SHORT COMMUNICATION

DETERMINATION OF NITRITE, NITRATE AND TOTAL NITROGEN IN VEGETABLE SAMPLES

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ABSTRACT. Yellow diazonium cation formed by reaction of nitrite with 6-amino-1-naphthol-3-sulphonic acid is coupled with β-naphthol in strong alkaline medium to yield a pink coloured azo dye. The azo-dyes shows absorption maximum at 510 nm with molar absorptivity of 2.5×10^4 M^{-1} cm^{-1}. The dye product obeys Beer's law (correlation coefficient = 0.997), in terms of nitrite concentration, up to 2.7 µg NO_2 mL^{-1}. The above colour reaction system has been applied successfully for the determination of nitrite, nitrate and total nitrogen in vegetable samples. Unreduced samples give direct measure for nitrite whilst reduction of samples by copperized-cadmium column gives total nitrogen content and their difference shows nitrate content in the samples. Variety of vegetables have been tested for their N-content (NO_2^-/NO_3^-/total-N) with % RSD ranging between 1.5 to 2.5 % for nitrite determination. The effects of foreign ions in the determination of the nitrite, nitrate, and total nitrogen have been studied. Statistical comparison of the results with those of reported method shows good agreement and indicates no significant difference in precision.

KEY WORDS: Yellow diazonium cation, 6-Amino-1-naphthol-3-sulphonic acid, Vegetables, Nitrite in vegetables, Nitrate in vegetables, Total nitrogen in vegetables

INTRODUCTION

Nitrate itself is relatively non-toxic, but when ingested in food or water, it may be reduced to nitrite by bacteria in the mouth and gut. Nitrite is powerful oxidizing agent and converts iron in the hemoglobin from ferrous to ferric form and due to this hemoglobin loses its property to carry oxygen. The nitrite and nitrate concentration in vegetable, especially in the green house ones, have been a topic of great concern for research in several countries [1]. High level of nitrogen in soils commonly lead to high concentration of nitrate in various vegetables, and toxic levels of nitrite may then be produced by microorganism activity in the gastrointestinal tract. There has been concern over the potential health danger from nitrite in foods because the possibility of nitrate reacting with secondary amines present in the body to form carcinogenic nitrosoamines [2].

Various instrumental methods such as polarography [3], voltammetry [4], fluorometry [5], biamperometry [6] and flow injection spectrometry [7] have been used for nitrite determination. Nitrite is determined spectrophotometrically based on diazo coupling reaction [8], extraction of the azo dye in to suitable organic solvent provides a much lower detection limit and improved sensitivity [9]. Most of the methods based on Griess reaction, *i.e.* the formation of an azo dye by diazotization of an aromatic amine or phenol gives good sensitivity and selectivity but require close control of pH and temperature during the diazotization step and relatively longer coupling time. Literature available on use of specific reagents for spectrophotometric determination of nitrite in various environmental samples include 2-sec-butylphenol [10], neutral red [11], phloroglucinol [12], *N*-1-naphthylethylenediammonium [13], 4-aminosalicylic acid [14], 4,5-dihydroxy coumarin [15] and p-aminophenyl mercaptoacetic acid [16].

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In the present work, we have utilized a new diazotization system (i.e. reaction of NO_2^- with 6-amino-1-naphthol-3-sulphonic acid (J-acid) to form yellow colour diazonium cation) for microgram level determination of nitrite, nitrate and total nitrogen in vegetables. The reliability of the present method was established by parallel determinations by reported method and by recovery studies of added nitrite and nitrate [16].

EXPERIMENTAL

Apparatus and reagents

A Carl-Zeiss Jena (Jena, Germany) spectrophotometer fitted with EK-5 unit and matched quartz cells of 1-cm path length was used for all absorbance measurements. A Systronic digital pH meter type-335 (India) was employed for the measurement of pH value of solutions.

All chemicals used were of analytical reagent grade, BDH/E. Merck/Glaxo/SD Fine Chem. The standard solution of nitrite was prepared by dissolving 0.15 g of dried sodium nitrite (105 ^{o}C for 1 h) in 100 mL of double distilled water. The nitrite content of solution was 1000 µg mL^{-1}. Little chloroform was added as a stabilizer. A 4.2 x 10^{-3} M, i.e. 0.1 % w/v solution of 6-amino-1-naphthol-3-sulphonic acid (J-acid) was prepared in concentrated sulfuric acid. A 0.35 M solution, i.e. 5 % w/v solution of β-naphthol was prepared in acetone. A 2 M aqueous solution of NH_4Cl was prepared in double distilled water.

Copperized cadmium column

In order to estimate NO_2^--N, NO_3^--N and total-N, samples were reduced to NO_2^--N state by passing samples through a copperized cadmium columns. To prepare copperized cadmium column, 40 to 60 meshes size of copperized cadmium granules were taken, stirred for 2 min with distilled water and 100 mL of 2 % copper sulfate solutions, respectively, and then the supernatant liquid was removed. Metallic cadmium was washed with distilled water till the washings were free from precipitated copper. A glass column (5 mm internal diameter) was carefully filled with prepared material up to 10 cm height on a glass wool support without any air bubbles. The column was washed with 1 M hydrochloric acid after every four runs of nitrite reduction and reused.

Procedure for diazotization and determination of nitrite

An aliquot of sample containing 1-25 µg mL^{-1} NO_2^- was taken in a 25 mL Nessler tube and to this 0.2 mL of 6-amino-1-naphthol-3-sulphonic acid was added, a yellow colour system was formed. The solution was thoroughly shaken for 1 min to allow the diazotization reaction to complete and then 0.5 mL of β-naphthol solution was added and shaken for a min. Then solution was made alkaline with addition of 5 mL of 2 M ammonium chloride solutions. To this 6 mL of acetone was added to make a clear solution. Then the solution was made up to 20 mL with (1:2) ammonia solution. While soon after the addition of ammonia solution the yellow colour changed into pink. The absorbance of the resulting pink colour dye was measured at 510 nm against the reagent blank. The amount of NO_2^- was computed from a calibration curve prepared by linear least square method.

Determination of nitrite and nitrate in vegetable samples

The raw vegetables were chopped on an ordinary wooden plate and then ground in a porcelain mortar. A 2.5 g of the slurry was transferred into a 25 mL beaker using 17.5 mL of distilled

water and 0.025 mL of 4 % sodium hydroxide solution. The solution was warmed on a water-bath at 80 °C for 20 min, shaking occasionally, and then cooled to room temperature. This solution was filtered through a fluted filter paper into a 100 mL calibrated volumetric flask and diluted to the mark with distilled water. The solution was centrifuged manually at normal speed using a '6-tube holder' manual centrifuge machine and clear supernatant liquid was taken for nitrite determination.

Nitrite determination. 2 mL of the above solution was taken and analyzed for nitrite following the procedure describe under the procedure.

Nitrate determination. 10 mL of the made up solution was taken and passed through copperized cadmium reductor column at the rate of 1 mL min^{-1}. Total nitrate content was determined following procedure described under the analysis of vegetable samples.

Procedure for studying the effects of other ions

An aliquot containing 2.0 μg mL^{-1} standard NO_2^- was taken in a 25 mL Nessler tube and to this varied known amounts of the other ions in solution form (1-2 mL), as listed in Table 1, were added individually for the separate study of effect of each and every foreign species. To this mixed solution, all reagents were added and diazotization process was done according to the procedure as described for diazotization and determination of nitrite earlier. The absorbance of the resulting pink colour dye was measured at 510 nm against the reagent blank.

RESULTS AND DISCUSSION

Spectral studies

The absorption spectrum of the coloured system showed maximum absorption at 510 nm. All absorbance measurements were carried out against reagent blank. Reagent blank showed negligible absorbance at this region.

Diazotization reactions

The 6-amino-1-naphthol-3-sulphonic acid when treated with a solution of nitrite (taken as sodium nitrite) in concentrated H_2SO_4 medium gives a yellow diazonium cation, which in turn produced a bright purple coloured azo-dye when treated with naphthol in ammoniacal medium. Further, this reaction is well supported by similar system reported by Saitoh *et al.* [10].

Effect of diluents

The effect of various diluents, which are water-soluble and form a homogeneous mixture with system, was studied. The values of molar absorptivity at wavelength of maximum absorption of system in different solvents are as follows: methanol, $\varepsilon = 1.9 \times 10^4$ M^{-1} cm^{-1}; λ_{max} 510 nm, ethanol, $\varepsilon = 1.6 \times 10^4$ M^{-1} cm^{-1}; λ_{max} 510 nm and acetone, $\varepsilon = 2.5 \times 10^4$ M^{-1} cm^{-1}; λ_{max} 510 nm. The maximum absorbance of the complex is observed with acetone as compared to the other diluents and therefore it was preferred in all subsequent analysis.

Effect of 6-amino-1-naphthol-3-sulphonic acid

The result obtained indicated that at least $(4.1-7.3) \times 10^{-5}$ M of 6-amino-1-naphthol-3-sulphonic acid in concentrated H_2SO_4 was required for diazotization reaction. In subsequent studies, 5.2×10^{-5} M was used.

Effect of β-naphthol

The concentration range of β-naphthol was varied from 8.5 x 10^{-3} - 1.1 x 10^{-2} M. Therefore, 9.5 x 10^{-3} M was used for completion of coupling reaction.

Effect of ammonium chloride

Appropriate volume, which showed maximum absorbance at 510 nm, was 4.0-6.0 mL of 2 M ammonium chloride. 5 mL of ammonium chloride solution was employed throughout the reaction.

Effect of ammonia solution

1:2 ratio of ammonia solution with distilled water shows maximum absorbance at 510 nm with reaction mixture.

Effect of temperature

The temperature dependency of the nitrite-6-amino-1-naphthol-3-sulphonic acid-β-naphthol system was observed in terms of absorbance values in the temperature range 10-50 ˚C. No significant change was observed in the absorbance value in temperature range 18-40 ˚C.

Statistical evaluation

The system obeyed Beer's law up to 2.8 μg NO_2^- mL^{-1} with an excellent linearity in terms of correlation coefficient value of 0.997. The sensitivity of nitrite-6-amino-1-naphthol-3-sulphonic acid-β-naphthol system calculated in terms of molar absorptivity is 2.5 x 10^4 M^{-1} cm^{-1} at λ_{max} 510 nm. The precision of the method in terms of relative standard deviation (n = 6) for the determination of 2.0 μg NO_2^- mL^{-1} is 1.1 %. The method has a relative mean error of 1.1 % and a relative mean accuracy of 98.9 % for six replicate analyses at a level of 2.0 μg NO_2^- mL^{-1}.

Effect of other ions

In order to evaluate the stability of the method for the determination of nitrite in vegetable and water samples, the interference of several species in the determination was studied. Tolerable limits of various species, after adding suitable masking agents were established in presence of 2.0 μg NO_2^- mL^{-1}. The method is free from the interference of large number of anions/cations and foreign species, Table.1.

Table 1. Tolerance limit of other species in the determination of 20 μg NO_2^-/25 mL.

Anion/cation/foreign species added	Tolerable amount mg/25 mL
Cl^-, I^-	1.6
CO_3^{2-}, PO_4^{3-}, SO_3^{2-}	2.0
SO_4^{2-}	2.8
Hg^{2+}	0.10
Mn^{2+}	0.50
Co^{2+}	0.80
Cr^{6+}, Mo^{6+}, Ni^{2+}, Pb^{2+}	1.0
Cd^{2+}, W^{6+}, Na^{2+}, Ca^{2+}, Zn^{2+}, Fe^{3+}, Pd^{4+}, Pt^{4+}	1.5
Mg^{2+}	20
L(+) Tartaric acid, L(-) ascorbic acid	40
Citric acid, oxalic acid, EDTA	40
Formaldehyde	50

Application of the method

The proposed method has been applied for the determination of nitrite, nitrate and total nitrogen in vegetable samples. The result obtained by this method agreed well with those obtained by an earlier reported method [16]. The nitrite, nitrate, and total nitrogen concentration in the samples and % RSD of the two methods for are given in Table 2.

Table 2. Determination of nitrite, nitrate and total nitrogen in vegetable samples.

Samples[a]	Total nitrite (A)		Total nitrate (B-A)	Total nitrogen (B)
	Present method µg/mL (% RSD, n = 5)	Reported [16] method µg/mL (% RSD, n = 3)		
Spinach	2.16 (±2.0)	2.0 (±1.2)	2.90	5.06
	3.3 (±1.5)	3.5 (±1.5)	1.77	5.07
Tomato	1.6 (±1.8)	1.8 (±2.0)	1.2	2.8
	2.0 (±2.5)	2.3 (±1.8)	2.0	4.0
Potato	0.72 (±2.0)	0.80(±1.8)	1.08	1.80
	1.09 (±1.8)	2.0 (±2.1)	0.09	1.18
Carrot	1.09 (±1.6)	1.15 (±1.7)	4.36	5.55
	0.36 (±1.7)	0.50 (±2.0)	5.46	5.82
Radish	2.20 (±1.9)	2.0 (±1.9)	1.45	3.65
	0.36 (±1.9)	0.45 (±2.0)	1.82	2.18
Asparagus	3.60 (±2.3)	3.0 (±1.8)	2.90	6.50
	3.20 (±1.3)	3.15 (±2.2)	2.30	5.50

[a]The raw vegetable samples, grown in the agricultural fields from the surrounding areas of the city, were obtained from the local market. Total nitrite (NO_2^-) = without reduction (A). Total nitrogen ($NO_2^- + NO_3^-$) = after reduction (B). Total nitrate (NO_3^-)= (B-A).

CONCLUSIONS

The spectrophotometric determination of nitrite is based on diazotization reaction of 6-amino-1-naphthol-3-sulphonic acid with β-naphthol. The present method is suitable for the determination of nitrite and nitrate in environmental samples.

AKNOWLEDGEMENTS

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