Spectrophotometric Determination of Hydroxylamine and Its Derivatives in Pharmaceuticals

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A sensitive spectrophotometric method for the determination of hydroxylamine is described. The method is based on the oxidation of hydroxylamine to nitrite using sodium arsenate under alkaline condition. The formed nitrite is determined based on the diazo coupling reaction between *p*-nitroaniline and *N*-(1-naphthyl)ethylenediamine dihydrochloride [NEDA]. The system obeys Beer's law over the concentration range 0—7 μg of hydroxylamine at 545 nm and the colour is stable for 3 h. The molar absorptivity of the colour system is found to be 6.7×10^4 l mol^{-1} cm^{-1}. The relative standard deviation is 1.2% for ten determinations at 4 μg of hydroxylamine. Interferences due to various foreign ions have been studied and the method has been applied to the determination of hydroxylamine and its derivatives used in pharmaceutical formulations after hydrolysis.

Key words hydroxylamine; spectrophotometry; diazo coupling; *N*-(1-naphthyl)ethylenediamine dihydrochloride

Hydroxylamine and its derivatives lead to the formation of methemoglobin in man and animals.[1)] Hydroxylamine has been identified as an intermediate in the nitrogen cycle[2)] and in nitrogen fixation.[3)] Heterotropic nitrification[4)] is involved in the production of hydroxylamine. Hydroxylamine has also been detected in bacterial media and in tissues of a number of organisms.[5)] It induces point mutation by reaction with cytosine, but in the presence of trace metal ions and oxygen it also produces radicals which rapidly inactivate DNA.[6)] Hydroxyl ammonium salts are also used in many branches of chemical industries like paints, pharmaceuticals, plastics, textiles, nuclear industries and photography.[7)] Hence the determination of hydroxylamine in low concentration levels is very important both in industrial and biological samples.

The known method for the spectrophotometric determination of low concentration of hydroxylamine is the Blom method,[8)] which is based on oxidation of hydroxylamine to nitrite by iodine. Excess iodine is removed by dropwise addition of thiosulphate followed by diazocoupling reaction between sulphanilic acid and α-napthylamine. Methods based on the modification of Blom's method have also been reported.[9,10)] The most important modification of this method by Pratima and Gupta[11)] involves the use of *N*-(1-naphthyl)ethylenediamine dihydrochloride [NEDA] as coupling agent instead of α-napthylamine. A method by Breg and Beker[12)] based on direct reaction of hydroxylamine with oxine in alkaline medium to form an intensely green coloured compound indo-oxine is also reported.

The use of iodine as oxidizing agent for hydroxylamine to nitrite has an inherent disadvantage: the excess iodine on reduction with thiosulphate leads to the formation of iodide. The formed nitrite can react with iodide to regenerate iodine, leading to the loss of nitrite and resulting in lowering the sensitivity of the method.

The present paper deals with the oxidation of hydroxylamine to nitrite by sodium arsenate under alkaline condition. The formed nitrite is determined based on diazo coupling reaction between *p*-nitroaniline and NEDA in acid medium. There is no loss of nitrite due to the interaction of formed arsenite. Because of this there is an enhancement in sensitivity in the determination of hydroxylamine ($\varepsilon=6.7\times10^4$ l mol^{-1} cm^{-1}) compared to the method based on iodine oxidation ($\varepsilon=4.1\times10^4$ l mol^{-1} cm^{-1}).

Experimental

Apparatus All absorbance measurements were made using an Elico SL177 scanning spectrophotometer with 1 cm glass cells.

Reagents All chemicals used were of analytical reagent grade and distilled water was used in their preparation.

Standard hydroxylamine (1000 ppm) was prepared by diluting 0.1242 g of hydroxyl ammonium sulphate in 100 ml of water. A suitable aliquot of this solution was diluted to give 1 ppm of hydroxylamine.

Sodium arsenate (Note: should be handled with care) (0.5 M) was prepared by dissolving 15.6 g in water and diluting it to 100 ml. Sodium hydroxide solution (4%) was prepared by dissolving 4 g in water and diluting it to 100 ml. *p*-Nitroaniline (0.05%) was prepared by dissolving 0.05 g of the reagent in 100 ml of 1 : 1 hydrochloric acid. *N*-(1-naphthyl)ethylenediamine dihydrochloride (0.1%) was prepared by dissolving 0.1 g in water and diluting it to 100 ml.

Procedures Preparation of Calibration Graph: Place 0—7 ml of standard hydroxylamine solution containing not more than 7 μg in a 25 ml volumetric flask containing 2 ml of 4% NaOH and 1 ml of 0.5 M sodium arsenate solution. Mix well and allow to stand for 3—5 min. Add 1 ml of 0.05% *p*-nitroaniline and 1 ml of 0.1% NEDA and dilute to 25 ml. Measure the absorbance at 545 nm against the reagent blank.

Determination of Hydroxylamine Derivatives (Oximes, Hydroxamic Acids): Take 10 ml of sample containing not more than 100 μg of the sample and add 1 ml of 7 N HCl and heat it in a boiling water bath for 90 min to hydrolyze it to hydroxylamine.[13)] Neutralise the resulting solution with 1 ml of 5 N NaOH and make up to 25 ml in a 25 ml volumetric flask. Take 3 ml aliquots of the made up solution and determine hydroxylamine content as described using a calibration graph.

Determination of Hydroxylamine in Pralidoxime Iodide (PAM) Injection [Pyridine-2-aldoxime-methyl Iodide]: Dilute a volume equivalent to 500 mg of pralidoxime iodide with 100 ml of water in a standard flask. Further dilute the solution to get a 10 ppm solution. Take a sample volume of 10 ml containing not more than 100 μg of the pralidoxime, which is equivalent to 12.50 μg of hydroxylamine, and hydrolyse it as described under determination of hydroxylamine derivatives.

Determination of Hydroxylamine in Oxyrea Capsules (Hydroxy Urea): To the mixed contents of twenty capsules, weigh the amount equivalent to 500 mg of hydroxy urea and dissolve in 50 ml of distilled water, shake for 15 min and filter, then make the filtrate up to 100 ml in a standard flask. Further dilute the solution to obtain 10 ppm of hydroxy urea. Take a sample volume of 10 ml containing not more than 100 μg of the hydroxy urea which

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is equivalent to 43.38 μg of hydroxylamine after hydrolysis. Hydrolyse the sample following the procedure described under determination of hydroxylamine derivatives.

Results and Discussion

The developed method is based on the oxidation of hydroxylamine to nitrite and subsequent determination of the formed nitrite by diazo coupling reaction. The selection of oxidising agent is very critical in this study. A strong oxidising agent oxidises hydroxylamine to nitrate instead of nitrite. In some cases nitrite is produced as an intermediate and the use of excess oxidising agent converts the nitrite to nitrate. Hence the destruction of excess oxidising agent is essential prior to the diazo coupling reaction.

Iodine used as a weak oxidising agent, is destroyed by thiosulphate thus generating a stronger reducing agent iodide. The formed iodide is partially oxidised to iodine by the nitrite resulting in the loss of nitrite and leading to lower sensitivity of the method. In the present study sodium arsenate was used instead of iodine for the quantitative oxidation of hydroxylamine to nitrite. No interference was observed due to the formation of arsenite as a result of the reduction of arsenate by hydroxylamine. In fact, sodium hydroxide (0.1 N) containing sodium arsenite (0.1%) is used as a trapping solution[14] for the determination of nitrogen dioxide in air by diazo coupling reaction.

Several mediums were investigated for the oxidation of hydroxylamine by sodium arsenate. The formation of nitrite was not quantitative in the presence of acid medium, sodium acetate, sodium bicarbonate or sodium carbonate. However, quantitative conversion was achieved in the presence of sodium hydroxide. The formed nitrite was determined based on diazo coupling reaction between *p*-nitroaniline and NEDA in acid medium.

All parameters influencing the colour development were investigated and the optimum values obtained were incorporated in the recommended procedure. Under these conditions the system obeys Beer's law over the concentration range 0—7 μg of hydroxylamine with a correlation coefficient of 0.9999. The molar absorptivity of the colour system was 6.7×10^4 l mol^{-1} cm^{-1}. The relative standard deviation is 1.2% for ten determinations at 4 μg of hydroxylamine.

Nature of Species Responsible for Colour Hydroxylamine on oxidation with arsenate under alkaline condition forms nitrite. Nitrite on treatment with *p*-nitroaniline undergoes diazotisation in acidic medium to give diazonium chloride, which couples with NEDA to form the azo dye 4-(4-nitrophenylazo)-*N*-(1-napthyl)ethylenediamine dihydrochloride, which has an absorption maximum at 545 nm. The developed colour is stable for 3 h after which a slow decrease in absorbance was observed.

Effect of Interfering Species The tolerance limits of

Table 1. Determination of Hydroxylamine in Derivatives of Hydroxylamine (Oximes and Hydroxamic Acids)

Derivatives of NH_2OH	Weight of the sample (μg)	Amount of NH_2OH added (μg)	Amount of NH_2OH formed after hydrolysed (μg)[a]		Amount as derivatives (μg)[a]		Recovery of added NH_2OH (%)[a]
			Developed method	Gupta's method	Developed method	Gupta's method	
Benzhydroxamic acid	100	—	24.00	23.60	99.74	98.07	—
		20	43.76	—	—	—	98.8
α-Benzoin oxime	100	—	14.50	14.20	99.86	97.79	—
		10	24.42	—	—	—	99.2
Dimethyl-glyoxime	100	—	55.75	55.00	98.08	96.76	—
		25	80.50	—	—	—	99.00

a) Average of three determinations.

Table 2. Determination of Hydroxylamine in Pharmaceutical Formulations

Drugs	Label claim (mg)	Weight of the drug taken (μg)	Amount of hydroxylamine added (μg)	Amount of hydroxylamine formed after hydrolysis (μg)[a]		Amount as drug (μg)		Recovery of added hydroxylamine (%)[a]
				Developed method	Gupta's method	Developed method	Gupta's method	
PAM injection SPM Pharma Bhavani, Tamil Nadu, India	500/20 ml	100	—	12.46	12.38	99.66	99.04	—
			10	22.43	—	—	—	99.70
Oxyrea Capsules Cadila Pharma Ahmedabad, India	500	100	—	42.84	42.43	98.75	97.83	—
			20	62.57	—	—	—	98.63

a) Average of three determinations.

interfering species were established as those concentrations, which did not cause more than ±1.5% error in the recovery of hydroxylamine at 5 μg level. The interferences of several anions and cations were evaluated to check the suitability of the method for the determination of hydroxylamine and its derivatives. Various concentrations of these interfering species were introduced into 10 ml of water containing 5 μg of hydroxylamine and the recovery of hydroxylamine was established. Anions like oxalate, citrate, tartrate, phosphate, borate, nitrate and bromide up to the 1000 μg level did not interfere nor did cations like Ba^{2+}, Pb^{2+} and Mg^{2+} up to this level. However, Cu^{2+}, Fe^{3+} and Hg^{2+} were tolerated only up to 25 μg levels. Ethanol and aniline were tolerated up to 1000 μg and acetone up to 100 μg level. Glucose and hydrazine interfered at the 1000 μg level by decreasing the absorbance. This was overcome by increasing the addition of 0.5 M sodium arsenate to 2 ml from 1 ml.

Applications Derivatives of Hydroxylamine: The proposed method was applied for the determination of hydroxylamine derivatives like benzhydroxamic acid, α-benzoin oxime and DMG. The present analytical method was evaluated by Gupta's method[11] and by the recovery studies of added hydroxylamine. The results are given in Table 1.

Hydroxylamine in Pharmaceutical Formulations: The proposed method was used for the determination of hydroxylamine derivatives in anti-cholinesterase and anti-tumor agents.[15] The suitability of the method was checked by Gupta's method[11] and by the recovery studies of added hydroxylamine. The results are presented in Table 2.

Conclusions

The proposed method for the determination of hydroxylamine is simple, sensitive, rapid and precise. The calibration graph is linear over the concentration range 0—7 μg of hydroxylamine. The correlation coefficient is 0.9999. Application of the proposed technique for the determination of hydroxylamine and its derivatives in pharmaceutical formulations has demonstrated the utility of the method.

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