DEVELOPMENT OF SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF HYDROXYLAMINE, HYDRAZINE AND IODINE SPECIES

Synopsis of the thesis to be submitted in partial fulfillment of the requirements for the degree of

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CONTRIBUTIONS

A. In Journals

1. **Mary George**, N.Balasubramanian and K.S.Nagaraja, Spectrophotometric determination of hydroxylamine and its derivatives in drug formulation using methyl red, *Ind. J.Chem.Technol.*, **14** (2007) 412-416.

2. **Mary George**, N.Balasubramanian and K.S.Nagaraja, Spectrophotometric determination of hydrazine using bromine and methyl red, *Ind.J.Chem.*,**46A** (2007) 1621 – 1624.

3. **Mary George**, N.Balasubramanian and K.S.Nagaraja, Spectrophotometric Determination of Hydrazine, *Anal.Lett.*, **40** (2007) 2597 – 2605.

4. **Mary George**, N.Balasubramanian and K.S.Nagaraja, Spectrophotometric Determination of Hydrazine, *Talanta* (in press) 2007.

5. **Mary George**, N.Balasubramanian and K.S.Nagaraja, Spectrophotometric Determination of Hydroxylamine and its Derivatives in Pharmaceutical Formulations, *Chem. Anal.*, (accepted) 2007.

6. **Mary George**, N.Balasubramanian and K.S.Nagaraja, Spectrophotometric Determination of Iodine Species in Table Salt and Pharmaceutical Preparations, *Chem.Pharm.Bull.*,(revised paper sent) 2008

7. **Mary George**, N.Balasubramanian and K.S.Nagaraja, Spectrophotometric Determination of Iodine Species in Table Salt and Pharmaceutical Preparations, *Ind.J.Chem.Technol.*, (communicated) 2008

B In Conference Proceedings

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INTRODUCTION

Modern analytical chemistry focuses on generating new methods of analysis that increase the specificity and sensitivity. However, advances in technology place greater emphasis on quality control and manufacturers have started seeking sensitive, reliable, faster and simpler methods of analysis. The late 20^{th} century has seen an expansion of application of analytical chemistry in the fields of environment, forensic, industrial and medical fields. Besides industry and agriculture, pollutants that are present in trace quantities that affect the public health require rapid and accurate determination. Hence there is an increased demand for trace analysis since these studies involve estimation in ppm or ppb levels.

Although modern analytical chemistry is dominated by sophisticated instrumentation, the basis of analyses and some of the principles used in modern instruments are from the traditional techniques many of which are still used today aided by computer technology. Spectrophotometric analysis continues to be one of the most widely used analytical technique in view of its easier accessibility. UV-Visible spectrophotometric methods find wide industrial applications, which include process monitoring and control. UV/Visible spectrophotometry remains an important tool in the area of environmental monitoring, biomedical applications, forensic science, pharmaceutical production, process monitoring and product assay. Hence development of newer procedures using spectrophotometric method would serve as an important tool in chemical analysis.

Our literature search indicated that there is a demand for the development of analytical methods for the determination of hydroxylamine, hydrazine and iodine containing species.

Hydroxylamine is determined using the diazo coupling method which involves oxidation of hydroxylamine to nitrite by iodine. The formed nitrite is determined based on the diazo coupling reaction [1-3]. Although these methods are sensitive for

determining the formed nitrite, the hydroxylamine oxidation is neither simple nor specific [4]. Hydroxamic acid when present along with hydroxylamine causes positive interference by undergoing oxidation to nitrite [5]. The use of iodine as oxidant to convert hydroxylamine to nitrite has an inherent defect in all these methods. The iodide formed as a result of reduction of iodine interacts with the formed nitrite and regenerates iodine, resulting in the loss of nitrite.

The well known method for the determination of hydrazine is the p-dimethylaminobenzaldehyde (p-DAB) method. The method is based on the formation of hydrazone with p-dimethylaminobenzaldehyde (p-DAB) [6]. Though the method is sensitive, since the reaction is temperature dependent, it is necessary to maintain a constant temperature to achieve accuracy [7] and the method is not specific to hydrazine. Hydrazine derivatives and amines interfere [8] in this method.

Literature shows several spectrophotometric methods developed for the determination of traces of iodine species like iodide, iodine, iodate and periodate. The popular method for the determination of iodide is based on its reaction with iodate in acidic medium liberating iodine. The liberated iodine reacts with excess iodide to form tri iodide [9,10]. Iodine reacts with starch to form an intense blue colour due to the formation of tri iodide starch complex [11]. These methods suffer from drawbacks such as instability of colour, insufficient sensitivity and interference of diverse ions. Many methods for the determination of iodate and periodate have been reported with chromogenic reagents, such as 2-oximinodimedonedithiosemicarbazone [12], triphenyl tetrazolium chloride [13], Alizarin Navy blue [14], tetramethylammonium iodide [15],amodiaquine dihydrochloride[16], ferroin [17],o-dianisidine [18], 4-bromo-N,N – bis(2-hydroxypropyl) – o- phenylenediamine[19], methylene blue[20], and thionin [21]. Some of these reagents are reported to be carcinogenic, while others are less selective, less sensitive, time consuming, suffer from interference of diverse ions.

SCOPE OF THE PRESENT WORK

Inherent drawbacks associated with the existing methods necessitate the development of a new set of analytical procedures, which could overcome the existing inadequacies for the determination of hydroxylamine, hydrazine and iodine species like iodide, iodine, iodate and periodate

The present investigation was aimed at the development of the following spectrophotometric methods,

1. Spectrophotometric determination of hydroxylamine after converting it to nitrite, followed by diazo coupling reaction.
2. Spectrophotometric determination of hydroxylamine, based on the bleaching of methyl red in the presence of a fixed concentration of bromine.
3. Spectrophotometric determination of hydrazine, based on the reduction of nitrate to nitrite followed by diazo coupling reaction.
4. Spectrophotometric determination of hydrazine after converting to 2,4-dinitrophenylhydrazine with 2,4-dinitrochlorobenzene.
5. Spectrophotometric determination of hydrazine, based on bleaching of methyl red in the presence of a fixed concentration of bromine.
6. Spectrophotometric determination of iodine species after converting to iodate, followed by nitrite determination generated in the presence of hydroxylamine.
7. Spectrophotometric determination of iodine species after converting to ICl_2^- followed by the bleaching of methyl red.

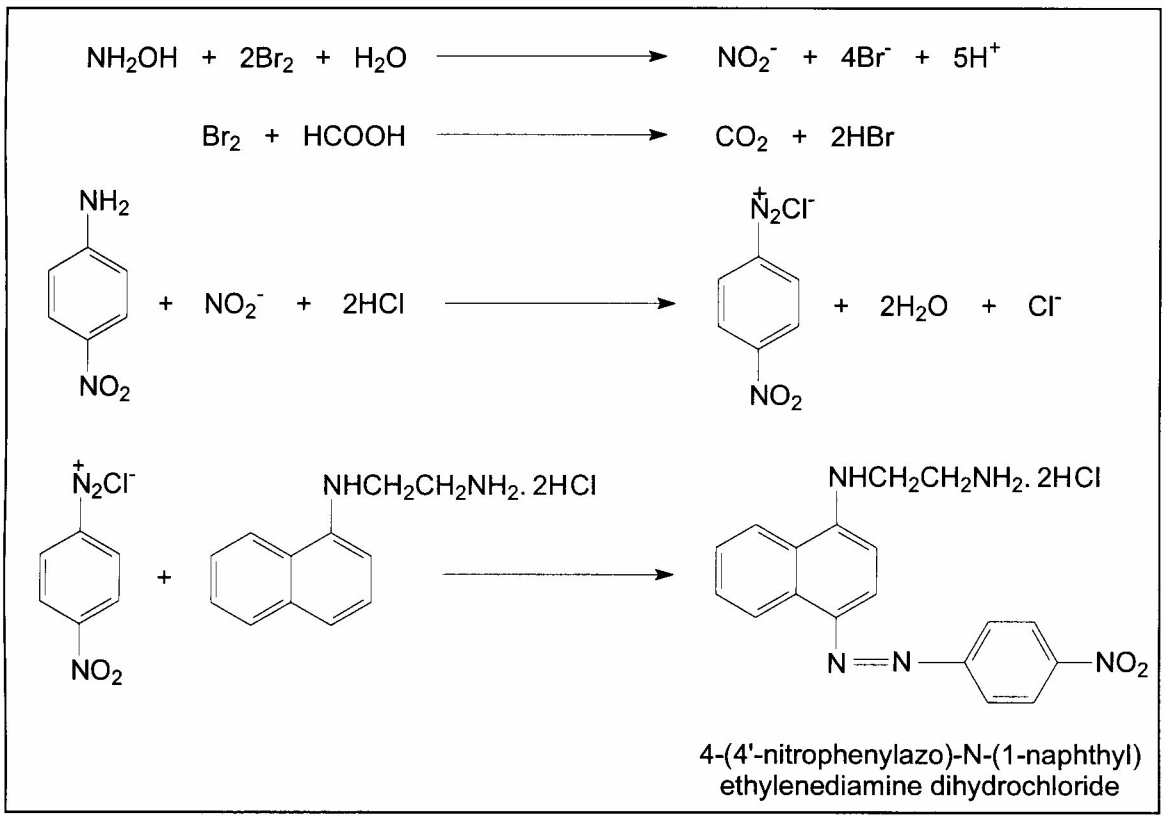
RESULTS AND DISCUSSION

1. Spectrophotometric determination of hydroxylamine after converting it to nitrite followed by diazo coupling reaction.

The well known spectrophotometric method for the determination of hydroxylamine is based on the oxidation to nitrite by iodine. But this method involving iodine as oxidizing agent has an inherent defect. The iodide formed as a result of reduction of iodine interacts with the formed nitrite and regenerates iodine, resulting in the loss of the analyte nitrite. Recently Deepa *et al* [22] reported a method using sodium arsenate under alkaline condition as oxidizing agent to convert hydroxylamine to nitrite. Hence an attempt was made to develop a simple and sensitive method for determination of hydroxylamine. The method is based on the oxidation of hydroxylamine to nitrite with excess of bromine solution under acidic condition. The formed nitrite is determined based on the diazo coupling reaction between p-nitroaniline(PNA) and N-(1-naphthyl) ethylenediamine dihydrochloride [NEDA](Scheme 1). The method obeys Beer's law in

the concentration range 0-10μg of hydroxylamine at 545 nm and the colour is stable for 3h. The molar absorptivity is 5.5 x 10^4 L mol^{-1} cm^{-1} with a relative standard deviation of 2.5 % (n=10) at 6 μg of hydroxylamine.

Detailed interference studies were carried out. The developed method was applied for the determination of hydroxylamine and its derivatives in drug formulations such as benzohydroxamic acid, α-benzoin oxime, dimethylglyoxime, hydroxy urea (oxyrea) and pyridine 2-aldoxime methyl chloride (praldoxime iodide, PAM injection) after acid hydrolysis. Added hydroxylamine showed recovery greater than 98 %.The results obtained were comparable with those obtained with Verma and Gupta's method [2].

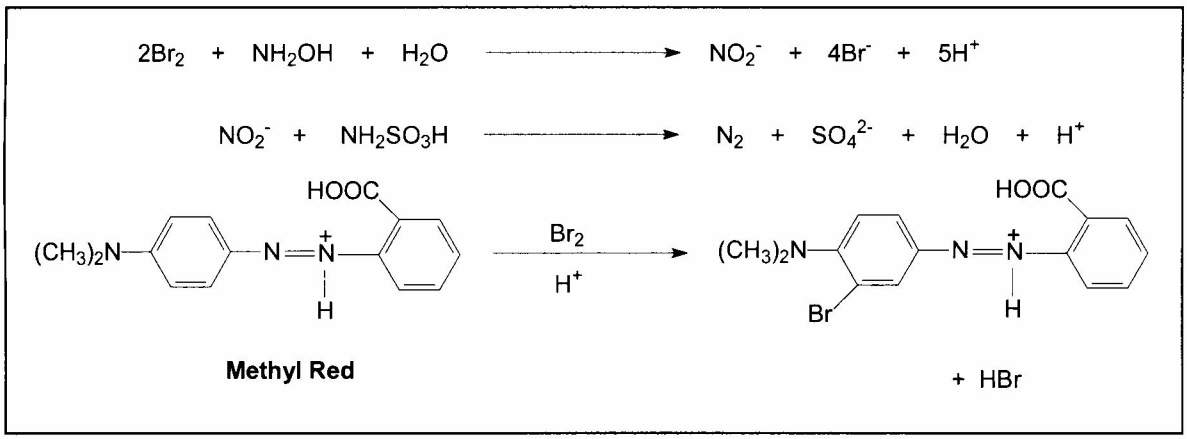


Scheme 1: Determination of hydroxylamine after converting it to nitrite followed by diazo coupling reaction

2. Spectrophotometric determination of hydroxylamine, based on the bleaching of methyl red in the presence of a fixed concentration of bromine

An attempt was made to develop a more sensitive method for the determination of hydroxylamine, based on the bleaching of methyl red in the presence of a fixed concentration of bromine. Bromine in acidic medium bleaches the methyl red dye. A known concentration of bromine when treated with hydroxylamine is reduced to bromide and the unreacted bromine is determined using methyl red [Scheme 2]. The method obeys Beer's law in the concentration range of 0-5 μg of hydroxylamine in an overall aqueous volume of 25 ml at 520nm. The relative standard deviation is 2.7% (n=10) at 3 μg of hydroxylamine. The molar absorptivity is 9.8×10^4 $Lmol^{-1}cm^{-1}$.

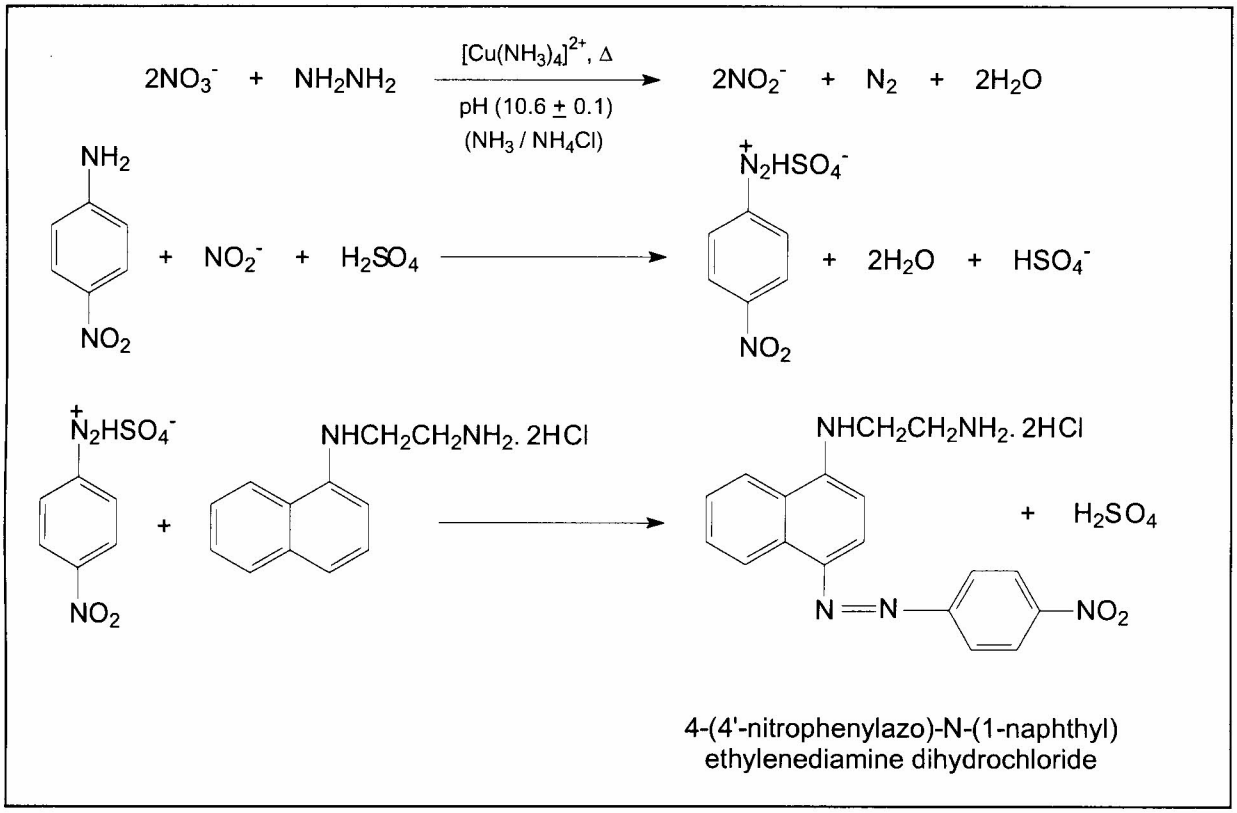
Detailed interference studies were carried out on the determination of hydroxylamine. The developed method was applied for the determination of hydroxylamine and its derivatives in drug formulations such as benzohydroxamic acid, α-benzoin oxime, dimethylglyoxime, hydroxy urea (oxyrea) and pyridine 2-aldoxime methyl chloride (praldoxime iodide, PAM injection) after acid hydrolysis. Added hydroxylamine showed recovery greater than 98 %. The results obtained were comparable with those obtained with Verma and Gupta's method [2].



Scheme 2: Determination of hydroxylamine based on the bleaching of methyl red.

3. Spectrophotometric determination of hydrazine based on the reduction of nitrate to nitrite followed by diazo coupling reaction.

An attempt was made to develop a sensitive method for the determination of hydrazine, based on the reduction of nitrate to nitrite followed by diazo coupling reaction. Hydrazine in ammoniacal medium is used as reducing agent with copper(II) as catalyst. The formed nitrite is determined based on the diazo coupling reaction between p-nitroaniline(PNA) and N-(1-naphthyl) ethylenediamine dihydrochloride (NEDA). [Scheme 3]. The method obeys Beer's law in the concentration range of 0-15 μg of hydrazine in a sample volume of 10 mL at 545 nm and the colour is stable for 3 h. The molar absorptivity is 3.83 x 10^4 L mol^{-1} cm^{-1} with a relative standard deviation of 1.8 % (n=10) at 12 μg of hydrazine.

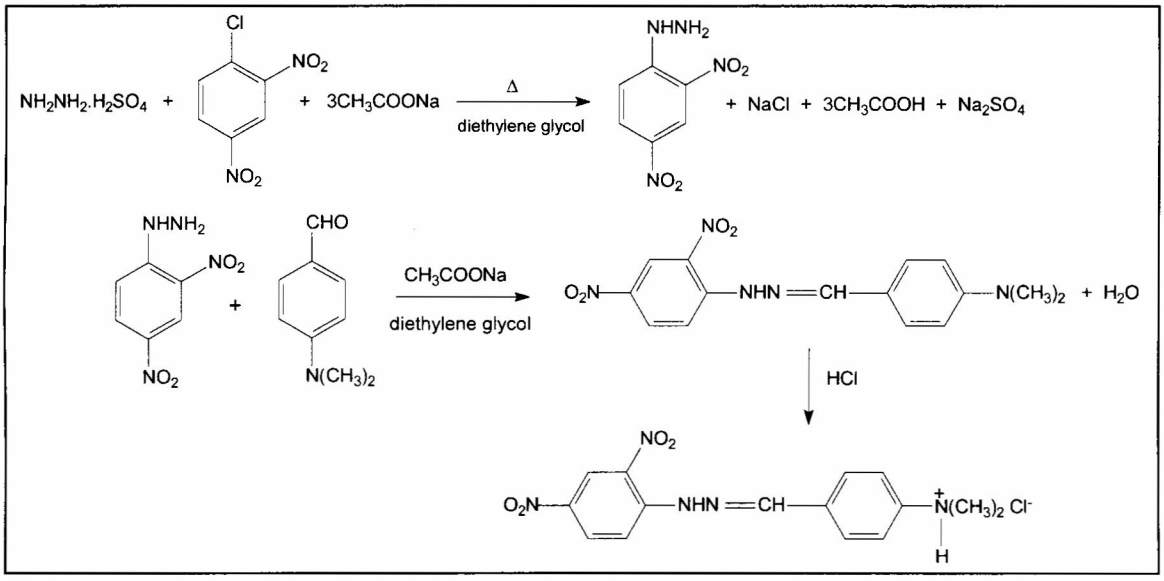


Scheme 3: Determination of hydrazine based on the reduction of nitrate to nitrite followed by diazo coupling reaction

Detailed interference studies were carried out on the determination of hydrazine. The method was applied for the determination of residual hydrazine in feed waters for high pressure steam generating boilers. Added hydrazine showed recovery greater than 98 %. The results were compared with p-dimethylaminobenzaldehyde (p-DAB) method used by Deepa *et al* [7,23] .

4. Spectrophotometric determination of hydrazine after converting to 2,4-dinitro phenylhydrazine with 2,4-dinitrochlorobenzene.

2,4-dinitrophenylhydrazine serves as an excellent reagent for the characterization of carbonyl compounds. The formed hydrazone is colored, often more crystalline and less prone to oxidation and cyclisation[24] Hence an attempt was made to develop a more sensitive method for the determination of hydrazine after converting to 2,4-dinitro phenylhydrazine(2,4-DNP) with 2,4-dinitrochlorobenzene, in the presence of sodium acetate and diethylene glycol [24] by heating on a hot plate. The formed 2,4-DNP undergoes condensation reaction to form the hydrazone with p-dimethylaminobenzaldehyde (p-DAB) [Scheme 4].



Scheme 4: Determination of hydrazine after converting to 2, 4-dinitro phenylhydrazine with 2, 4-dinitrochlorobenzene

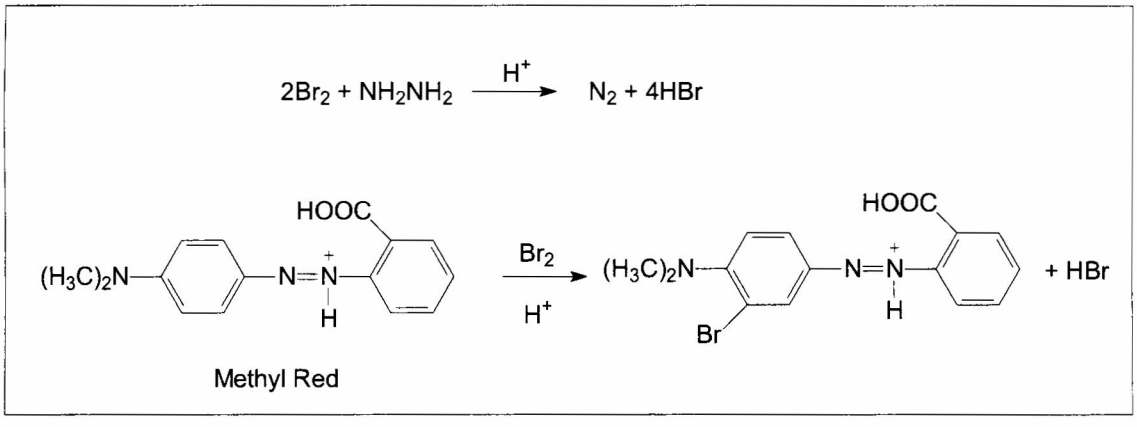
The resulting yellow colored product is stable in acidic medium and has a maximum absorption at 458 nm. The colour system obeys Beer's law in the concentration range 0-7 μg of hydrazine in an overall volume of 25 mL. The molar

absorptivity is 8.1 x10^4 L mol^{-1} cm^{-1} with a relative standard deviation of 1.7 % (n=10) at 6 μg of hydrazine.

Detailed interference studies were carried out on the determination of hydrazine. The utility of the method was demonstrated for the determination of residual hydrazine in feed waters used in high pressure steam generating boilers. Added hydrazine showed recovery greater than 98 %. The results obtained using the proposed method was validated by comparison with the p-dimethylaminobenzaldehyde (p-DAB) method used by Deepa *et al* [7, 23].

5. Spectrophotometric determination of hydrazine, based on bleaching of methyl red in the presence of a fixed concentration of bromine

The sensitive method developed for hyoxylamine was extended to the determination of hydrazine. The method involves the oxidation of hydrazine to nitrogen with a fixed concentration of bromine. Bromine in acidic medium bleaches methyl red dye. A fixed concentration of bromine when treated with hydrazine is reduced to bromide and the unreacted bromine is determined using methyl red [Scheme 5]. The method obeys Beer's law in the concentration range 0-6 μg of hydrazine in an overall aqueous volume of 25ml at 520 nm. The molar absorptivity is 9.95 x10^4 $Lmol^{-1}cm^{-1}$ with a relative standard deviation of 2.7% (n=10) at 3 μg of hydrazine.



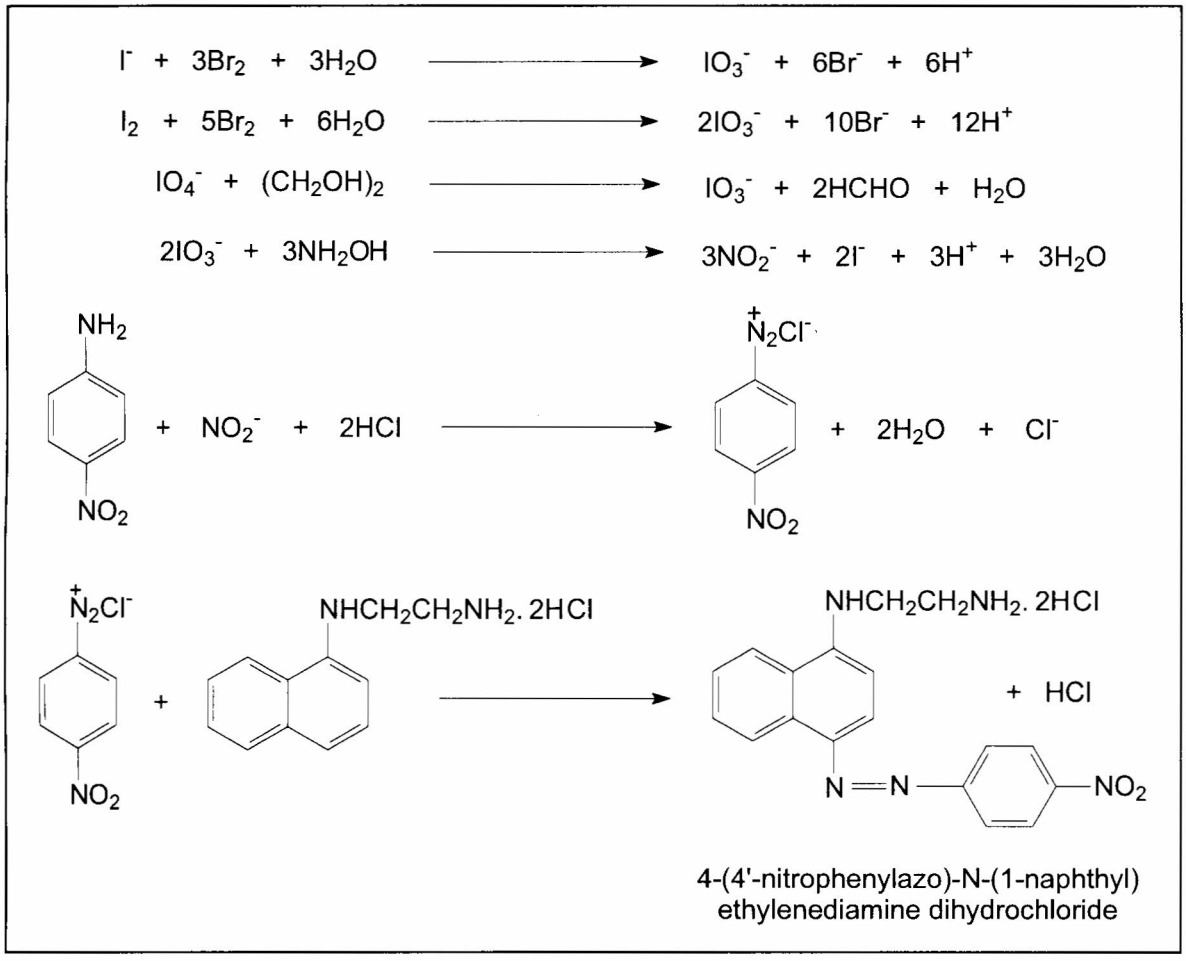
Scheme 5: Determination of hydrazine based on bleaching of methyl red.

Detailed interference studies were carried out on the determination of hydrazine. The method was applied for the determination of residual hydrazine in feed waters used in high-pressure steam generating boilers. The results were compared with p-dimethylaminobenzaldehyde (p-DAB) method used by Deepa *et al* [7,23] and by recovery studies of added hydrazine. Spike addition of hydrazine showed recovery greater than 98 %.

6. Spectrophotometric determination of iodine species after converting to iodate, followed by nitrite determination generated in the presence of hydroxylamine.

The varied applications of iodine and iodine species like iodate and periodate and the toxicological effects of iodine have made the determination of iodine species at very low concentration very important. An attempt was made to develop a simple sensitive spectrophotometric method for the determination of iodine species like iodide, iodate and periodate. Iodide,iodine and periodate are converted to iodate prior to its determination. The method involves the oxidation of hydroxylamine to nitrite with iodate under acidic condition. The formed nitrite is determined based on the diazo coupling reaction between p-nitroaniline(PNA) and N-(1-naphthyl)ethylenediamine dihydrochloride [NEDA] [Scheme 6]. The method obeys Beer's law in the concentration range 0-15μg of iodate in an overall aqueous volume of 10 mL at 545 nm and the color is stable for 3h. The molar absorptivity is 8.33 x 10^4 L $mol^{-1}cm^{-1}$ with a relative standard deviation of 1.7 % (n=10) at 12 μg of iodate.

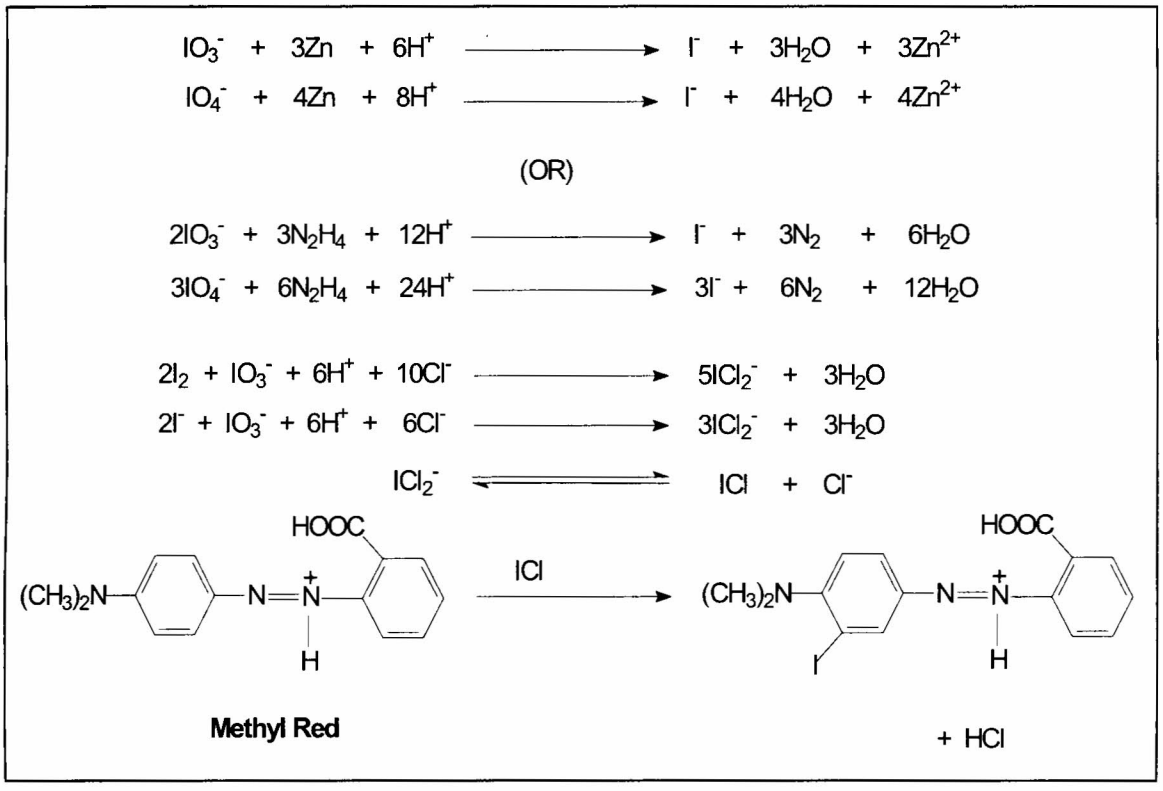
Detailed interference studies were carried out on the determination of iodate by the developed method. The developed method can be applied directly to samples containing iodate, iodide and iodine after oxidation to iodate with bromine solution and periodate after prereduction to iodate with ethylene glycol under acidic condition. It was successfully applied to the determination of iodide and iodate in salt samples, iodine in pharmaceutical preparations (wokadine and betadine ointment, betadine solution and collosol oral solution) and periodate in sea water samples. The results obtained by the developed method compare well with p-phenylenediamine (ppda) method [25] based on the oxidation of the diamine with iodate. The results obtained in the case of table salt and pharmaceutical preparations are well within the manufacturers claim.



Scheme 6: Determination of iodine species after converting to iodate, followed by nitrite determination generated in the presence of hydroxylamine.

7. Spectrophotometric determination of iodine species after converting to ICl_2^- followed by the bleaching of methyl red.

An attempt was made to develop a more sensitive method for the determination of iodine species. The method involves the oxidation of iodide to ICl_2^- in the presence of iodate and chloride in acidic medium. The formed ICl_2^- bleaches methyl red dye [Scheme 7]. The decrease in the intensity of the colour of the dye is measured at 520nm. Beer's law is obeyed in the concentration range 0-3.5 μg of iodide in an overall volume of 10 mL. The molar absorptivity of the colour system is 1.73×10^5 L $mol^{-1}cm^{-1}$ with a relative standard deviation of 3.6 % (n=10) at 2 μg of iodide.



Scheme 7: Reaction scheme for determination of iodine species after converting to ICl_2^- followed by the bleaching of methyl red.

Detailed interference studies were carried out on the determination of iodide by the developed method. The developed method can be applied directly to samples containing iodide and iodine and after prereduction of iodate and periodate to iodide using Zn/H^+ or NH_2NH_2/H^+. The proposed method has been successfully applied to the determination of iodide and iodate in salt samples and iodine in pharmaceutical preparations (wokadine and betadine ointment, betadine solution and collosol oral solution). The results obtained by the developed method compare well with *p*-phenylenediamine (ppda) method [25]. The results obtained in the case of table salt and pharmaceutical preparations are well within the manufacturers claim

CONCLUSIONS

The wide application of hydroxylamine ,hydrazine ,iodine and iodine species like iodate and periodate and their toxicological effects have emphasized the need to develop reliable ,rapid and sensitive methods for their determination at trace levels. The present investigations have demonstrated the feasibility of such an approach for their determination. The developed methods are precise and reproducible in terms of sensitivity and working range as is evident from Table 1.

Table 1. Comparison of Spectrophotometric Methods

S.No	Analyte	Method	Working Range (μg)	Sample Volume (mL)	Final Volume (mL)	Molar absorptivity $Lmol^{-1}cm^{-1}$
1.	Hydroxylamine	Br_2 - PNA –NEDA*	0 - 10	10	25	5.5×10^4
		Br_2 - Methyl Red*	0 - 5	10	25	9.8×10^4
		I_2 - PNA -NEDA**	0- 8	10	25	4.0×10^4
2.	Hydrazine	NO_2^- - PNA – NEDA*	0 – 15	10	25	3.83×10^4
		2,4-DNP - p-DAB*	0 - 7	10	25	8.1×10^4
		Br_2 - Methyl Red*	0 - 6	10	25	9.95×10^4
		p-dimethylaminobenzaldehyde** (p-DAB)	0 - 10	10	25	3.6×10^4
3.	Iodine species	IO_3^- - PNA –NEDA*	0 - 15	5	10	8.33×10^4
		I^- - ICl_2^- - Methyl Red*	0 – 3.5	3	10	1.73×10^5
		IO_3^- - p-phenylenediamine** (ppda)	20 - 400	16	25	5.14×10^3

* Method developed in the present investigation

** Methods used for comparison

The developed methods compare well with the existing methods and can serve as an alternate. In addition the developed methods can tolerate higher concentration of interfering species. In some cases, the interference was overcome or the tolerance limit was increased by suitable chemical modification. The utility of the developed methods is demonstrated by application to practical samples.

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