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Phosphomolybate and Vanadomolybdate Methods for the Determination of Nitrite in Soil, Water and Root Nodules of Diancha, Cowpea, Greengram Leguminous Plants

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Abstract: The new methods designed works on the principle that the thiourea reduced phosphomolybdenum blue complex formed is oxidized by nitrite with λ_{max} 790 nm, system 1. The other method is based on the decolorization of the vanadomolybdate complex by nitrite in acidic medium 410 nm, system 2. The decrease in the color intensity of the dye products of the system 1 and 2 are proportional to the concentration of nitrite with its quantification range 0.3-6.0 µg ml^{-1}. The molar absorptive values for system 1 and 2 are found to be 4.314 x $10^3$and 4.067 x 10^3 $lmol^{-1}cm^{-1}$ with the corresponding sensitivity values 0.0106 and 0.0113 μgcm^{-2}. The composition between molybdate and phosphate were determined by Jobs method of continuous variation along with mole ratio method and was found to be in the ratio1:2. The system 1 and 2 have been effectively applied for the nitrite determination in the samples of soil, water and root nodules of diancha, cowpea and greengram leguminous plants. The results of nitrite obtained from both systems were good and also found to be analogous with those of the nitrite results determined separately following the official method.

Key words: Phosphomolybate, vanadomolybdate, thiourea, soil, leguminous plant

1. Introduction

Inorganic nitrogen species are ubiquitous within the environment, in food manufacturing, other industrial processes and biological fluids. Determination and speciation of nitrite and nitrate in drinking waters and foodstuffs have gained increasing attention in recent years because of their potential harmful impact on human health[1]. Nitrite and nitrate are commonly monitored for purposes of environmental protection, in water, agriculture and food control[2]. Nitrite ion formation is an important step in the nitrogen cycle. It is formed during the biodegradation of domestic or industrial nitrogenous wastes as well as some fertilizers. Air-borne nitrogen oxides are converted into nitrite ion, which is also a component of acid rains[3]. The monitoring of nitrite in environmental samples is being practiced by most health authorities worldwide, with legislation being levied on its permitted levels in drinking water, at present the maximum contamination level in drinking water is 1μgml^{-1}[4].

Excessive concentration of nitrite in drinking water could be hazardous to health, especially for infants and pregnant women. Nitrite oxidizes iron in hemoglobin of the red blood cells to form methemoglobin, which loses its oxygen carrying ability. This creates the condition known as methemoglobinemia[5]. The nitrite ion is an important intermediate in the biological nitrogen cycle and is present in soils and surface water [6]. The important role of nitrite represents its toxicity, which is primarily due to the fact that it can produce nitrosamines compounds which are known to be carcinogens[7], teratogenic and mutagenic[8] *via* its reaction with secondary or tertiary amines present inhuman being. Nitrite is considered as an important widespread contaminant of aqueous environment and serves as a significant indicator of natural water quality[9] where it is found that, high concentration of nitrite in water generally indicates the poor quality of water[10]. Nowadays, analytical methodologies are well established for environmental monitoring. However, a paradoxical situation has emerged as majority of the analytical methods employed to investigate environmental problems generate chemical wastes, which contribute to environmental pollution[11,12]. As a consequence the determination of nitrite is of vital importance and methodologies which is less harmful to human population and the environment has to be developed, hence the proposed method[13].Among several methods spectrophotometric methods is the majority and extensivelyused for nitrite determination due to the excellent limits of detection obtained and the simplicity of the protocol. Paradoxically, this analytical methodology uses reagents or generates chemical wastes, which are more toxicthan the species being monitored[14]. Various methodsarereported for quantitative determination of nitrite including chromatograpy[15,16], potentiometry[16,17] flow injection analysis[18,19] spectrofluorometry[20,21], capillary electrophoresis[22,23]. These methods[14-22] suffer from complicated and expensive instrumentation, use of expensive chemicals and need skilled person to handle[12]. Hence, the aim of the present research was to develop simple spectrophotometric methods for the determination of nitrite

which is based on the reduction of phosphomolybdic acid to phosphomolybdenum blue complex by thiourea[24] . The reduced phosphomolybdenum blue complex formed is oxidized by addingnitrite which causes a reduction in the intensity of the blue color. The absolute decrease in the absorbance of the blue color is found to be directly proportional to the amount of nitrite added. The absorbance of the phosphomolybdenum blue complex is scrutinized spectrophotometrically at 790 nm, system 1. The other proposed method is based on the decolorization of the vanadomolybdate complex, by nitrite in acidic medium and the decrease in color intensity, is supervised spectrophotometrically at 410 nm, system 2.The developed methods are sensitive, require no control of pH or temperature and do not suffer from most of the potential interferences. The proposed methods(system1/2) were successfully applied individually for the determination of nitrite in the samples of soil, water and root nodules of diancha, cowpea, and greengramleguminous plants.

2. Experimental

2.1. Apparatus

Systronics Visiscan spectrophotometer model SL 167 with 1 cm matched quartz cells, Acculab digital balance readable 0.0001g was used.

2.2.Reagents and Solutions

Chemicals employed in each of the experiments conducted were of analytical grade reagents. Also, distilled water was used for each experiment

2.2.1. Standard nitrite solution 30 µg ml^{-1}

Weighed amount, 0.1 g of sodium nitrite was poured into a 100 ml volumetric flask. The solution thus obtained after dissolving was then diluted using water up until the mark. The working solutions as and when required were prepared by appropriate dilution to get 30µgml^{-1}.

2.2.2. Ammonium heptamolybdate solution5.0 x 10^{-3}M

Weighed amount, 1.544 g of ammonium molybdate was dissolved in about 150 ml warm water. A slightly milky solution was obtained. Further, the temperature of the solution was brought down tomatch the room temperature. It was transferred into a 250 ml volumetric flask and diluted to the mark with water.

2.2.3. Ammonium metavanadate solution 2.5 x 10^{-3}M

Weighed amount,0.731gm of ammonium metavanadate was dissolved in 250ml volumetric flask and diluted to the mark with distilled water. 2.5 x 10^{-3} M of ammonium metavanadate solution was readied by further dilution from the available stock solution

2.2.4. Sodium dihydrogen phosphate solution of 0.065 M

Weighed amount, 2.535 g of sodium dihydrogenphosphate was transferred into a clean 250 ml volumetric flask, it was dissolved and the solution was diluted to the mark with water.

2.2.5. Thiourea 2 %

Weighed amount, 2 g of thiourea was transferred into a clean 100 ml beaker. It was dissolved in about 100 ml volumetric flask and diluted to the mark with water.

2.2.6. Sulfuric acid/ Nitric acid

Sulfuric acid (0.25 N) was prepared by diluting the concentrated sulfuric acid (36 N) and nitric acid (16 N) with water.

2.2.7. Recommended procedure (system 1/system 2)

Twoseries comprising of 10 ml volumetric flaskswere arranged, to one series known aliquots of solutions, 0.1, 0.2, 0.5,1.0, 1.5, and 2.0 ml 30 µg ml^{-1} nitrite (0.3-6.0 µg ml^{-1}), 2.5 ml 5.0 x 10^{-3} M ammonium molybdate, 1.5 ml 0.065 M sodium dihydrogen phosphate, 2 ml 2 % Thiourea and 1 ml 0.25 N sulfuric acid(system 1) was added. To the other series of 10 ml volumetric flasks (0.3-6.0 µg ml^{-1}) 30 µg ml^{-1} nitrite, 2.5 ml 5.0 x 10^{-3}ammonium molybdatefollowed with1 ml 2.5 x 10^{-3} M ammonium metavanadate, 0.5 ml of 0.1 N nitric acid(system 2)were transferred to each flask. After 10 minutes

further dilution was done to the mark with water.Absorbance of each of the solution in addition to the blank (the same test solution containing no sodium nitrite) was taken after 30 min at 790 nm(system 1) /10 minutes at 410 nm (system 2)against water. The calibration graph is obtained by plotting absorbance values of the solutions against their nitrite concentration.

2.3. Applications

2.3.1. Determination of nitrite in soil from agriculture fields

The soil samples from the agriculture field were collected using standard procedures[24]. Soil samples were dried at100^{o}C in an electronic oven for 24 h, cooled to room temperatureand ground to fine dust with the mortar. 10 g of each soil sample was mixed with 25 ml of distilled waterand shaken vigorously for 20 min and the resulting solution was filtered through a Whatman No. 41 filter paper[13]. The soil was washed with distilledwater until about 50 ml of the filtrate was collected. The filtratewas made up to the mark with distilled water and known aliquots were analyzedby the proposed methods for nitrite following the recommended procedure following standard addition method.

2.3.2. Determination of nitrite in roots of leguminous plants[13]

Weighed amount, 25 g of roots of each leguminous plants of diancha (Sesbaniabispinosa), cowpea (Vignaunguiculata) and greengram (Vigna radiate) containing bacterial nodules was crushed and ground in a mortar by adding 10 ml distilled water and transferred that into a 250 ml conical flask. 20 ml of water was added into that container and were stirred, and filtered through a Whatman No. 1 filter paper. The filtrate was transferred into a 50ml volumetric flask and made up to the mark with water. Known aliquots were analyzed proposed methods as given in the recommended procedurefollowing standard addition.

2.3.3. Determination of nitrite in water samples

Water samples were collected from different lakes around Mysore city and analyzed within 24 h without adding any additive. The water samples were filtered through a Whatman No. 1 filter paper before analysis, and then an aliquot of the filtrate was taken for the analysis by the proposed methods through the recommended procedures following standard addition.

3. Result and discussion

The new methods for spectrophotometric determination of nitrite are based on the reduction of phosphomolybdic acid to phosphomolybdenum blue complex by thiourea. The reduced phosphomolybdenum blue complex formed is oxidized by the addition of nitrite which causes a reduction in intensity of the blue color (system 1). The other method is based on the decolorizing effect of nitrite on the vanadomolybdate system in nitric acid medium (system 2). Under the specified experimental conditions and fixed concentration of phosphate, molybdate, thiourea,vanadate, the addition of nitrite is inversely proportional to the color intensity supervised at 790/410 nm (system1/system 2). The reaction circumstances as well as the various experimental factors affecting the progress and stability of the colored complexes were cautiously investigated and optimized for the quantitative determination of nitrite in soil from agricultural field, lake watersamples, roots nodules of diancha (Sesbaniabispinosa), cowpea (Vignaunguiculata) and greengram(Vignaradiate)leguminous plants. The experimental parameters such as various concentrations of Sodium dihydrogen phosphate, ammonium molybdate, thioureaand types of acids were optimized and the tolerance quantity of other ions and stability of the complex with time have been reported. The Stoichiometry between molybdate and phosphate (system 1) were determined by Jobs method of continuous variation[25]and by mole ratio method which accounts for 1:2 between molybdate and phosphate. Both the methods are independently producing satisfactory and complimenting results for nitrite present in samples of soil, water and roots of leguminous plants and are also comparable with the results determined from the official method[13].The nitrite results so obtained by different methods(system1/2) are given in Table 1. The developed methods are very simple, sensitive and selective method for nitritedetermination, requires no control of temperature nor pH[26], requires no extraction[2627], does not require sophisticated instruments[2021]and does not suffer from most of the potential interferences[28]. Therefore, the developed phosphomolybate and the vanadomolybdate methods designed for the nitrite determination offers a simple, selective, economic, rapid and alternative to existing methods, Table 2.

Table 1 Determination of nitrite in a variety of samples

Sample	Nitrite added (µg ml^{-1})	Proposed methods				Official method [13]	
		System 1		System 2			
		Nitrite found (µg ml^{-1})	% Recovery	Nitrite found (µg ml^{-1})	% Recovery	Nitrite found (µg ml^{-1})	% Recovery
Soil[1]	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	3.0 ± 0.02	100	2.9 ± 0.02	96.66	2.9 ± 0.02	96.66
Soil[2]	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	3.0 ± 0.03	100	3.0 ± 0.03	100	3.0 ± 0.03	100
Soil[3]	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	3.0 ± 0.01	100	2.8 ± 0.01	93.33	2.9 ± 0.04	96.66
Soil[4]	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	2.9 ± 0.01	96.66	2.9 ± 0.01	96.66	2.8 ± 0.04	93.33
Soil[5]	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	3.0 ± 0.01	100	3.0 ± 0.01	100	3.1 ± 0.04	103.33
Soil[6]	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	3.3 ± 0.03	110	3.33 ± 0.02	111	3.32 ± 0.03	110.66
Soil[7]	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	3.6 ± 0.04	120	3.8 ± 0.02	126.66	3.8 ± 0.03	126.66
Soil[8]	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	3.1 ± 0.02	103.33	2.9 ± 0.01	96.66	3.1 ± 0.02	103.33
Soil[9]	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	3.0 ± 0.02	100	2.9 ± 0.02	96.66	3.0 ± 0.02	100
Root nodules of diancha(Sesba niabispinosa)							
	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	4.0 ± 0.01	133.33	3.8 ± 0.01	126.66	3.8 ± 0.01	126.66
cowpea(*Vignaunguiculata)*	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	3.2 ± 0.01	106.66	3.3 ± 0.03	110	3.3 ± 0.01	110
Greengram	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	3.8 ± 0.01	126.66	3.7 ± 0.02	123.33	3.8 ± 0.01	126.66
Karanjilake water[a]	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	3.0 ± 0.02	100	2.8 ± 0.02	93.33	2.8 ± 0.02	93.33
Kokkarehalli lake water	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	2.9 ± 0.03	96.66	2.9 ± 0.02	96.66	2.9 ± 0.02	96.66
Devi lake water	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	3.0 ± 0.02	100	2.8 ± 0.02	93.33	3.0 ± 0.02	100
Lingabudhi lake water	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	3.0 ± 0.02	100	3.0 ± 0.02	100	3.0 ± 0.03	100
Tap water 1	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	3.0 ± 0.02	100	3.0 ± 0.02	100	3.0 ± 0.01	100
Tap water 2	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	3.0 ± 0.03	100	3.0 ± 0.02	100	3.0 ± 0.01	100
Tap water 3	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3.0	3.0 ± 0.02	100	2.8 ± 0.02	93.33	2.8 ± 0.01	93.33

1-Pegion pea, 2-Cotton, 3-Sorghum, 4-turmeric, 5-black gram, 6-Cow pea, 7-Green gram, 8-Paddy, 9- Coffee,

4. Optimiztion

Table 2: Assessment of various spectrophotometric methods for nitrite with the proposed methods

Reagent	λmax nm	Beers law range (µg/mL)	Molar absorptivity (L mol−1 cm−1)	Remarks
p-nitroaniline +sulfanilamide + ethyl acetoacetate	356	0.2–3.0	1.22×10^4	Fe3+interference, narrow determination range[29]
p-Nitroaniline+2-methyl-8-quinolinol	585	0.002– 0.40	4.72×10^4	Most cations and anions interfered[30]
4-(1-Metyl-1-mesitylcyclo butan-3-yl)-2-aminothiazole + N,N-dimethyl aniline	482	0.05–2.00	2.03×10^4	Time consuming and requires extraction[31]
Sulphanilic acid + 1-naphthol	418	0.02–0.87	1.70×10^4	Time consuming[32]
p-Nitroaniline + 8-quinolinol	550	0.01–0.06	5.85×10^4	pH-dependent and extractive[26]
Peroxovanadate complex	470	6.67–66.7	0.276×10^3	Very less sensitive[13]
P-Aminophenylmercaptoacetic acid + *N*-(1-naphthyl)ethylenediaminedihydrochloride.	565	0.02–0.80	4.65×10^4	Time consuming[28]
Acetyl acetone + *p*-nitroaniline	490	0.05–1.40	3.20×10^4	Interferences of many ions[33]
p-Rosanilinium chloride + N-(1-naphthyl)ethylinediamine hydrochloride.	560	0.04–00.4	8.33×10^3	time consuming, more interference by metal ions [34]
Phosphomolybdenum blue	814	0.2–03.6	1.1×10^4	time consuming[35]
Sulfanilic acid + methylanthranilate	493	0.2-08.0	$1.03\text{x}10^4$	time consuming [36]
p-nitroaniline and N-(1-naphthyl) ethylenediammedihydrochloride	545	00–3.0	5.7×10^4	Requires extraction[27]
fluorescein amine isomer	495	00-0.4	6.67×10^4	Requires extraction [37]
Proposed methods				Simple, non extractive, no temperature control, independent of pH, common ions do not interfere.
System1	790	0.3-6.0	$4.341\text{ x }10^3$	
System 2	410	0.3-6.0	$4.067\text{ x }10^3$	

4.1. Result of various volumes of 0.065 M Sodium dihydrogen phosphate solution.

The effect of concentration of sodium dihydrogen phosphate on absorbance was been examined to achieve high absorbance by takingvarious volumes (0.5-2.5 ml)0.065 M Sodium dihydrogen phosphate, 1 ml 30 µg ml^{-1} nitrite,2.5 ml 5.0 x 10^{-3} M ammonium molybdate, 2 ml thiourea 2 % and 1 ml 0.25 N sulfuric acid into a series of labeled 10 ml volumetric flasks. Then the solutions in each of these flaskswere diluted to the mark with water. Absorbance of solutions was measured at 790 nm. The results tabulated in Fig.1indicate that the solution containing 1 ml of0.065 M Sodium dihydrogen phosphate appeared to be more sensitive with high absorbance value. As a result, 1 ml 0.065 M Sodium dihydrogen phosphate was selected to as the optimized volume throughout the work.

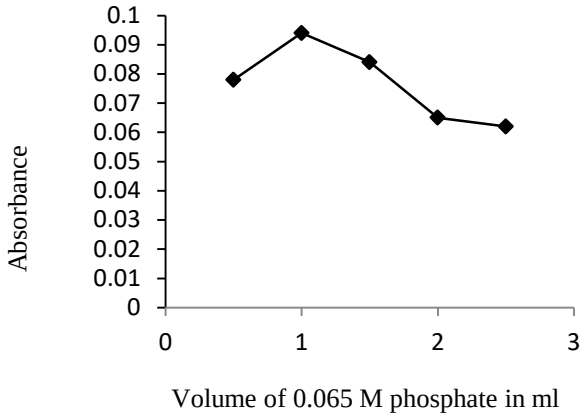

Fig. 1. Effect of different volumes (0.5-2.5 ml) 0.065 M Sodium dihydrogen phosphate + 1 ml 30 µg ml^{-1} nitrite + 2.5 ml 5.0 x 10^{-3} M ammonium molybdate + 2 ml 2 % thiourea + 1 ml 0.25 N sulfuric acid solutions and made to 10 ml with water.

4.2. Result of various volumes of 5.0 x 10^{-3} M*ammonium molybdate solution.*

The result for various volumes of ammonium molybdate solution on absorbance was investigated to achieve high absorbance via taking 1 ml 30 µg ml^{-1}nitrite,1 ml 0.065 M Sodium dihydrogen phosphate, various volumes (1.0-3.5 ml) 5.0 x 10^{-3} M ammonium molybdate solution, 2 ml thiourea 2 % and 1 ml 0.25 N sulfuric acid in a series of labeled 10 ml volumetric flasks. Then the solution in each flask was diluted to the mark with water. Absorbance of the solutions was measured at 790 nm. The results obtained are presented in Fig. 2. The results clearly indicate that the solution containing 2.5 ml 5.0 x 10^{-3} M ammonium molybdate has highest absorbance readings andtherefore has been chosen as an optimal volume for the construction of the calibration graph for the determination of nitrite throughout the work.

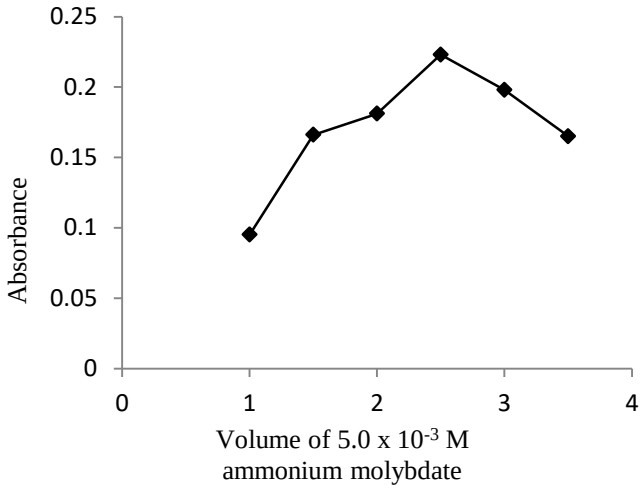

Fig. 2. Effect of different volumes (1.0-3.5 ml) 5.0 x 10^{-3} M ammonium molybdate + 1 ml 30 µg ml^{-1} nitrite + 1ml 0.065 M sodium dihydrogen phosphate + 2 ml 2 % thiourea + 1 ml 0.25 N sulfuric acid solutions and made up to 10 ml with water.

4.3. Effect of different volumes of thiourea

The influence of the concentration of the reducing agent, thiourea on the phosphomolybdate complex was examinedsimilarly as has been done above but with varying volumes of (0.5-3.0 ml) of 2% thiourea solution per 10 ml of total volume. Absorbance values of the solutions were found to be increasing with an increasing volume of 2% thiourea solution up to 2 ml. It was observed that absorbance values remained approximately the same for later readingFig. 3.Hence 2 ml 2% thiourea was selected as an optimized volume for the construction of the calibration graph to determine nitrite.

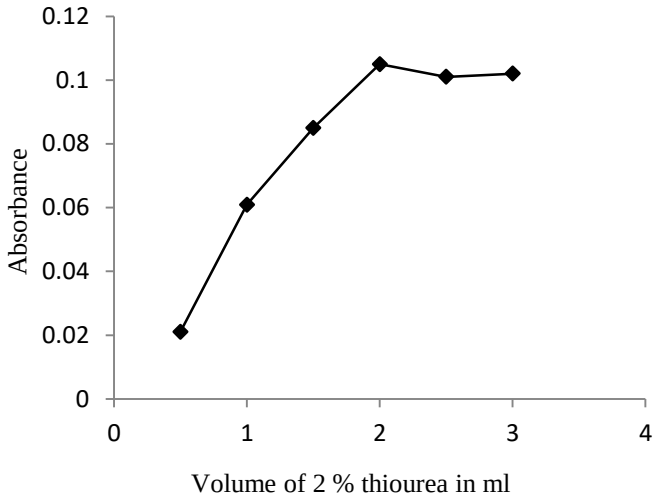

Fig. 3. Effect of different volumes of (0.5-3.0 ml) 2% thiourea + 1 ml 30 µg ml^{-1} nitrite 2.5 ml 5.0 x 10^{-3} M ammonium molybdate solution + 1ml 0.065 M sodium dihydrogen phosphate + 1 ml 0.25 N sulfuric acid solutions and made up to 10 ml with water.

4.4. Effect of different volumes 0.25 N Sulfuric acid.

This experiment was also undertaken similar to the previous one but with different volumes of (0.25-2.0 ml) 0.25 N sulfuric acid. The absorbance values as shown in Fig. 4 indicates that the solution containing 1.0 ml 0.25 N sulfuric acid solution appeared to be more sensitive and hence was selected as an optimized volume for the construction of the calibration graph to determine nitrite.

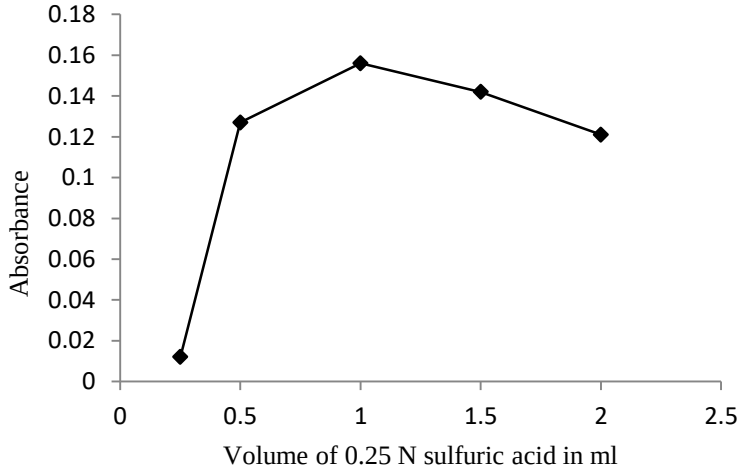

Fig. 4. Effect of different volumes (0.25-2.0 ml) 0.25 N sulfuric acid + 1 ml 30 µg ml^{-1} nitrite + 2.5 ml 5.0 x 10^{-3} M ammonium molybdate solution + 1ml 0.065 M sodium dihydrogen phosphate solutions and made up to 10 ml with water.

4.5. Effect of various acids on system 2

The effect of types of acids on the reaction system 2 was studied to get the best sensitivity and linearity. The various acids namely, Nitric acid, sulfuric acid, hydrochloric acid and acetic acid were investigated by using 0.5 ml 0.1 N at a time following the recommended procedure, system 2. The results thus found are plotted in Fig. 5 which indicates, in case of nitric acid and acetic acid, the reaction show highestand almost the same absorbance with good linearity. Although hydrochloric acid and sulfuric acidshow good linearity, it is observed that the absorbance values are lesser when compared to the other two acids (nitric acid and acetic acid). Hence 0.5 ml 0.1N nitric acid is chosen as the most favorable optimal acidic medium for the determination of nitrite, system 2.

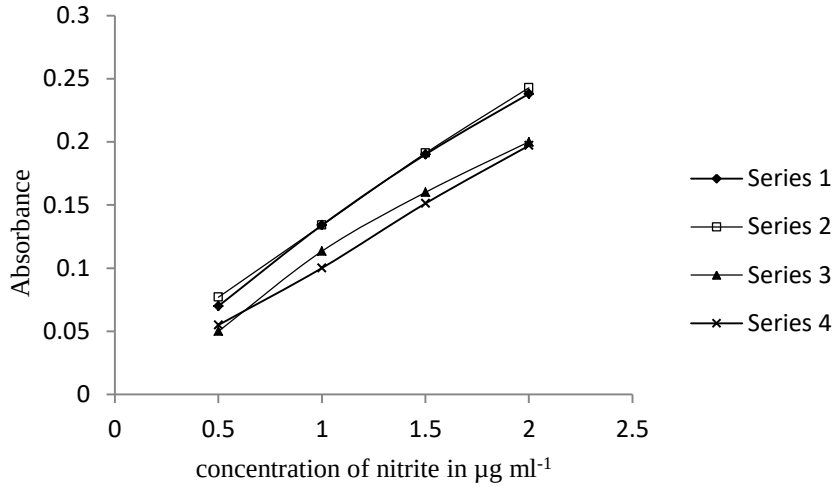

Fig. 5. Effect of different types of acids for the determination of nitrite of system 2 with 0.1-1.0 ml 30 µg ml^{-1}nitrite + 2.5 ml 5.0 x 10^{-3} M ammonium molybdate + 1 ml of 2.5 x 10^{-3} M ammonium vanadate + 0.5 ml 0.1 N HNO_3 series 1/ 0.5 ml 0.1 N CH_3COOH series 2/ 0.5 ml 0.1 N HCl series 3/ 0.5 ml 0.1 N H_2SO_4 series 4.

4.6. Calibration graph

Over the optimized experimental condition, the calibration graph established for the nitrite determination in its concentration range 0.3–6.0 µgml $^{-1}$ of system 1/ system 2 isrevealed in Fig. 6. The analytical parameters are represented in Table 3.

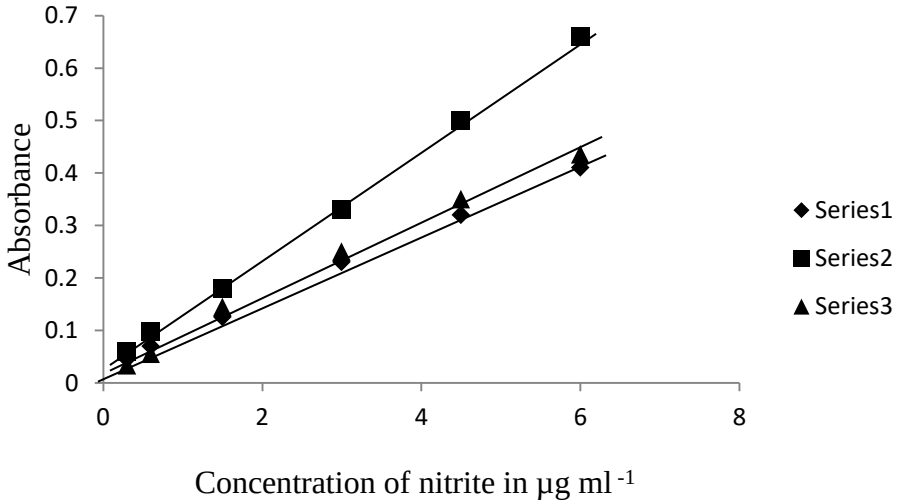

Fig.6.series 1, 0.3-6.0 µg ml $^{-1}$ nitrite + 2.5 ml 5.0 x 10^{-3} M ammonium molybdate + 1.5 ml 0.065 M sodium dihydrogen phosphate + 2 ml 2 % Thiourea + 1 ml 0.25 N sulfuric acid , system 1 .
Series 2, System 1 after 60 minutes,
Series 3, 0.3-6.0 µg ml $^{-1}$ nitrite + 2.5 ml 5.0 x 10^{-3} M ammonium molybdate + 1 ml 2.5 x 10^{-3} M ammonium vanadate + 0.5 ml 0.1 N nitric acid were added and made up to mark with water, system 2.

Parameters	System 1	System 2
λ max (nm)	790	410
Linear range (µg ml $^{-1}$)	0.3-6.0	0.3-6.0
Molar absorptivity l mol^{-1} cm^{-1}	4.341 x 10^3	4.067 x 10^3
Sandell sensitivity (µg cm^{-2})	0.0106	0.0113
Regression equation		
Intercept	0.0303	0.0235
Slope	0.0640	0.0705
Correlation coefficient	0.9995	0.9959

Table 3 Optical parameters for the determination of nitrite system1/2

4.7. Color stability

The color stability of the reaction systems was studied cautiously owing to the importance of the parameter and its effect on reproducibility of the method. It was investigated by performing the experiment using the suggested volumes and concentrations of all the reagents from the recommended procedure of system 1 and 2 except1.5µg ml^{-1}nitriteper 10 ml of the reaction mixture. The results in Fig7, illustrate that, system 2 is very much stableup to 2 hours and found to be stable even after 24 hours. While the system 1 emergeless stability, as the color of the reaction mixture keep increasing from 0 min to 120 min[3524]. However, the absorbance readings were taken after 30 min after the preparation of the solutions for the system 1 throughout the completion of the work[24].

FIGURE 7

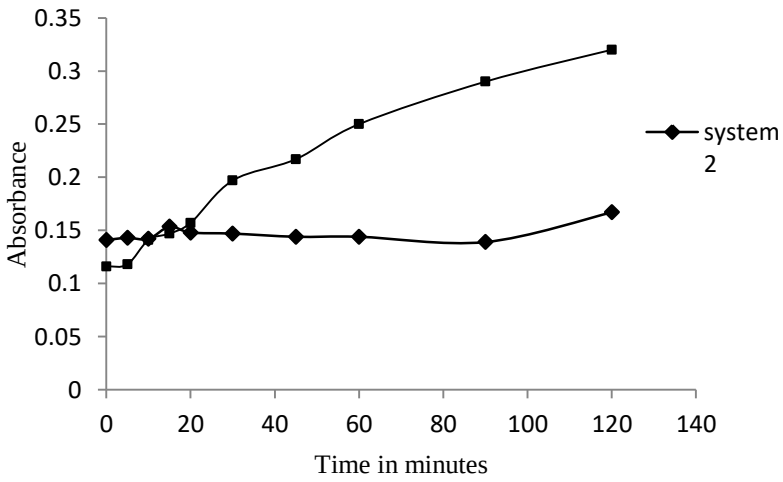

Fig. 7. Result of time on the color stability of system 1 and system 2

4.8. Stoichiometry of the system 1

4.8.1. Job's method of continuous variation[38,39]

The experiment was performed by using a sequence of solutions of equimolar concentration of 0.001Mmolybdateand 0.001 M phosphate. The solutions were prepared by mixing complementary proportions to a final volume of 10 ml. various volumes (0-10 ml) of 0.001 M phosphate, various volumes 0.001 M ammonium molybdate (10-0 ml), 1 ml 0.25 N sulfuric acid, 2 ml 2% thiourea were added into a sequence of labeled 25 ml volumetric flasks and allowed to stay undisturbed for 20 minutes. Then the solution in each flask was made up with water to the mark. The results are revealed in Fig. 8 and accounts for 1:2 stoichiometry between molybdate and phosphate at790 nm

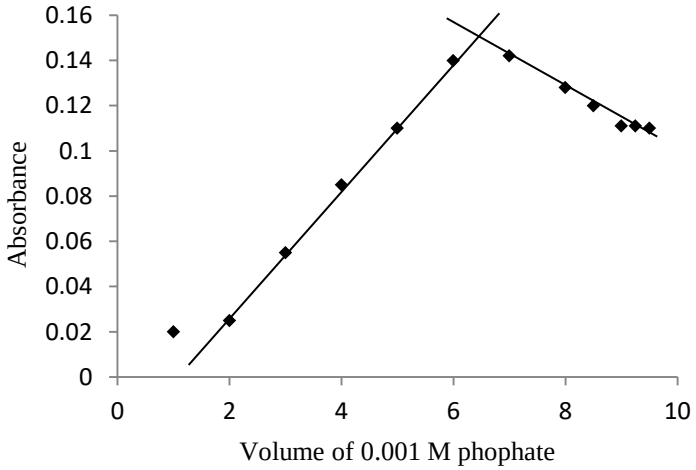

Fig. 8. Job's method of continuous variation for the study of stoichiometry between molybdate and phosphate involving the solutions amounted to 10 ml and diluted to 25 ml with water.

4.8.2. Mole ratio method

This method was carried out using same concentrations of phosphate and molybdate used for Job's method but keeping the 0.001 M solution 4 mlmolybdate a constant volume with varying volumes (2-10 ml) 0.001 M phosphate, 1 ml 0.25 N sulfuric acid, 2 ml 2% thiourea, each solution in 25 volumetric flask that were made up to the mark with water. The absorbance was monitored at 790 nm. The results obtained were used in plotting the graph, shown in Fig. 9, accounts for 1:2 stoichiometry between molybdate and phosphate.

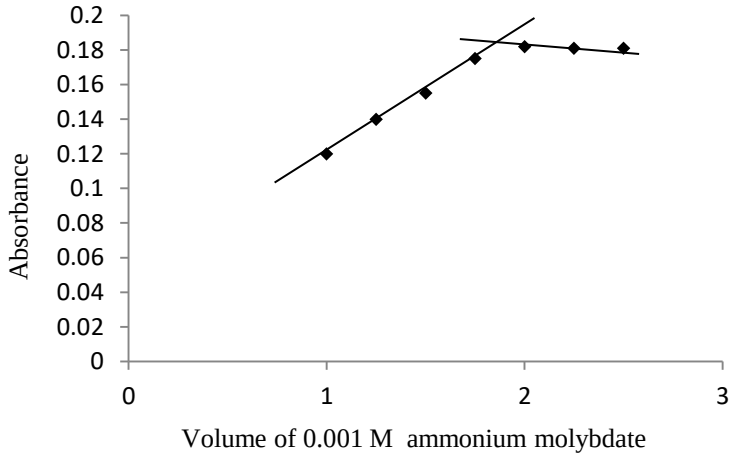

Fig. 9. Mole ratio method for the study of stoichiometry between phosphate and molybdate involving the solutions amounted to 10 ml and diluted to 25 ml with water.

4.9. Study of interference ions

For understanding the reaction selectivity, interferences of common ions which often accompany nitrite were investigated in the determination of 1.5µg ml^{-1}under the optimized experimental condition given in the recommended procedure system 1 and 2. The results obtained are summarized in Table 4.

Table 4 Interference study of other ions.

Interfering ions	Concentration of interfering ions in µg ml^{-1}	
	System 1	System 2
Cu^{2+}	038.00	126.00
Fe^{2+}	003.20	016.00
K^{+}	261.50	261.00
Cl^{-}	237.50	237.00
Cr^{3+}	013.40	065.00
V^{5+}	004.40	-
NO^{3-}	218.70	218.00
Na^{+}	196.00	235.00
Zn^{2+}	067.80	113.00
Fe^{3+}	023.20	011.60
Pb^{2+}	093.00	186.00
Co^{2+}	037.00	061.50
Cd^{2+}	152.00	152.00
PO_4^{3-}	-	003.04
F^{-}	003.00	003.00

5. Conclusion

The new methods (system 1/2) constructed for the nitrite determination in soil, ground water and root nodules of diancha (Sesbaniabispinosa), cowpea (Vignaunguiculata) and greengram (Vigna radiate) plants are straightforward,fairly rapid, sensitive and economic as it employs cheaper chemicals and instruments. These rewards of the methods is superior compared to some of the reported methods for its determination Table 2, which engross solvent extraction, critical control of pH and temperature, employs sophisticated instruments, utilize toxic reagents. In addition, the developed new methods are working satisfactorily for the determination of nitrite in a variety of samples investigated hereTable 1. The nitrite results thus obtained from the new methods also correspond and are consistent with those of the nitrite results determined discretely from official method[13].The absorbance of the phosphomolybdenum blue method (system 1) requires firm management of time while the vanadomolybdate method (system 2) is exceptionally stable and found to be stable even after 24 hours. However, the nitrite results obtained for the samples are reproducible when the determination of nitrite was carried out through the recommended procedures.As a result, the developed Phosphomolybate and vanadomolybdate methods for the determination of nitrite could be used either as an independent method or as an alternate method to the official method[13] with very less sensitivity.

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