that the maximum wavelength for the adsorption is 420 nm against to the reagents blank and the reagents gave a falt absorption at 420 nm against distilled water as shown in fig. (1).

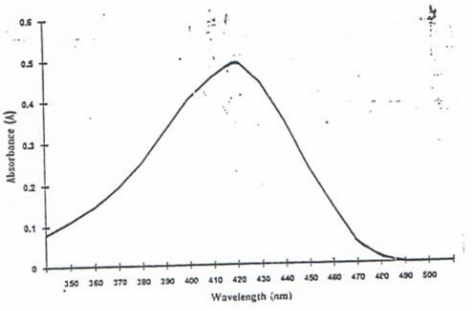


Fig. (1) Absorption spectra of the azo dye $\lambda_{max} = 420$ nm.

Effect of amounts of diazo component

In order to obtain the best conditions for the formation of intense azo dye color, many variation were studied. For instence, the effect of concentration of P-aminobenzoic acid reaget were performed by treating identical aliquot standard solution containing 10 µg of nitrite with 0.2, 0.5, 1.0, 1.5, 2, 2.5 and 3 ml of P-aminobenzoic reagent seperately. It was found that 2 ml of the reagent giving maximum and constant absorbance as shown in Fig. (2). Therefore 2 ml was recommended for the procedure.

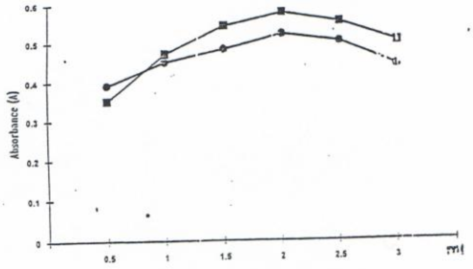


Fig. (2) The effect of the volume

(4) P-aminobenzoic acid.

(1) 2-thiobarbituric acid.

The best quantity of 2-thiobarbituric acid that gives intense azo dye color was achieved, according to treat different solutions each containing 10 µg nitrite with 2 ml of P-aminobenzoic acid solution in 1.5 ml of conc. HCl and variation volumes of 2-thiobarbituric acid were added with 0.5, 1.0, 1.5, 2, 2.5 and 3 ml respectively. It was found that the optimum volume of this reagent was 2 ml as shown in Fig. (2).

Finally, the effect of different mineral acids on the absorbance was studied, including conc. H_2SO_4, HNO_3, $HClO_4$ and HCl. Thus the results were appeared that the most effective acid was 1.5 ml (12 M) conc. HCl per 100 ml volume of amine solution was the optimum amounts.

The conditions of the formation of the azo dye:

The yellowish azo dye of 5-(P-carboxy phenyl azo)-2- thiobarbutric acid was formed instantaneously and it was found that the intensity of the color remained stable under the optimum condition.

The order of addition of the reagents for the maximum absorption should be followed as in the recommended procedure, otherwise a decrease in the intensity of the color was observed.

Calibration curves

After the optimum conditions had fixed, the linear calibration curve obtained for yellowish azo dye solution with the variation in the amount of nitrite, Beer's law is obeyed (0.02 – 1.5) ppm in final volume of 10 ml. The absorbances were measured at λ_{max} 420 nm as shown in Fig. (3).

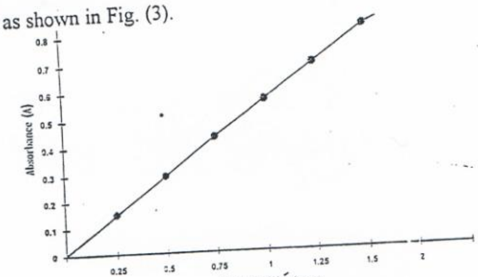


Fig. (3) Conformity to Beer's law for nitrite ion.

The molar absorptivity is 5.8 x 10^4 l. mol^{-1}. cm^{-1} (Ayers, 1961) (corresponding to the absorbance of 1 ppm in a cuvette with optical path length of 1 cm) and Sandell function for the sensitivity is 0.005 μg cm^{-2} (Sandell, 1959) (represent the number of micrograms of the determinand per ml of solution having an absorbance of 0.001 for a path length of 1 cm).

Interferences

The inteferences of a number of species on the absorption of the yellowish azo dye color were studied, by measuring the absorbance of 10 μg of nitrite ion in the presence of 200 μg (20 fold) of interfering species and the results represent as % interfernce as shown in the table below.

Intefering species	% Intefernce
Citrate	+1.7
Tertarate	+0.8
Phosphate	zero
EDTA	-31
Al^{+3}	-4.5
Bi^{+3}	+7.2
Fe^{+2}	+0.18
Fe^{+3}	+9.9
Mg^{+2}	-0.9
Mn^{+2}	-2.7
$NaHCO_3$	+5.6
Na_2CO_3	+2.1

Precision

The precision of the method was measured by using 5 μg of nitrite ion (6 determination). The relative standard deviation (%RSD) is 0.62.

CONCLUSIONS

This method of determination of nitrite by formation azo dye is occurred in aqueous medium without needing to solvent extraction and it is characterized by highly sensitive, rapid and accurate with best precision.

REFERENCES

1- Ayers, G. H. and Naragng, B. D., Anal. Chim Act., 24, 1961, 241.

2- C. A. Streuli and P. R. Averell, Anal. Chemistry of Nitrogen and its compounds, Part 1, Wiley Interscience, NewYork, 1970, p.117.

3- C. N. Sawyer and P. L. McCarty, Chemistry for Envirnomental Engineering MaGraw – Hill, NewYork 1978, p. 440.

4- E. Sawicki, J. Ppaff and T. W. Stanley, Rev. Univ. Ind. Santander, 5, 1963, 337.

5- FACC, Report on Nitrites and Nitrates in Cured meat and cheese, FACC/REP /27 London : HMSO, 1978.

6- F. A. Abeed, R. I. AL-Bayati, B. H. Salem, Mu'tah Lil – Buhooth Wa – AL – Dirasat, Vol. 7, No. 1, 1992.

7- G. Norwite and P. W. Keliher, Analyst, 1984, 109, 1281.

8- I. M. Kolthoff and P. J. Elving, Treatise on Anal. Chemistry Part II Vol. 5, Interscince, NewYork, 1961, p. 275.

9- Lechowich, R. V., Brown, W. L., Deibel, R. H. and Somers, I. I., 1978, Food Technology, 32 (5), 45.

10- Nesynov, E. P., Besprozvannaya, M. M. (Inst. Org. Khim., Kiev, USSR). Khim. Geterosikl. Soedin. 1971, 7 (9), 1271 – 5. (Chem. Abst. V76, 1972, 153700x).

11- Nakamura, Motoslai and Murata, Analyst (London), 1979, 104 (1243), 985 – 988.

12- Sandell, E. B., Colorimetric deterimination of traces of metals, 3rd Ed., Interscience N.Y., 1959.

13- S. Flamerz and W. A. Bashir, Analyst, 1981, 106, 243.

14- W. A. Bashir and S. Flamerz, Talanta, 28, 697 – 699, 1981.

الخلاصة

تم انجاز طريقة طيفية لتقدير النتريت طيفيا عن طريق تكوين صبغة الازو ٥- (بارا كاربوكسي فينل آزو) -٢- ثايو حامض الباربيتيورك ذات اللون المصفر والعالية الذوبان في الماء وذلك بتفاعل ايون النتريت مع بارا – امين حامض البنزوبك المحمض بحامض الهيدروكلوريك لتكوين ملح الدايزونيوم والذي يرتبط مع ٢ – ثايو حامض الباربيتيورك. لقد تم الحصول على مدى من تركيز النتريت يطاوع قانون بير للامتصاص عند القياس بطول موجي اعظم ٤٢٠ نامتر بحدود (٠,٠٢- ١,٥) جزء من المليون. كما وجد إن قيمة الامتصاصية المولارية هي ٥،٨ x $١٠^{٤}$ لتر.مول$^{-١}$ سم$^{-١}$ مع دالة ساندل قيمتها ٠،٠٠٥ مايكروغرام.سم$^{-٢}$ ، كما درست الظروف المثلى للتقدير وبعض التداخلات الايونية.