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## **RESEARCH ARTICLE**

# GREEN SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF NITRITE IN ENVIRONMENTAL SAMPLES USING SULFADIAZINE

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ARTICLE INFO	ABSTRACT
Article History:	New spectro photometric method based on electrophilic coupling reaction for the determination of trace amounts of nitrite in environmental samples had been developed. Replacement of toxic reagents was
Received 05 <sup>th</sup> August, 2015	explored to attain the standards of clean chemistry. This method utilizes phenoxazine and sulfadiazine
Received in revised form	(SZX), the well-established drug in the presence of limited amount of hydrochloric acid. The method is
08 <sup>th</sup> September, 2015	based on the oxidation of sulfadiazine (SZX), by nitrite in hydrochloric acid medium and coupling with
Accepted 10 <sup>th</sup> October, 2015	phenoxazine (PNZ), which yielded red colored derivative having absorbance maximum at 525 nm and is
Published online 28st November,	stable for about 1h. Beer's law was obeyed for nitrite in the concentration range $0.08 - 0.68 \mu g \text{ ml}^{-1}$ . The
2015	reaction conditions and other important analytical parameters were optimized to get maximum sensitivity
	of the method. Interference if any, by non-target ions was also investigated. The method can be applied
Kev words:	successfully for determining nitrite in environmental samples. The performance of this method was
	evaluated by applying student's t-test and variance ratio F-test to find out the significance of proposed
Spectro photometry, Nitrite,	method over the reference spectro photometric method.

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## **INTRODUCTION**

Sulfadiazine, Phenoxazine, environmental Samples.

The green chemistry - its principles and the concepts of sustainability are becoming part of explicit policy to prevent pollution from hazardous and or toxic substances (Anastas et al., 2000; Anastas and Warner, 1998). Consequently, the emergence of green analytical chemistry as an approach to address one of the twelve basic principles of green chemistry is gaining importance in recent years (Anastas, 1999). However, behind the research in green analytical chemistry there might be an intellectual motivation towards the paradoxical situation, which has emerged because most of the analytical methodologies and methods employed to investigate environmental problem(s) generate chemical wastes, which are most hazardous and toxic than the species being monitored thus, resulting an environmental impact (Rocha et al., 2001). An example of a reference method, which is environmentally not acceptable, is the procedure for the determination of nitrite employing Griessdiazo coupling reaction with sulfanilamide and N-(l-napthyl) ethylenediamine hydrochloride (NEDA), which produces colored azo compounds besides other end product(s), which may be carcinogenic (Alien, 1989). This

Monitoring of nitrite in environmental samples is of paramount importance because of its harmful effects on human health (BIBRA, 1990). Nitrite salts are versatile chemical agents, which find varied applications such as dye manufacture, food industry and corrosion inhibition on industrial process water (Lijinsky and Epstein, 1970; Kross *et al.*, 1993). The nitrite ion is an important intermediate in the biological nitrogen cycle and is present in soils and surface water.

Excess Concentration of nitrite in drinking water above the prescribed limit could be hazardous to health, especially to infants and pregnant women. Nitrite oxidizes iron present in hemoglobin of the red blood cells to form methemoglobin; as a

method suffers from the shortcoming that it requires careful control of acidity at each step and the entire process takes a long time to complete. As a consequence, the approach in analytical chemistry is focused on the development of methods and procedures less harmful to humans and to the environment. Hence, in the development of any new analytical methodology or a method the amount and toxicity of wastes generated in the process are as important as any other analytical feature.

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result it loses its oxygen carrying ability. This leads to the condition known as methemoglobinemia (Bruning-fann and Kaneene, 1993; Wolff and Wasserman, 1972). Nitrite has also direct impact on the health as its reaction with amines or amides in human body to produces different types of nitrosamines, which are very powerful carcinogens (Lijinsky and Epstein, 1970; Wolff and Wasserman, 1972; Choi and Fung, 1980; Patty, 1948). Therefore, elucidation of nitrite concentration in different materials is necessary from the stand point of environmental analytical chemistry. This nascent branch contributes significantly to the growth of environmental responsibility for sustainable development through environmental monitoring, which in turn led to the development of Green Analytical Chemistry.

Many contemporary analytical methodologies including (i) pulse polarography (Fogg and Alonso, 1988; Li et al., 2002), (ii) membrane sensor (Hassan et al., 2003; Rocha et al., 2002) (iii) liquid chromatography (Lee and Field, 1984), (iv) capillary zone electrophoreses (Okemgbo et al., 1999; Fukushi et al., 2000; Tagliaro et al., 2002), (v) flow injection analysis (Chaurasia and Verma, 1994; Chen et al., 2000; Andrade et al., 2003; Haghighi, and Tavassoli, 2002; Wei et al., 2002), and (vi) visible spectrophotometry (Flamerz, and Bashir, 1981; Kaveeshwar et al., 1991; Baveja et al., 1981; Sunita, and Gupta, 1984; Kesari, and Gupta, 1988; Revanasiddappa, and Bilwa, 2001; Johannes et al., 2000; Abdul Galila Mahadevaiaha, 2007; AOAC, 1995; Horita et al., 1997) and UV spectrophotometry (Aydin et al., 2005) have been employed to determine the nitrite ion in different matrices. However, these methods proved to have limitations with respect to specificity, sensitivity, simplicity and or short analysis time. For example, pulse polarography demands extensive selectivity regarding the solvent and choice of the electrode material is not only costly but also cumbersome. Membrane sensors lack reproducibility and they have low sensitivity. Liquid chromatography methods are valuable techniques for identification in physiological samples, rather than for routine environmental analysis. Further the cost of the instrument is relatively high and maintenance demands sophistication. Thus, for routine environmental analysis, spectrophotometry seems to be the most attractive and suitable analytical approach and it happens to be convenient and simple and relatively inexpensive also.

Visible Spectrophotometric methods used for the determination of nitrite may be broadly classified into eight types. Methods under Type 1 are based on the diazotization of aromatic amines by means of nitrite under special conditions and the intermediate is allowed to react with a selected aromatic compound to couple (Flamerz, and Bashir, 1981; Kaveeshwar et al., 1991; Baveja et al., 1981; Sunita and Gupta, 1984; Kesari and Gupta, 1988; Revanasiddappa and Bilwa, 2001; AOAC, 1995). The type II methods involve aromatic partners for diazonium compound selected mostly from phenolic compounds (Haghighi and Tavassoli, 2002; Kaveeshwar et al., 1991). Although type III methods are similar to type I and II, they use diazotization coupling reaction involving active methylene compounds (Revanasiddappa and Bilwa, 2001). Type IV methods use the reactions of nitrite to give nitrosocompounds of (Johannes et al., 2000). The fluorescence methods come under Types V (Johannes et al., 2000).

Type VI methods are based on bleaching property of nitrite which utilizes decolourisation of coloured complex (Abdul Galila and Mahadevaiaha, 2007). Type VII methods are based on the catalyst effect of nitrite on the oxidation of organic compound by various oxidants under acidic conditions (AOAC, 1995). Type VIII methods are based on oxidations of electrophilic coupling reagents in acidic medium and coupling with phenoxazines as new class of Spectro photometric reagents (Al-Okab and Syed, 2007). Methods falling under types I, II and III are based on azo dye formations which are characterized by high sensitivity, but, often associated with drawbacks as pH dependence, diazotization temperature, coupling time and large sample volumes. Besides, these procedures often require large volumes of samples of carcinogenic reagent(s), which keep those methods outside of the standards of clean chemistry (Alien, 1989). In some process, either the extraction by a solvent (Kaveeshwar et al., 1991; Baveja et al., 1981) or concentration on a solid support (Horita et al., 1997) of azo dye has been proposed to get higher sensitivity. These methods suffer from the interference of cations and anions, such as Cu<sup>2+</sup> Co<sup>2+</sup>, Fe<sup>3+</sup>, Cr<sup>3+</sup>, Al<sup>3+</sup>, sulphite, thiosulphate and iodide (Flamerz, and Bashir, 1981; Baveja et al., 1981). Nevertheless the use of masking agents for cations has been reported. Type IV and type V methods are susceptible to the influence of various foreign ions. Type VI methods are

 Table 1 Comparison of Spectro photometric methods for the determination of nitrite.

Reagent	Range	λ-max (nm)	Remarks	Reference
4-Aminobezoic acid	0.10-1.30	519	Less sensitive	(Flamerz, and Bashir, 1981)
o-Nitroaniline and l -aminonapthalene	0.08-0.68	454	Extractive	(Kaveeshwar et al., 1991)
4-Nitroaniline + l-napthol	0.02-0.14	610	Extractive, many interferes	(Baveja et al., 1981)
PNA + guaiacol	0.03-0.15	540	Extractive, NO3-, A13+ interferes	(Sunita and Gupta, 1984)
PNA + phloroglucinol	0.004-0.04	420	Fe(III) and Cu(II) interfere	(Kesari and Gupta, 1988)
PNA + acethylacetone	0.50-14.00	490	Less sensitive Cu(II), Co(II), and Hg(II) interfere	(Revanasiddappa and Bilwa, 2001)
Peroxovanadate complex	6.67-66.70	470	Less sensitive	(Abdul Galila Mahadevaiaha, 2007)
SAA+NEDA <sup>a</sup>	.05-4.00	540	p <sup>H</sup> control and development of the color takes 30 min	(AOAC, 1995)
Proposed method PNZ+SZX	0.08-0.68	525	No p <sup>H</sup> control and color develops in 3 min	
A, N- (l-napthyl) ethylenediamine hydrochl	oride; PNA, p-	nitroaniline; SA.	A, Sulfanilamide <sup>a</sup> AOAC Reference method	

sensitive, but require careful control of temperature, acidity and reaction time. Besides, the procedures are highly influenced by a large number of ions, particularly  $Fe^{2+}$ ,  $Fe^{3+}$ ,  $Ag^+$ ,  $SO_3^{2-}$ ,  $Br^-$ , F, SCN<sup>-</sup> and V<sup>5+</sup>. A comparison of a few selected procedures, their spectral characteristics and draw backs are encountered in Table 1. With the objective of utilizing the non toxic chemicals which is a 'control exercise' for the formation of hazardous substances, oxidative coupling reaction using phenoxazine (PNZ) as chromogen and sulfadiazine (SZX), as electrophilic coupling reagent for the determination of nitrite to yield red color derivative have been developed. Based on this, highly sensitive, selective and rapid method was developed and applied for the determination of nitrite in drinking and environmental water samples.

#### Experimental

#### Apparatus

All spectral and absorbance measurements were carried out on a Specord 50 UV-vis spectrophotometer with 1.0-cm silica quartz matched cell.



#### **Reagents and solutions**

SZX and PNZ (Aldrich), and Sodium nitrite (Ranbaxy, India), were used without further purification. All reagents used were of analytical grade chemicals unless specified otherwise. Stock sodium nitrite solution of  $2.176 \times 10^{-2}$  M (1000µg nitrite ml<sup>-1</sup>) in 1000 ml volumetric flask was prepared by dissolving 1.50g sodium nitrite (predried at 110°C for 4 h) in water. A small amount of sodium hydroxide to prevent nitrite decomposition and a few drops of chloroform to prevent bacterial growth were also added. Required standard solution of (2µg nitrite/ml) was prepared by diluting with water 2 ml of standard sodium nitrite solution to 1000 ml.

A stock solution (0.05% w/v) of PNZ was prepared by dissolving 500 mg of the compound in distilled ethyl alcohol and further diluting quantitatively to 100 ml with distilled ethyl alcohol. Solution (0.05% w/v) of SZX was prepared by dissolving 500 mg of the compound and diluting quantitatively to 100 ml with water. Hydrochloric acid (2M 3 ml) was added during the preparation of sulfadiazine to increase its solubility. Hydrochloric acid solution of 2M was prepared by diluting quantitatively 176.99 nil of 35% HC1 (Merck, Germany) to 1L with distilled water.

#### Analytical procedure

To a series of 25-ml calibrated flasks; 1 ml of PNZ (0.05% w/v) reagent, 1 ml of Sulfadiazine (0.05% w/v), 2 ml of 4M HC1 and nitrite solution were added and the mixture shaken

thoroughly and allowed to stand for 3 min and the volume was made up with water. Absorbance at 525 run for PNZ-SZX method was measured in 1.0-cm quartz cell against reagent blank which was prepared without nitrite. The optical characteristics for the determination of RSC with PNZ using SZX are detailed in the Table 2.

**Table 2** Spectral data for determination of nitrite using sulfadiazine as electrophilic coupling agent and phenoxazine as chromogen.

Parameters	PNZ
Color	Red
$\lambda_{max}$ (nm)	525
Stability (h)	1
Beer's law (jag ml <sup>-1</sup> )	0.08-0.68
Recommended ion concentration (µg ml <sup>-1</sup> )	0.20
Molar absorptivity (L mol <sup>-1</sup> cm <sup>-1</sup> ) x 10 <sup>4</sup>	$4.98 \text{ x} 10^4$
Sandell's sensitivity (µg cm <sup>"2</sup> )	0.00092
Regression equation:	
Slope (a)	0.9743
Intercept (b)	0.3343
Correlation coefficient	0.9927
Reaction time (min)	3
R.S.D % (n=5)	0.67

\*y=ax + b where x is the concentration of nitrite in  $\mu gmL^{-1}$ \*\* Relative Standard Deviation, n - number of the replicates.

Table 3 Tolerance limits of some diverse ions on the
reaction of nitrite (0.2ug ml" <sup>1</sup> ) with sulfadiazine using
PNZ

Foreign ions	Tolerance limit (µgmL <sup>1</sup> )
Hg <sup>2+</sup> , K <sup>+</sup> , Mg <sup>2+</sup> , Zn <sup>2+</sup> , Pb <sup>2+</sup> ,	400
EDTA,F <sup>-</sup> ,Br <sup>-</sup> ,Bi <sup>3+</sup> ,Ba <sup>2+</sup> ,Ti <sup>4+</sup> ,Cr <sup>-</sup> ,Co <sup>2+</sup>	200
$Na^{+},Al^{3+},Bi^{2+}$	100
NO <sub>3</sub> <sup>-</sup> ,Br <sup>-</sup> ,SO <sub>4</sub> <sup>2-</sup> ,NH <sup>4+</sup> ,CO <sub>3</sub> <sup>2</sup> -,Cu <sup>2+</sup> ,COO <sup>-</sup>	50
Fe <sup>3+</sup>	0.5
IO <sup>3-</sup> ,IO <sup>4-</sup> ,BrO <sub>3</sub> <sup>-</sup>	0.4

#### Parameters

The physical parameters in visible spectrophotometric procedure control the extent of the reaction and responsible for the optimization of the mixing of the reagent and the substrate. Since the proposed method relies on the measurement of the absorbance anything that affect the absorbance will have a pronounced effect on the performance of the method.

#### Wavelength determination

In order to have minimum interferences, it was necessary to identify optimum wavelength for nitrite determination in the method. This wavelength must be specific for the quantitative and specific monitoring of the nitrite-SZX -PNZ. The



Figure 1 Absorption spectrum of nitrite-PNZ- SZX.

wavelength of maximum absorbance was identified by scanning the product of nitrite- PNZ-SZX over the range 300-800 nm with a specord 50 UV-vis spectrophotometer. A wavelength of 525 nm was found optimum for getting best results (Figure.1)

#### Effect of reagents and acid concentration

The effect of PNZ reagent was studied in the range 0.10 - 10.00 ml (0.05% w/v) solution of each to achieve the maximum color intensity; volume of 0.50 - 3.00 ml of the solution gave good result. Hence, 1 ml (0.05 % w/v) PNZ solution in 25-ml standard flask was selected for further studies, under optimized conditions. The maximum intensity of the red color was achieved in hydrochloric acid medium. Preliminary investigations showed that hydrochloric acid was better than sulphuric, phosphoric or acetic acid. Maximum intensity of the red color was achieved in the range of 2-5 ml of 4M HC1. Therefore, 2 ml of 4M HC1 in 25 ml was used for getting the best results.

Similarly, the same procedure was adopted to ascertain the amount of SZX required for getting constant and maximum color intensity. It was found that 0.50 - 3.00 ml of the solution was needed to get good result. Hence, 1 ml of (0.05% w/v) SZX solution is sufficient to get reproducible results. Experiments were carried out to optimize temperature and time of the reaction. It was found that the maximum color developed within 3 min at room temperature and remained almost stable for about 1 h. Increase in the temperature decreased the intensity of the red color. Hence, 3 min reaction at room temperature was sufficient for the routine analysis.

#### Order of addition of react ants

During the course of the investigation it was observed that the sequence of addition of reactants was also important as it influence the intensity and the stability of the color of the product to great extent. The sequence (i) PNZ-HCl-nitrite-SZX and (ii) nitrite-HCl-PNZ, SZX gave less intense and unstable color. While, (iii) PNZ- SZX- HCl- nitrite gave more intense and stable red color.

#### Analytical figures of merit

The Spectrophotometric methods were evaluated under the optimum conditions with respect to linearity, accuracy, precision, molar absorptivity and Sandell's sensitivity.

#### Linearity (Beer's law application), accuracy, precision

The linearity of the Spectrophotometric method for the determination of nitrite was evaluated under optimum conditions. The regression calibration equation obtained under optimum conditions for nitrite, PNZ and SSA was: Y=-0.0334+0.9741X; r=0.9927 and n=9 Where, Y is the absorbance and X is the nitrite concentration as mg /L. The calibration curve was linear over the range 0.08-0.68 mg /L.

Sandell's sensitivity (S) represents the number of micrograms of the determinant per milliliter of a solution having an absorbance (A) of 0.001 for a path length (1) of 1-cm. Thus, S =  $10^{-3}$  / a =  $\mu$ g cm<sup>-2</sup> where a is the specific absorptivity and its value (in ml g<sup>-1</sup> cm<sup>-1</sup>) corresponds to the determinant in a cuvette with an optical length of 1-cm. Also,

a = b / molecular weight of nitrite ion x 1000

b = molar absorptivity = A/C 1 where C is the molar concentration of the determinant and 1 = 1-cm path length.

The accuracy of the method was evaluated by comparing the results obtained for real environmental samples (obtained from lake, well and tap water and soil) with the proposed Spectro photometric methods and with the result of standard Spectro photometric method. The results obtained in the proposed Spectrophotometric methods compared very well with those from the standard method. The % R.S.D. was found to be <0.8 (n = 5). The proposed method was found as accurate and precise as that of the official method (Table .4).

To further confirm the validity and accuracy of the proposed method recovery tests were performed by standard addition method. Each test was repeated five times. The results presented in Tables 4 indicate very good recoveries and noninterference from commonly encountered constituents normally

 Table 4 Determination of nitrite in different environmental samples using sulfadiazine as electrophilic coupling agent and PNZ

 as chromogen

	us enfontogen									
Sample	Nitrite added Hgrnl <sup>-1</sup>	Propos	sed method	Reference method (AOAC method, 1995)						
		Nitrite recovered µgml <sup>-1</sup>	Recovery % ±R.S.D <sup>a</sup>	Nitrite recovered µgml <sup>-1</sup>	Recovery % ±R.S.D <sup>a</sup>	T-value <sup>b</sup>	F-value <sup>c</sup>			
Lake	0.300	0.291	97.06±0.16	0.297	99.34±0.11	111	1.58			
Water	0.500	0.494	98.88±0.23	0.498	99.68±0.18	1.15	1.62			
Tap water	0.300 0.500	0.301 0.493	100.16*0.19 98.62±0.25	0.301 0.501	100.10±0.21 100.22±0.51	2.20 1.87	2.19 2.61			
Mineral	0.300	0.298	99.22±0.15	0.298	99.60±0.11	1.55	2.35			
water	0.500	0.510	101.94±0.11	0.491	98.82±0.17	1.96	2.07			
Well	0.300	0.304	101.20±0.13	0.305	101.50±0.16	2.30	1.66			
water	0.500	0.493	98.60±0.14	0.521	100.42±0.22	1.69	2.35			
Manured	0.300	0.304	101.14±0.05	0.297	99.08±0.84	2.51	2.29			
garden soil	0.500	0.507	101.46iO.20	0.5052	$101.04 \pm 0.18$	0.97	1.22			
Farmland	0.300	0.306	102.10*0.1 1	0.2993	99.78±0.08	1.52	1.69			
soil	0.500	0.496	99.22±0.15	0.5016	100.32±0.18	1.77	1.48			
Roadside	0.300	0.302	100.5±0.09	0.296	98.62±0.08	2.01	1.42			
Soil	0.500	0.505	101.04±0.11	0.497	99.48±0.13	1.43	1.32			

<sup>a</sup>Average of 5 determinations ± Relative Standard Deviation ; <sup>b</sup>Tabulated t-value at 95% confidence level is 2.78 ; <sup>c</sup>Tabulated F-value at 95% confidence level is 6.39

#### Effect of diverse ions

The interference from various cations and anions on the determination of nitrite was studied by adding known amounts of foreign species to a solution of known concentration of nitrite as per the proposed method. The tolerance limit for various ions is defined as the concentration of added ion causing less than  $\pm 3\%$  relative error for the nitrite determination. The maximum tolerable concentrations are given in the Table. 3

#### Method validation

To validate the proposed Spectrophotometric method, Student's /test was performed on the results of five real samples (Table .4). Comparison was made between the proposed Spectro photometric method and the standard method to find out whether the two methods give the same results at the 95% confidence level. The /test with multiple samples was applied to examine whether the two methods for nitrite determination differ significantly at the 95% confidence level. The calculated Student's /-value and *F*-value did not exceed the tabulated value indicating that the proposed method is as accurate and precise as the official method (AOAC, 1997).

#### Application of the method to polluted water and soil

Samples of potable water were collected in wide-mouthed plastic vessels from different taps and different packaged water bottles. The samples were frozen at O°C within 1 h of collection. Samples were filtered through Whatman No. 41 paper before analysis. Samples of manured garden soil, farmland soil and roadside soil were collected. Each sample was broken into lumps, and 5-g portion was dried at 55°C hi an oven for 12- 16 h. The dried sample was ground, passed through a 2-mm mesh sieve and transferred to What man No. 50 filter paper on a Buchner funnel. Sufficient water (containing 1 or 2 drops of concentrated sulphuric acid) was poured on to soak the soil completely. After a few minutes, gentle suction was applied and the soil was washed with double distilled water until about 250 ml of filtrate was collected. The filtrate was made up to a standard volume and aliquots were analyzed (Chaube *et al.*, 1984).

## **RESULTS AND DISCUSSION**

The optical characteristic for the determination of nitrite with PNZ using SZX is detailed in the Table 2. Phenoxazine is an isolog of phenothiazine and is known since 1887. It is a part of the chemical structure of actinomycin D, which is known to exert intensive anticancer activity on malignant tumors in children (Japp and Davidson, 1895) and is reported to be more potent and less toxic chemosensitizer (Thimmaiah et al., 1990). PNZ derivatives exist in neutral form, as monocations, as dications and even as trications depending on the environment (Gvishi et al., 1989). Their molecular structure and luminescent properties have been studied extensively (Grofcsik et al., 2000). Besides, they have impressive applications as biological stains (Channu et al., 1999), as laser dyes (Schafer, 1973) and as redox indicators (Stransky and Gruz, 1972). PNZ derivatives are nervous system depressants particularly with sedative, antiepileptic, tranquillizing activity (Ribbentrop and Schaumann, 1964) spasmalytic activity (Gal and Avakian, 1963] antitubercular activity (Girard, A. 1962) and anthelmentic activity (Rogers et al., 1955]. In recent years PNZ

derivatives are reported to be potential chromophoric compounds in host-guest artificial photonic antenna systems (Calzaferri *et al.*, 2002). The sulfonamides are analogues of *p*-aminobenzoic acid and are known since 1932. Though a large number of sulfonamides are synthesized and reported in the literature, only about two dozens of them have been used in clinical practice (Calzaferri *et al.*, 2002). They differ only slightly in their antimicrobial activity but vary greatly in their pharmacokinetic properties, or rate of excretion. Accordingly, they are classified as short-, medium-, long- and ultra-long acting drugs (Reynolds, 1993) Sulfadiazine (SZX) belongs to short-acting sulfonamides.

Electrophilic coupling reaction has attracted considerable attention for quantitave analysis of many environmental active compounds (Suresha *et al.*, 2002; Rekha *et al.*, 2007). In the present investigations SZX is oxidized quantitatively by nitrite ions and coupled with PNZ to form red color. A general reaction mechanism proposed is prescribed in Fig 2. The standard method for the determination of nitrite in water samples uses the reddish purple azo dye produced at pH 2.0-2.5 by coupling diazotized sulfanilamide with NEDA. The aim of this study was to avoid diazotisation reaction to make the method greener. Consequently, we have exploited the quantitative property of nitriteto oxidize sulfadiazine and subsequently couple with PNZ to yield red color which forms the basis for Spectro photometric determination.

The following experiments were carried out to prove this point; the diazotized sulfadiazine was made to react with PNZ instead of NED A which did not develop any color. Alternatively, instead of nitrite, chloramine-T was used to oxidize sulfadiazine and subsequently coupled with PNZ, which developed red color. These experiments confirmed that the proposed reactions were not based on conventional diazotization reaction; instead, they followed an alternate route to produce color based on oxidative electrophilic coupling reactions. Key parameters that influenced the performance of the method were studied to arrive at the optimum working configurations. All the data given and % R.S.D. in the optimization steps for both physical and chemical parameters are the mean values from successive determinations. All the optimization steps were carried out with a chosen nitrite concentration as mentioned in Table 2. The effects of possible interference and the maximum tolerable concentration are given in the Table 3.

## CONCLUSION

Green analytical chemistry refers to the development of methodologies, methods and procedure that reduce or eliminate the use and generation of hazardous and or toxic substances, while keeping economics, analytical future as well as environmental viability in mind. By virtue of more efficient use of selective reagents that follow green chemistry principle are more profitable and vital to the feature of green analytical chemistry. Now a day, the toxicity of the chemicals must be taken into accent in the development of new analytical methods and procedure. However, there are many analytically reliable methods of procedure that are not environmental benign many of them are recommend as standard or reference methods (Eaton *et al.*,1995Lawrence, L.H. 1996). Counting the effect of analytical chemist to develop the current assay it is not realist to hope that the replacement of all hazardous and or toxic chemicals can be made within the near

future (De la Guardia and Ruzicka, 1995). However, they should be one of the many goals of analytical chemistry. This paper is a step in this direction.

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