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Spectrophotometric determination of nitrite using new coupling agents

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A method is described for the spectrophotometric determination of nitrite. It is based on the reaction of nitrite with *p*-nitroaniline (PNA) in acid medium to form diazonium ion, which is coupled with frusemide (FRU) or 5-methyl-4-{[(1*E*)-phenylmethylene]amino}-2,4-dihydro-3H-1,2,4-triazole-3-thione (MPAT) in basic medium to form azo dyes, showing absorption maxima at 680 or 395 nm respectively. The range of linearity for PNA-MPAT and PNA-FRU system are 0.4-2.0 µg/mL, 0.02-0.6 µg/mL of nitrite respectively. Molar absorptivity, Sandell sensitivity, Detection limit, Quantitation limit for PNA-MPAT and PNA-FRU system were found to be 3.314×10^3 $Lmol^{-1}cm^{-1}$, 0.014 µg cm^{-2}, 0.559 µg mL^{-1}, 1.695 µg $mL^{-1}$and 1.46×10^3 $Lmol^{-1}cm^{-1}$, 0.032 µg cm^{-2}, 0.485 µg mL^{-1}, 1.470 µg mL^{-1} respectively. The method has been applied to various water samples, soil samples and tablets.

Keywords: Spectrophotometry, Diazotization, Nitrite, Frusemide, 5-methyl-4-{[(1*E*)-phenylmethylene] amino}-2, 4-dihydro-3H-1,2, 4-triazole-3-thione

Nitrogen compounds are of interest to scientists because they are essential nutrients for living organisms, but at the same time they can be pollutants in the environment with harmful effects[1,2]. Nitrite is of major concern because it is significantly more toxic than ammonia or nitrate[3, 4]. The main source of nitrite in the environment is the microbial oxidation of ammonium ions, particularly of the genus nitrosomonas, or the reduction of nitrates in water bodies by the denitrification bacteria (*Pseudomonas* and *Achromobactor*)[5]. Nitrites are mainly used as food preservative. Nitrite combines with myoglobin to form nitrosohaemoglobin, which is responsible for the characteristic red colour in cured meat and hence it is sometime used as meat preservative[6]. Nitrite is not a direct toxicant, but may be dangerous in the form of *N*-nitroso compounds produced upon its interaction with proteins. In the recent years there has been an increasing concern about the role of nitrite, as important precursors in the formations of *N*-nitrosamines that possesses tetratogenic and mutagenic activities and hence are potential carcinogens[7].

It was discovered that vascular endothelial cells could synthesize the free- radical gas nitric oxide and since that discovery[8], it has been possible to demonstrate the considerable physiological importance of NO, for example in the control of human blood flow and pressure. Therefore, the determination of nitrite ion is very important in various fields, such as environmental studies, biology and clinical chemistry. Marczenco[9] and Fox[10] have reviewed spectrophotometric methods for the determination of nitrite. In an acid medium, nitrite reacts with primary aromatic amines to form diazonium salts, the salt is then coupled with a suitable aromatic compound to yield an azo dye, which is the basis of the UV-visible spectrophotometric determination[11,12]. The classical Griess method is based on this principle[12-14]. More than fifty different combinations based on formation of azo dyes have been reported. Obviously, the more the reaction step involved greater is the chance of loss of nitrite (or reaction intermediates) due to possible side reactions, leading to incorrect determination of nitrite. Despite the simplicity and ease of application of the spectrophotometric technique it is essentially time consuming, which makes it unsuitable for routine analysis of large numbers of samples. In addition to spectrophotometry, polarography[15], voltammetry[16], fluorimetry[17], biamperometry[18], and flow injection spectrophotometry[19] have also been used for determination of nitrite. However, some of these methods suffer from complicated and expensive instrumentation and costly chemicals, while others involve difficult and time consuming separation procedure whereas, certain methods require high temperatures. Some kinetic methods have also been reported for the determination of nitrite[19-21].

In the present investigation, nitrite is determined spectrophotometrically using new coupling agents frusemide and 5 - methyl- 4-{[(1*E*)-phenylmethylene] amino}-2,4-dihydro-3H-1, 2,4-triazole-3-thione (MPAT). Reagent PNA was diazotized in acidic

medium, and coupled with FRU or MPAT to give coloured dyes in alkaline medium having absorption maxima 680 and 395 nm respectively. The developed method has been successfully applied to the determination of nitrite in water samples, soil samples and tablets.

Experimental Procedure

Apparatus

A SHIMADZU UV- 2550 UV-Vis spectrophotometer with 1 cm matching quartz cells was used for the absorbance measurements.

Reagents

All reagents used were of analytical reagent grade and distilled water was used throughout the study. A stock solution of nitrite (1000 μg mL^{-1}) was prepared by dissolving 0.150 g of the dried sodium nitrite in 100 mL water. To this 2 mL sodium hydroxide (~ 1 M) solution was added to prevent nitrite decomposition and 1 mL of chloroform to prevent bacterial growth. Other reagents used were *p*-nitroaniline (0.05% in 2.5M HCl), frusemide (0.2%) in 50% ethanol, 5-methyl-4-{[(1*E*)-phenylmethylene]amino}-2,4-dihydro-3H-1,2,4-triazole-3-thione (0.2%) in 50% ethanol, sodium hydroxide (2 M), EDTA (0.02 M) and sodium carbonate (1%).

General procedure

To an aliquot of sample containing 0.02-0.6 μg/mL of nitrite for PNA-FRU method and 0.4-2.0 μg/mL of nitrite for PNA-MPAT method was transferred in to a series of 10 mL calibrated flasks. To this solution 1 mL of 0.05% PNA was added and the solution was shaken thoroughly for 2 min to allow to complete the diazotization reaction. Then, 1 mL of 0.2% FRU or MPAT and 1 mL of ~2 M NaOH solution were added and the contents were diluted to 10 mL with distilled water in a standard flask. The absorbance of the coloured dyes was measured at 680 and 395 nm respectively against the reagent blank.

Nitrite in water samples

Aliquots of water samples (>3 mL) were treated with 0.5 mL 1 M NaOH and 0.5 mL 0.2 M EDTA and shaken well. The precipitate formed was removed by after centrifugation. The centrifugate was transferred to a 10 mL calibrated flasks. They all tested negative. To these samples a known amount of nitrite (containing not more than 0.6 μg/mL of nitrite using PNA and FRU, 2 μg/mL of nitrite for PNA-MPAT method, respectively) was added. Aliquots of the made up solution containing nitrite was determined directly according to the proposed method (using, PNA-FRU and PNA-MPAT) and also by the reference method[22].

Determination of nitrite in tablets

Isosorbidinitrate tablet sample was taken and was reduced to nitrite using Zn\NaCl, and the clear solution was made up to 100 mL using distilled water. Known amount of this solution was taken and analyzed for nitrite content following the procedure described for the analysis of water sample.

Determination of nitrite in soil samples

About 1 g of the soil sample was weighed and placed in a 50 mL beaker and extracted three times with 5 mL portions of 1% sodium carbonate solution. The extract was filtered through Whatman 41 filter paper. Pipetted out 1-2 μg/mL of nitrite for PNA-MPAT system and 0.4-0.6 μg/mL of nitrite for PNA-FRU system were transferred into a 10 mL calibrated flasks and nitrite content was analyzed according to the proposed method.

Results and Discussion

The method involves the diazotization of PNA followed by coupling with FRU or MPAT in alkaline medium. The formed PNA-FRU and PNA-MPAT dye system has absorption maximum at 680 and 395 nm respectively. Preliminary investigations showed that hydrochloric acid was better than sulphuric, phosphoric or acetic acid. The effect of acidity on the diazotization reaction was studied in the range 0.1-0.5 M of HCl, and constant absorbance was observed in this range. It was found that the maximum colour developed within 2 min and remained almost stable for about 1 h. Diazotization was carried out at room temperature and the optimum acidity for the formation of diazonium chloride was fixed to be 0.2 M.

It was found that 1 mL of 0.05% solutions of PNA was sufficient for complete diazotization. FRU or MPAT was used as a 0.2% solution and 1 mL of FRU or MPAT was sufficient for maximum colour development. There was a decrease in absorbance at lower concentration of frusemide or 5-methyl-4-{[(1*E*)-phenylmethylene] amino}-2,4-dihydro-3H-1,2,4-triazole-3-thione, whereas higher concentration did not give good results. The effect of sodium hydroxide concentration on the absorbance was also studied, effects of addition of 0.5-2.0 mL of 2 M sodium hydroxide were examined. The investigation showed that 1-2 mL of sodium hydroxide gave

maximum absorbance and 1 mL was chosen for the procedure. The interaction of nitrite with amino group of *p*-nitroaniline to form diazonium salt is almost specific, which provides the determination of nitrite with good sensitivity. The introduction of frusemide and 5-methyl-4-{[(1*E*)-phenylmethylene] amino}-2,4-dihydro-3*H*-1,2,4-triazole-3-thione, as a new coupling agent provides a simple, rapid and selective method for the determination of nitrite. The method has the advantage of simplicity, rapidity and selectivity over the other existing methods in the literature. No extraction step is required hence the use of organic solvents which are generally toxic pollutant is avoided.

Effect of interfering species

The selectivity of the proposed methods was studied by determining the effect of various chemical species on the estimation of nitrite. The tolerance limit was defined as the concentration of added ion causing less than ±2% relative error for the nitrite determination. The present method is based on the oxidation of *p*-nitroaniline with nitrite then coupled with coupling reagents. Therefore strong oxidizing or reducing species are expected to interfere. The results indicated that Hg^{2+}, Ce^{4+}, Cu^{2+} and Co^{2+} showed severe interference. However, the tolerance level of these ions may be increased by the addition of 2 mL of 2% EDTA. Molybdate, thiocyanate and hexacyanoferrate are found to interfere and are masked by masking agents.

Analytical data

Key parameters that influence the performance of the method were studied to arrive at the optimum working configurations. All the optimization steps were carried out with a chosen nitrite concentration. The range of linearity for PNA-FRU and PNA-MPAT system are found to be 0.02-0.6 and 0.4-2.0 µg/mL of nitrite respectively. Molar absorptivity, Sandell's sensitivity, detection limit, Quantitation limit for PNA-MPAT and PNA-FRU systems were found to be 3.314×10^3 $Lmol^{-1}cm^{-1}$, 0.014 µg cm^{-2}, 0.559 µg mL^{-1}, 1.695 µg mL^{-1} and 1.46×10^3 $Lmol^{-1}cm^{-1}$, 0.032 µg cm^{-2}, 0.485 µg mL^{-1}, 1.470 µg mL^{-1} respectively.

Applications

The proposed methods were applied to the

Table 1—Determination of nitrite in different water samples, soil samples and tablets. (*p*-nitroaniline with 5-methyl-4-{[(1*E*)-phenylmethylene] amino}-2, 4-dihydro-3*H*-1, 2, 4-triazole-3-thione and frusemide)

Sample	Nitrite added (µg/mL)	Standard method		Proposed method		[b]t test	[c]F test
		[a]Nitrite found, (µg/mL)	Relative error	[a]Nitrite found, (µg/mL)	Relative error		
Using MPAT as coupling agent							
Sample 1	0.4	0.402	0.50	0.401	0.25	2.230	1.00
	0.6	0.604	0.66	0.596	0.66	1.400	4.00
Sample 2	0.4	0.418	4.50	0.404	1.00	0.894	1.23
	0.6	0.606	1.00	0.598	-0.90	0.479	1.56
Soil sample	1.0	1.010	1.00	1.002	0.20	0.447	4.00
	2.0	1.983	-0.85	2.406	0.42	1.340	1.56
Tablets	1.0	0.990	-1.00	1.004	0.40	0.894	6.25
(ID)	2.0	2.060	3.00	2.039	1.95	2.900	2.25
Using FRU as coupling agent							
Sample1	0.2	0.198	-0.90	0.221	0.25	2.236	1.00
	0.4	0.418	4.50	0.407	1.80	2.000	1.20
Sample 2	0.2	0.208	4.00	0.200	0.20	0.089	1.00
	0.4	0.403	0.75	0.402	0.50	2.940	1.10
Soil sample	0.4	0.402	0.50	0.403	1.50	1.630	4.00
	0.6	0.606	1.00	0.596	0.66	1.400	2.70
Tablets	0.4	0.402	0.50	0.401	0.25	2.236	1.00
(ID)	0.6	0.602	0.66	0.602	0.50	2.790	1.00

[a]Average of 5 determinations.
[c]Tabulated F- value for (4,4) degrees of freedom at 95% probability level is 6.39;
[b]Tabulated *t* - value for 5 degrees of freedom at 95% probability level is 2.31.
ID – Isosorbidinitrate tablets, Nitrite content = Nitrite formed by reduction of nitrate, average of 5 values.

determination of nitrite in water samples, soil samples and tablets (Table 1). The water samples were collected from different sources, and were filtered before analysis. As the samples that were available were found to devoid of nitrite, synthetic samples were prepared by the addition of nitrite and then analyzed according to the proposed procedure. The performances of the proposed methods were compared statistically in terms of student's t-values and the variance ratio F-test. At 95% confidence level, the calculated t value and F value do not exceed the theoretical values for the two methods. The theoretical t-value was 2.31 (n=5) and F–value was 6.31 (n=5). The detection limit and quantitation limit were determined with good results.

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