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Spectrophotometric Determination of Zinc Nitrate and Nitrite

[1]Jadhav Rajesh Balkrishna, Dr. Utpal Saha and Dr. Pushpendra Sharma

[1]*Research Scholar,Department of Chemistry, Sri Satya Sai University of Technology & Medical Sciences, Sehore,M.P.*

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ABSTRACT

This work included the creation and testing of spectrophotometric methods from diverse sample matrices such as water, soil samples for simultaneous determination of zinc nitrate and nitrite. Several tests were conducted as follows in order to set optimal conditions for the spectrophotometric willpower of zinc nitrite. The potential method has been applied for zinc nitrite and nitrate determination in instances of blow liquid and insincere water. Through hastening the chloride with silver sulphate [1, 2], the chloride became removed from the meddling. Using the suggested methods and orientation, sufficient aliquots were obtained and analysed for zinc nitrite and nitrate. The reagents provide a simple and sensitive method for the determination of nitrite and nitrate by spectrophotometry for monitoring water pollution. The method developed does not involve strict reaction conditions and offers the advantages of color stability.

1. Introduction

$Zn (NO_3)_2$ is a mineral component contains with a chemical name Zinc nitrate. It is correspondingly called as Zinc dinitrate or Celloxan or Zinc Nitrate Hexahydrate. It is extensively uses as a reagent to production in pharmaceuticals, dyes and numerous further chemicals. A Spectrophotometry [3, 4] is involved in portioning the amount of light one object receives. The apparatus works by transitory a ray of bright finished an example and gage the intensity of a sensor attaining energy. Easy relation between the color of a substance and its microelectronic structure is the basis of spectrophotometric methods. A fragment or an ion shows preoccupation in the discernible or ultra-violet district when the radioactivity reasons an electronic change in particles covering one or additional chromophoric collections. The color of a particle may be strengthened by substituent called auxochromic group, which move the absorption most to lengthier wavelength (bathochromic shift). The hue decisive factors in numerous particles are the outline of conjugated dual promises by incomes of electron giver or electron acceptor groups. The quantitative application of the absorption process is grounded on the detail that the amount of photons engrossed is straight relative to the amount or attentiveness of particles, ions or fragments.

Recently evolved fluorometric HPLC process includes precolumn nitrite derivatization with 2, 3-diaminonapthalene and enzymatic nitrite conversion to nitrate [5]. The study focused on selecting and optimizing reversed-phase HPLC conditions, demonstrating method performance (sensitivity, accuracy, and accuracy), and investigating common inorga interferences.

2. Experimentation

Material and Methods used

- **Equipment**

Table 1.1: Instrumentation used in RP-HPLC Method Development

Sr. No.	Name of Instrument	Make	Model
1	HPLC System	Shimadzu, Japan	Software–LC Solution Pump-LC 2010 CHT Detector- UV
2	Analytical Balance	Shimadzu, Japan	AUY 120
3	Digital pH Meter	Electronic Corporation Ltd., India	pH 5651
4	Ultrasonicator	Servewell Instrument, India	RC System MU-1700
5	Degasser	Tarsons, India	Rockyvac-300
6	Milli-Q water purification system	Bedford, MA, USA	Millipore

- **Reagents and Materials**

A. Standards

1. Zinc nitrate (Merck, India)
2 Sodium chloride, sodium sulfate, potassium phosphate (monobasic), and sodium carbonate (Fisher Scientific, India)
3. Milli-Q water with 18.2 MΩ-cm resistances (Millipore water system)

B. Real Samples

1. Water samples- Tap; Ground well Surface water from local wells and river

2. Soil sample- From local farm

C. Chemicals and Reagents

1. Methanol (HPLC grade), Merck (India)
2. Potassium dihydrogen Phosphate AR Grade, Qualigens (India)
3. Orthophosphoric acid AR Grade, Qualigens (India)

3. Results and Discussion

3.1 Selection of Common Solvent (Diluent)

HPLC grade water was classified as a joint solvent for stock solution preparation and evolving spectral manifestations of nitrate and nitrite, additional dilutions from stock solutions were completed in the aqueous HPLC grade methanol mobile stage.

3.2 Determination of λ_{max} of Nitrate and Nitrite

Preparation of Standard Stock solution: Weighed exactly 0.01500 gm of sodium nitrite and 0.016 gm of potassium nitrate working normal in a 10.0 ml volumetric flask and dissolved in appropriate mobile phase by sonication, then mobile phase volume up. Dilute 1.0 ml of this solution with mobile level, i.e. methanol to 10.0 ml. The aliquot component of regular nitrate stock solutions, as well as nitrite, was sufficiently diluted with aqueous methanol to obtain concentration of 10 ppm of each analyte. The solutions in the 200-400 nm range is skimmed. The absorbance spectrum of nitrate and nitrite are exposed respectively. It is observed as the wavelengths of maximum absorption, λ_{max}, for nitrate as well nitrite were 230 and 233 nm, respectively. From the overlain spectra, the wavelength selected for simultaneous approximation of nitrate as well nitrite was 222 nm as an isoabsorptive point for nitrate and nitrite ions.

3.3 Chromatographic Conditions Optimization

The mobile phases have been allowed to align with stationary phases awaiting attainment of a steady pattern. The standard solution covering nitrate and nitrite mixture was route, and separate dissimilar solvents as well as diluent mixtures were strained to become a good separation and stable peak. Every time the mobile phase was drinkable by a filter membrane of 0.45 µm. Various mobile phase compositions were tested using chosen chromatographic conditions to achieve appropriate separation, based on sample solubility and stability.

The various parameters of the measured mobile stages, flow rate and pH buffer are shown in Table 1.2, and the chromatograms obtained in Trial 1, 2 & 3.

Table 1.2: Trials for Variable Chromatographic Parameters

Chromatographic Parameters	Trial 1	Trial 2	Trial 3
Column	C13	C13	C13
Wavelength	222 nm	222 nm	222 nm
Flow rate	0.4 ml/min	0.4 ml/min	0.7 ml/min
Injection volume	20.0 µl	20.0 µl	20.0 µl
Column oven Temperature	25°C	25°C	25°C
Run Time	less than 10 minutes	less than 10 minutes	less than 10 minutes
Mobile Phase	Methanol: Water (30:70)	Methanol: Water (20:80)	Methanol: Buffer (20:80)
pH buffer	not used	not used	Potassium dihydrogen phosphate (pH=3.0)

Test 1's chromatogram indicates very low resolution, and Test 2 also indicates less resolution between nitrate and nitrite; thus certain process parameters were not sufficient. Test 3 chromatographic conditions were calculated by trial and error and held constant during the process since the correct peak form, resolution, and suitability of the device were found within limits.

3.4 Mobile phase, pH and Flow Rate Optimization

On successive HPLC chromatograms, the various aqueous methanol concentrations (10, 20, 25 and 30 percent, v/v) and specific pH levels (2.0, 2.5, 3.0 and 3.5) of mobile phase responses at multiple flow degrees (0.4, 0.7 and 1.0 ml/min). When seen in Figure 3.1, 20 per cent of methanol provides the right resolution. On 10 % methanol the resolution is achieved but the retention period (RT) is long and 25 and 30 % methanol concentrations in mobile phase gave less resolution. Figure 3.2 show that 3.7 is the optimum pH for the best resolution in RT. This pH is intermediate between the pKa values of both analytes. In the circumstance of flow rate, Figure 3.3 shows that the 0.7 ml/min is the preferred flow rate. On 0.4 ml/min flow rate the RT is too long and at 1.0 ml/min flow rate, the resolution is not achieved. So the 20 % methanol at pH 3.0 and 0.7 ml/min flow rate is considered as set of optimized chromatographic conditions.

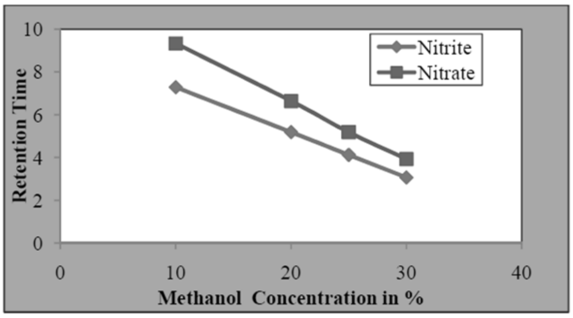

Figure 3.1: Effect of Methanol concentration variation in the mobile phase

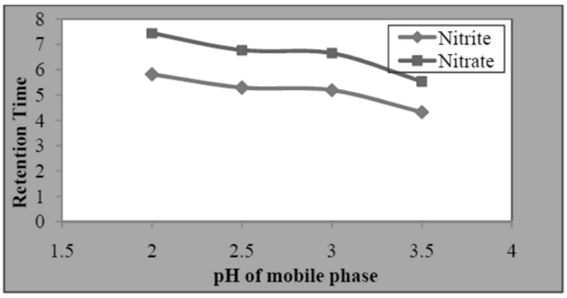

Figure 3.2: Effect of mobile-phase pH variations

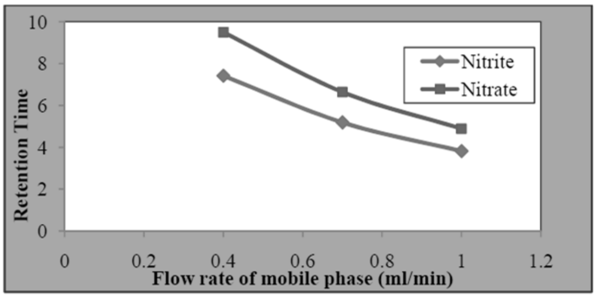

Figure 3.3: Effect of Mobile Phase Flow Rate Variation

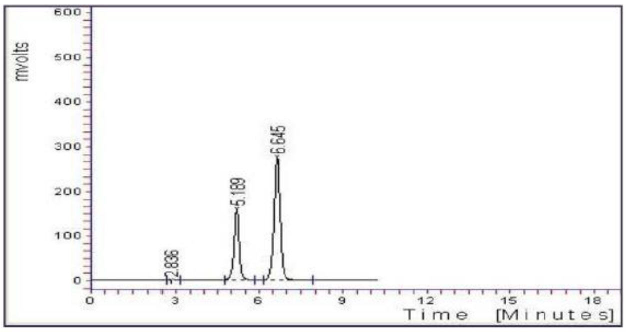

Figure 3.4: Zinc nitrite and nitrate separation in selected mobile phase, showing retention time for nitrite (1ppm) and nitrate (10ppm)

3.5 Wave Length of Maximum Spectrophotometric Detection Absorption

Zinc Nitrite Supply Solution Preparation (1000 µg / ml): An appropriately measured quantity of zinc nitrite 0.1500 g was dissolved in Milli-Q water and moved to the 100 ml volumetric container and mixed with Milli-Q water, and the final volume was filled with distilled water up to the location. The aliquot portions of nitrite stock solution were sufficiently diluted with Milli-Q water to get 0.2 µ.

3.5.1 Determination of λ_{max} of Azo Dye as Reagents using MSP-EAA

1 mL of 0.2 µg / ml Zinc nitrite solution was moved to 10 ml volumetric flask, 0.5 ml of 2 N HCl was applied to make it acidic, then diazonium ion was formed by adding 2 ml of 0.025 per cent mosapride reagent and shocked for 5 min. After this, 1 ml of 2% ethyl acetoacetate was used as a binding agent and 2 ml of 2 M NaOH solution was applied to produce a diluted to 10 ml by distilled water. The azo dye solution in the 200-800 nm range was then skimmed. From the spectrum, the average absorption wavelength chosen for nitrite determination was 429 nm. Figure 3.5 shows the absorbance spectrum of azo dye and diazonium ion using MSP.

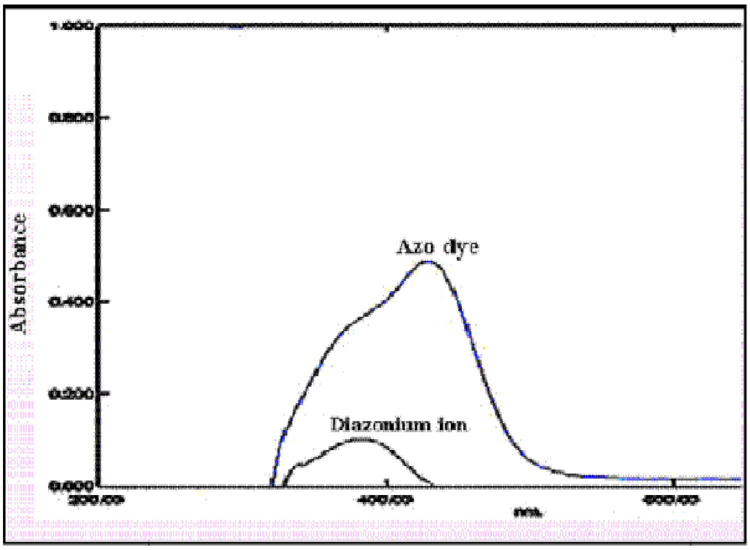

Figure 3.5: Absorbance Spectrum of Diazonium Ion and Azo Dye using MSP-EAA

3.5.2 Determination of λ_{max} of Azo Dye as Reagents using MTCP-EAA

1 ml of 0.2 µg / ml zinc nitrite solution was transferred to 10 ml volumetric flask, made acidic by inserting 1 ml of 0.5 M HCl to form a 0.1% metoclopramide reagent with diazonium ml and shocked for 5 minutes. Following this, 1 ml of 2% ethyl acetoacetate was used as a coupling agent and 2 ml of 2 was added to produce a yellow colored azo dye which is diluted to

10 ml with a dis azo dye solution was then skimmed in the variety of 200-800 nm. From the spectrum the wavelength of maximum absorption selected for willpower of nitrite was 437.5 nm. The absorbance spectrum of azo dye and diazonium ion using MTCP shown in Figure 3.6.

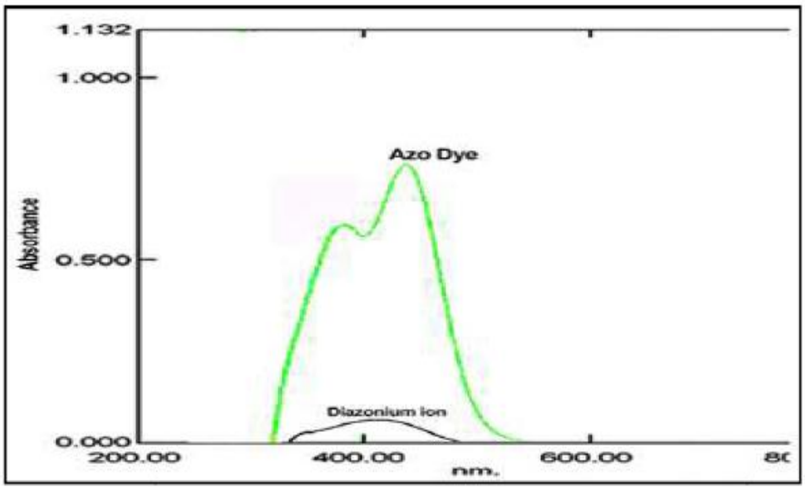

Figure 3.6: Absorbance Spectrum of Azo Dye and Diazonium Ion using MTCP-EAA

4. UV-visible spectrophotometric parameters optimization

Several tests were conducted as follows in order to set optimal conditions for the spectrophotometric willpower of zinc nitrite.

4.1 Effect of Acidity: Through the new method, hydrochloric acid provides a wide range of sourness for preserving the optimal pH. The result was to test solution of set zinc nitrite deliberation and variable HCl's concentration and efficiency. The findings revealed that 0.5 ml of 2 M HCl provided maximum absorbance for MSP-EAA reagents and that 0.5 ml of 1 M HCl provided maximum absorbance for the application of using MTCPEAA reagents. The absorbance was experiential in the attendance of 0.5-2.5 M hydrochloric acid in a total 10.0 ml of solution as shown in Figure 4.3 (for MSP-EAA) and Figure 4.4 (for MTCP-EAA). Overhead this variety a reduction in absorbance was experiential.

HCl 's best attentiveness was to be 2 M secure, and required HCl capacity was 0.5 ml for MSP-EAA reagents. HCl 's optimum attentiveness was to be 0.5 M secure and required HCl efficiency was 1 ml for MTCP-EAA reagents. This acidity was necessary for protonation in the nitrite diazotic reaction using reagents MSP-EAA and MTCP-EAA [1, 2, 7].

4.2 Effect of Substrate Concentration

- **Mosapride Substance Attentiveness:** In order to determine the amount of mosapride concentration needed for zinc nitrite diazotization and to reach optimum absorbance, tests were performed using various amounts (0.5 to 2.5 ml) of 0.025 per cent of mosapride using nitrite solutions in the 50-500 mg / L range for the diazonium salt technique.
- **Metoclopramide Reagent Concentration:** In order to set the amount of metoclopramide concentration needed for zinc nitrite diazotization and to reach optimum absorbance, tests were performed using specific amounts (0.5 to 1 ml) of 0.1 percent of metoclopramide in the 0.05 to 0.1 percent range for diazonium salty technique. The results are possible in Figure 3.6.

Mosapride with 2 ml of 250 mg / L of mosapride reagent solution was observed for optimum absorbance. For 0.55 ml of 0.1 per cent metoclopramide solution, the average absorbance was measured. The diazonium yellow colored salt is very reactive and behaves as an electrophile. Aryl diazonium cation undergoes electrophilic substitution reaction with coupling agent to create a derivative of azo [2].

4.3 Coupling Agent (EAA) Concentration Effects

Effect of EAA Concentration on Mosapride Diazonium Cation:

Mosapride diazonium cation pairs with equal concentrations and varying amounts of EAA solution to provide a base layer yellow colored azo dye. Such tests were performed using concentrations of 0.5-2.5 percent and quantities of EAA solutions of 0.5-2.5 ml, and the quantity of EAA solution required for optimum absorption was checked. The findings are listed in Figure 4.1.

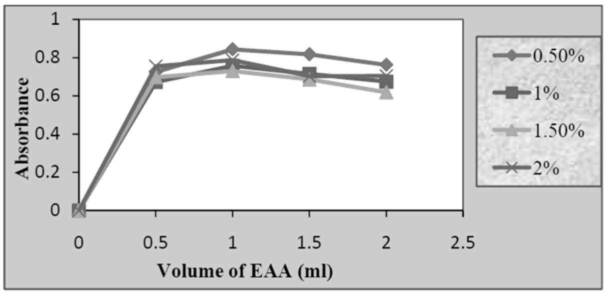

Figure 4.1: Absorbance Variation with varying Attentiveness and MSP EAA solutions volume

Effect of EAA Concentration on Metoclopramide Diazonium Cation:

This examined the quantity of EAA solution required for optimum absorbance. Experiments were performed using EAA solutions concentrations of 0.5-2.0 per cent and quantities of 0.5-2.0 ml. The findings are listed in Figure 4.2.

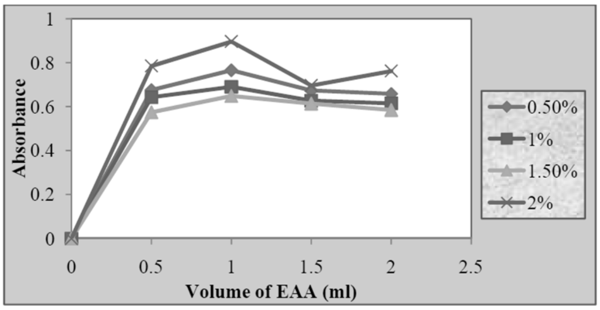

Figure 4.2: Absorbance Variation with varying Attentiveness and MTCP EAA solutions volume

1 ml of 0.5 percent EAA was found to be adequate to achieve optimal MSP absorption, and was used in all subsequent experiments. Diazonium cation in alkaline medium by mosapride pairs with EAA hydroxyl ring. The protons in EAA, alpha to carbonyl carbon are acidic, resulting in a nucleophilic replacement of the carbanion. EAA enolate acts as a bidentate ligand, thereby producing yellow azo dye. EAA enolate anion is a potent nucleophile. 1 ml of 2 per cent EAA was found to be adequate to achieve optimum MTCP absorption and was used in all subsequent experiments. Diazonium cation in alkaline medium of metoclopramid pairs with hydroxyl group EAA [1,2,7].

4.4 Effect of NaOH

MSP-EAA Dye

In the HCl average, zinc nitrite reacts with mosapride to form diazonium cation, which is coupled with ethyl acetoacetate in the base medium to form yellow colour. To shape the pigment, sodium hydroxide was used. Analysis of sodium hydroxide solutions has been observed at different concentrations and amounts. The results showed that, the use of 2 ml of 2 M sodium hydroxide was originate to give all-out absorbance with decent linearity and was charity for the willpower of nitrite in the optional process. The result of NaOH on the color development is exposed in Figure 4.9.

MTCP-EAA Dye

Nitrite responds to the treatment of diazonium cation with metoclopramide in the HCl average, which is combined with ethyl acetoacetate to create yellow color in the base medium. To shape the pigment, sodium hydroxide was used. Results of solutions for sodium hydroxide have been studied at varying concentrations and volumes. The results showed that, the use of 2 ml of 2 M sodium hydroxide was make to give all-out absorbance by dressed linearity and was cast-off for the willpower of nitrite in the recommended procedure. The effect of NaOH on the color development is shown in Figure 4.3.

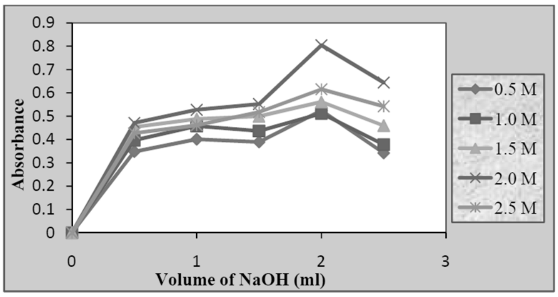

Figure 4.3: Absorbance Variation for MSP Reagent of varying concentration and NaOH volume

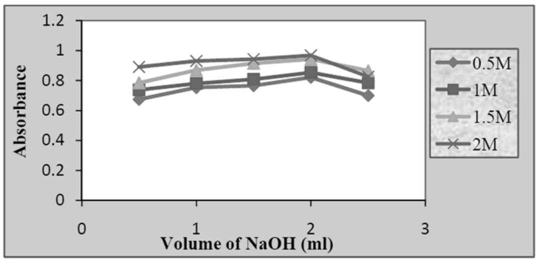

Figure 4.4: Absorbance Variation for MTCP Reagent of varying concentration and NaOH volume

It was observed from absorbance values that the color intensity and the stability maximum with 2 M NaOH. The diazonium cation couples with hydroxyl group in EAA. Isomeric structure of compound changes with change in properties of solvent. Formed azo dye shows increased absorption in NaOH because percentage of enolic form increases [2].

4.5 Effect of Reaction Time

The outcome of the diazotization reaction cycle has been studied. Variation of time from 2-10 minutes was tested. Figure 4.5 and Figure 4.6 show the results obtained.

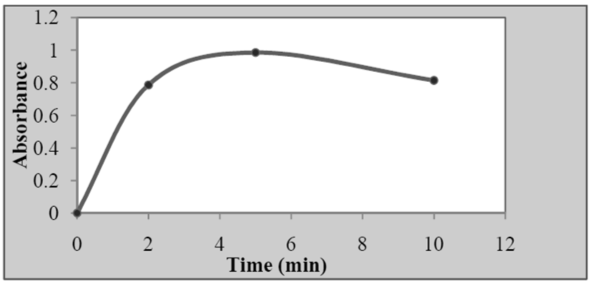

Figure 4.5: Absorbance Variation with Varying Time of Diazotization for MSP

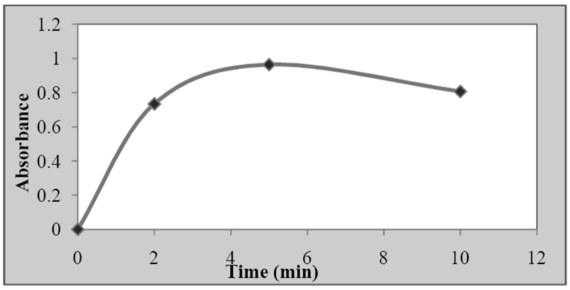

Figure 4.6: Absorbance Variation with Varying Time of Diazotization for MTCP

It was experiential that at room disease the required time for diazotization with trembling was 5 minutes, and the consistency of yellow tinted azo dye was up to 45 minutes. Increasing or decreasing the time for diazotic reaction affects the change in intensity of color and the absorbance value.

4.6 Proposed Spectrophotometric methods

Proposed Methods:

- MSP-EAA Method
- MTCP-EAA Method

Reference Method:

SAM-NEDA Method- Method Sulphanilamide (SAM) and N-(1-naphthyl) dihydrochloride ethylenediamine (NEDA)[1]. MSP-EAA and MTCP-EAA suggested methods and SAM-NEDA comparison system were used to handle filtered transparent specific samples for nitrite determination

4.6.1 Determination of Zinc Nitrate and Nitrite in Laboratory Mixture

Appropriate aliquots of nitrite and nitrate mixture were taken distinctly in 10 ml beakers at dissimilar mass connections, and the nitrate was simplified to nitrite with a touch of zinc dust / NaCl and conc granular mix. HCl. HCl. Excess zinc powder was drinkable and the remainder was processed to monitor the concentration of entire nitrate attractive nitrite by the expected and orientation afterwards. The absorbance of equivalent volume of nitrite and nitrate combination was slow deprived of discount of nitrate. This stretches the conc. of nitrite first current in the combination. Subtracting this from the total gives the concentration of nitrate present in the mixture [8].

4.6.2 Determination of Zinc Nitrate and Nitrite in Water and Soil Samples

The potential method has been applied for zinc nitrite and nitrate determination in instances of blow liquid and insincere water. Through hastening the chloride with silver sulphate [9, 10], the chloride became removed from the meddling. Using the suggested methods and orientation, sufficient aliquots were obtained and analysed for zinc nitrite and nitrate. About 1.0 g of soil samples was taken in a 25 ml beaker and extracted with 3 ml helpings of 0.5 % sodium carbonate answer. The excerpt was drinkable through Whatman No. 41 filter rag [9]. The filtrate was composed and dilute to 25 ml. suitable aliquots of 1-2 ml of the solution were transferred in to a 10 ml standardized bottles and examined rendering to the future process. They were tested negative for nitrite concentration. To these samples known amounts of zinc nitrite and nitrate solutions were additional and examined for nitrate and nitrite subsequent the planned and reference events.

4.7 Results of Spectrophotometric Determination of Zinc Nitrate and Nitrite

The acidic solution of the diazonium salt is usually added to the coupling part water. Because additional acid is released during coupling aside from the acid derived from the diazotization process, the optimum pH value must be adhered to by adding bases. Alkali hydroxide in metal e.g. For this purpose sodium hydroxide was used. The developed method avoids the use of sodium acetate or formate (weakly acid) or sodium phosphate (weakly alkaline) to buffer the acid. These are added to the reaction mixture before, during, or after combining the components. Because the pH value at the feed point of the diazonium salt solution is always different than that after thorough mixing has been completed, the type of stirrer and speed of stirring are also important in many instances.

Table 4.1: Determination of Zinc Nitrate and Nitrite in Laboratory Mixtures using MSP-EAA

Sr. No.	Nitrite taken µg/ml	Nitrate taken µg/ml	Nitrite found µg/ml	Nitrate found µg/ml	% Recovery	
					Nitrite	Nitrate
1	0.4	--	0.399	--	99.75	--
2	0.3	0.1	0.299	0.098	99.67	98
3	0.2	0.2	0.201	0.198	100.5	99
4	--	0.4	--	0.397	--	99.25

Table 4.2: Determination of Zinc Nitrate and Nitrite in Laboratory Mixtures using MTCPEAA

Sr. No.	Nitrite taken µg/ml	Nitrate taken µg/ml	Nitrite found µg/ml	Nitrate found µg/ml	% Recovery	
					Nitrite	Nitrate
1	0.35	--	0.344	--	98.29	--
2	0.3	0.05	0.296	0.051	98.67	102
3	0.25	0.1	0.247	0.099	98.80	99
4	--	0.35	--	0.347	--	99.14

Table 4.3: Determination of Zinc Nitrite in Water and Soil Samples using Proposed MSP-EAA Method and Reference Method (n=6)

Sample	**Nitrite added µg/ml**	**MSP-EAA method**		**Reference Method [27]**		**t-test**
		Nitrite found µg/ml±SD	% Recovery	Nitrite found µg/ml±SD	% Recovery	
Tap water	0.04	0.0397±0.0015	99.04	0.0396±0.001	98.96	0.4696
	0.06	0.059±0.002	98.19	0.058±0.002	97.25	0.3315
	0.08	0.079±0.0016	98.35	0.077±0.0028	95.71	0.1152
Surface water	0.04	0.039±0.0016	97.04	0.0393±.0019	98.42	0.3280
	0.06	0.058±0.002	96.69	0.059±0.002	98.22	0.3126
	0.08	0.077±0.0029	96.52	0.079±0.002	98.19	0.0399

Soil sample	0.04	0.039±0.002	97.92	0.0388±0.001	97.13	0.3742
	0.06	0.057±0.0015	95.78	0.0599±.0028	99.75	0.0658
	0.08	0.758±0.0017	94.71	0.0768±0018	96	0.1978

Table 4.4: Determination of Zinc Nitrite in Water and Soil Samples using Proposed MTCP-EAA Method and Reference Method (n=5)

Sample	**Nitrite added µg/ml**	**MTCP-EAA method**		**Reference Method [27]**		**t-test**
		Nitrite found µg/ml±SD	% Recovery	Nitrite found µg/ml±SD	% Recovery	
Tap water	0.1	0.093±0.003	93	0.095±0.004	95.8	0.0223
	0.2	0.189±0.005	94.3	0.190±0.012	95.2	0.395
	0.3	0.288±0.01	96	0.301±0.011	100.3	0.0689
Surface water	0.1	0.101±0.011	101.4	0.098±0.001	98.2	0.2636
	0.2	0.197±0.026	98.6	0.204±0.021	102.1	0.3593
	0.3	0.291±0.01	97.07	0.297±0.008	99.1	0.1331
Soil sample	0.1	0.092±0.003	92	0.092±0.004	91.6	0.466
	0.2	0.188±0.006	94.2	0.193±0.008	96.3	0.2684
	0.3	0.294±0.01	97.93	0.293±0.007	97.7	0.4695

5. Statistical Analysis

Student's t-test statistical analysis of the findings shows that there is close agreement between the results produced by the suggested approach and reference system for the water and soil sample tests. In the presence of Cu^{2+}, K^{+}, Na^{+}, Ca^{2} the recovery tests are carried out on water and soil samples.

Relevant results can be drawn from the analysis of the determination of nitrate and nitrite in water and soil samples with composite diazotic-coupling reagents containing mosapride (MSP) or metoclopramide (MTCP) as the diazotic aromatic amines and ethyl acetoacetate (EAA) as the coupling agent. Optimum coupling conditions depend on the nature of the diazo components used and their coupling. The spectrophotometric approach is simple, inexpensive and accurate but time intensive and laborious. It provides researchers with ample ability for the primary analysis of measuring nitrate and nitrite. (MSP) or (MTCP) react with nitrite in the acid medium to produce diazonium ion that is mixed with ethyl acetoacetate (EAA) in the basic medium to produce azo dye. MSPEAA is a complex of yellow colours, which gives the maximum absorption (πmax) at 429 nm. The MTCP-EAA is also yellow-colored complex, which at 437.5 nm gives the maximum absorption (πmax).

6. Conclusion

The work included the creation and testing of spectrophotometric and chromatographic methods from diverse sample matrices such as water, soil samples for simultaneous determination of nitrate and nitrite. The sequence of the combination of the two components can also greatly influence the result. The coupling reaction can be completed directly after mixing of the materials or several hours later. It is best to cool with ice and prevent exposure to artificial light if the reaction takes a longer time. To test if excess diazonium compounds are still present, a drop of the reaction solution is spotted on filter paper along with a portion that pairs readily (e.g. low alkaline H acid solution). If there is no coloring, the coupling is complete. The location of unconsumed coupling components can be determined by spotting with a diazonium salt solution. Attention often needs to be given to the coupling solution or suspension length. The physical condition is an important consideration when starting components with low solubility. The reagents provide a simple and sensitive method for the determination of nitrite and nitrate by spectrophotometry for monitoring water pollution.

The applied different ions do not interfere with the extraction up to maximum concentrations. T-test statistical analysis shows that there is no significant difference in accuracy and accuracy between the method proposed and the reference method. The precision of the proposed method is assessed by replicate analysis at five different concentrations of samples containing nitrite and nitrate. The proposed method of determining trace amounts of nitrite and nitrate in soil, water samples preparations has been successfully applied.

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