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Spectrophotometric determination of hydroxylamine and its derivatives in drug formulation using methyl red

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Hydroxylamine has been determined by its oxidation to nitrite with a known excess of bromine. Bromine in acidic medium bleaches the dye methyl red. A known excess of bromine when treated with hydroxylamine is reduced to bromide and the unreacted bromine is determined using methyl red. The method obeys Beer's law in the range 0-5 μg of hydroxylamine in an overall aqueous volume of 25 mL. The relative standard deviation is 2.7% (n=10) at 3 μg of hydroxylamine. The molar absorptivity is calculated to be 9.8×10^4 $Lmol^{-1}cm^{-1}$ with the correlation coefficient of 0.995. The developed method bas been applied for the determination of hydroxylamine and its derivatives after hydrolysis in drug formulations. The results obtained by the present method compare well with those obtained by the Verma-Gupta's method and with the standard addition of hydroxylamine and recovery studies.

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Hydroxylamine and its derivatives such as hydroxamic acid and oxime are widely used as anti cholinesterase and antitumor agents[1]. They are known to cause methemoglobinemia in man and animals[2]. Hydroxyl ammonium salts are also used in many branches of chemical industries like paints, pharmaceuticals, plastics, textiles, nuclear industries and photography[3]. Hence the determination of hydroxylamine is important both in industrial and biological samples.

Several methods have been suggested for the determination of hydroxylamine based on its redox reaction[4-8]. The well-known spectrophotometric method is based on the oxidation of hydroxylamine to nitrite by iodine, removal of excess iodine by thiosulphate followed by diazotization-coupling reaction involving sulphanilic acid and 1-naphthylamine to form an azo dye. Alternative methods for removal of excess iodine are the use of arsenite[6] in place of thiosulphate or by solvent extraction[7]. One more modification involves the use of (1-naphthyl) ethylenediamine dihydrochloride as a coupling agent instead of 1-naphthylamine[8]. When iodine is used as oxidant, there is a possibility of secondary reaction between iodide and nitrite leading to the loss of nitrite resulting in lowering the sensitivity of the method. Other methods include the reaction of hydroxylamine with *p*-nitrobenzaldehyde to form an yellow colored *p*-nitrobenzaldoxime[9], or with picryl chloride to form red color[10], conversion of hydroxylamine to ammonia using zinc reductor column and determination of ammonia by indophenol blue method[11], and the oxidation of hydroxylamine to nitrite by sodium arsenate under alkaline condition[12] followed by diazo coupling reaction in acid medium.

Bromine and its higher oxidation state compounds are widely used in analytical determinations[13]. The present paper deals with the oxidation of hydroxylamine to nitrite by using a known excess of bromine under acidic condition. The unreacted bromine is determined based on its ability to bleach the dye methyl red. Response of the proposed method in the presence of potential interferents has been evaluated.

Experimental Procedure

Apparatus

All absorbance measurements were made using Elico SL 177 Scanning Spectrophotometer with 1 cm glass cells.

Reagents

All chemicals used were analytical grade reagents and distilled water was used for the dilution of

reagents and the preparation of samples. A 0.2106 g of hydroxyl ammonium hydrochloride was dissolved in 1 L of water to give a stock solution equivalent to 100 mg/L of hydroxylamine. Bromate-bromide mixture for bromine generation was prepared by dissolving 0.05 g of potassium bromate and 0.5 g of potassium bromide and diluting to 500 mL with water. To generate 28 μgmL^{-1} of bromine stock solution 20 mL of bromate-bromide mixture was diluted to 100 mL. Fifty mL of this stock solution was transferred into a 100 mL calibrated flask containing 40 mL of 4.25 M sulphuric acid and diluted to 100 mL with water. This bromine solution (14 μgmL^{-1}) was prepared on the day of use. Solution of methyl red (0.01%) was prepared by dissolving 0.1 g of methyl red in 1 mL of 4.5 M sodium hydroxide and diluting to 100 mL with water to get 0.1% solution. Ten mL of this solution was diluted to 100 mL, after acidifying it by adding 1 mL of 4.25 M sulphuric acid. Sulphuric acid (4.25 M) was prepared by adding 59 mL of concentrated sulphuric acid (Sp. Gravity 1.84) to 150 mL of water, cooled and then diluting to 250 mL in a calibrated flask. Sulphamic acid solutions (0.1 and 0.5%) were prepared in the usual manner.

Method

To 10 mL aliquot of sample solution containing 0-5 μg of hydroxylamine, 1 mL of 0.1% sulphamic acid and 5 mL of 14 μgmL^{-1} bromine solution were added. The solutions were mixed well followed by the addition of 1 mL of 0.01% of methyl red before diluting to 25 mL with distilled water. The absorbance of the solution was measured at 520 nm against reagent blank using 1 cm glass cells.

Determination of hydroxylamine derivatives

Aliquot of sample (10 mL) containing not more than 100 μg of hydroxylamine derivative was mixed with 1 mL of 7 M hydrochloric acid taken in a beaker and digested in a boiling water bath for 90 min. The sample volume was maintained around 10 mL by the addition of water. After digestion the solution was cooled to room temperature, the volume was made upto the mark in a 25 mL calibrated flask. Hydroxylamine was determined by taking 10 mL of sample volume containing not more than 5 μg by the proposed method.

Results and Discussion

Quantitative bleaching of azo dyes by halogens like chlorine and bromine are widely used for their spectrophotometric determinations[14]. In the present investigation reaction between bromine and methyl red (4-dimethylaminoazobenzene-2-carboxylic acid) is made use for the spectrophotometric determination of hydroxylamine. Methyl red, an azodye, shows two absorption bands at 520 nm and 315 nm. The intensity of the band at 315 nm is very weak as compared with the band at 520 nm. In the absence of hydroxylamine, a known excess of bromine bleaches the dye and the absorbance is very low at 520 nm. In the presence of hydroxylamine, bromine is reduced to bromide and the unreacted bromine decolorizes the dye. Thus with the increasing concentration of hydroxylamine, more of bromine is reduced and this is observed by a linear increase in the absorbance due to the unbleached methyl red at 520 nm and 315 nm under acidic condition (Fig. 1). It was observed that with increasing bromine concentration, absorption decreased for the band at 520 nm whereas it increased for the band at 315 nm. The reaction of bromine with methyl red involves competition between aromatic ring substitution and azo link cleavage. Like methyl red, methyl orange is also bleached by bromine. It is established that the bleaching of methyl orange by bromine occurs greater than 95% by aromatic ring substitution[14]. Based on this the bleaching of methyl red can be explained as a result of aromatic ring substitution of bromine. The ring substitution of bromine in the dye molecule cause steric inhibition of resonance[14] resulting in the decrease of absorbance at 520 nm (Scheme 1).

The effect of variation in acidity for an effective reaction between bromine and methyl red was established and this was found to be 0.3-1.0 M with respect to sulphuric acid. Thus for all subsequent

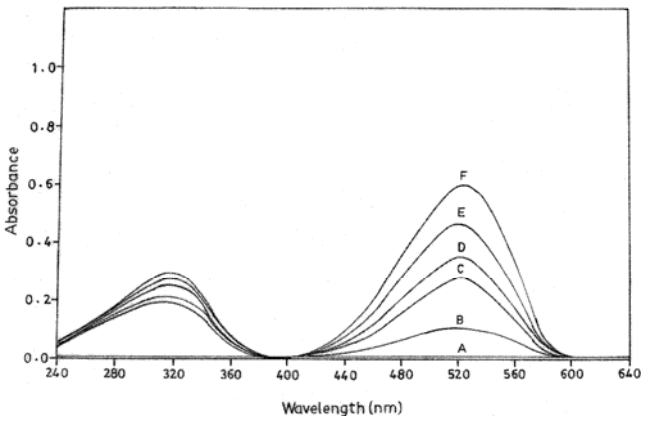
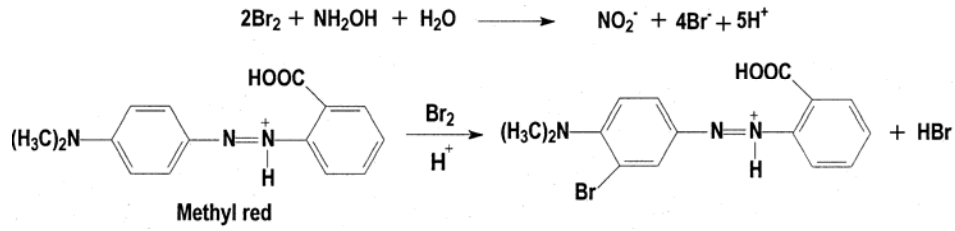


Fig. 1 — Absorption spectra

A — Reagent Blank; B — 1 μg NH_2OH; C — 2 μg NH_2OH; D — 3 μg NH_2OH; E — 4 μg NH_2OH; F — 5 μg NH_2OH (All measurements made against reagent blank).



Scheme 1 — Oxidation reaction of hydroxylamine

studies a reaction acidity of 0.34 M was maintained by taking 5 mL of bromine solution (14 ppm) with an overall acidity of 1.7 M.

Hydroxylamine derivatives such as oxime, hydroxamic acid and hydroxy urea can be hydrolyzed[15] to hydroxylamine by heating with 1 mL of 7 M HCl in a boiling water bath for 90 min. Derivatives of hydroxylamine in pharmaceutical preparations can be determined after hydrolysis by the proposed method.

The proposed method was useful for the determination of hydroxylamine in the concentration range of 0-5 μg in a sample volume of 10 mL. A sample containing hydroxylamine was treated with reagents and finally diluted to an overall aqueous volume of 25 mL. The molar absorptivity of the colored system was found to be 9.8×10^4 $Lmol^{-1}cm^{-1}$ and the dye colour remained stable upto 17 days. The correlation coefficient was 0.995 and the relative standard deviation was 2.7% (n = 10) for 3 μg of hydroxylamine.

Interferences

The interfering effects of common anions and cations, which may coexist with hydroxylamine, were evaluated. Any deviation in the absorbance value of ±0.01 to that obtained in the absence of the interfering ions in hydroxylamine determination was taken as a sign of interference. Varying concentrations of interfering species were added to 3 μg of hydroxylamine and the absorbance of hydroxylamine was compared to that in the absence of interference. Tolerance limits of various ions studied in hydroxylamine determination are summarized in Table 1.

Hydrazine is a potential interferent in most of the methods of hydroxylamine determination. In the proposed method, sodium tellurite solution was added to selectively oxidize hydrazine to nitrogen, and as high as 1000 μg of hydrazine could be tolerated in the determination of 3 μg of hydroxylamine. The sample was heated with 1 mL of 0.2% sodium tellurite. The solution was centrifuged to remove the reduced tellurium before the addition of bromine solution.

Table 1 — Effect of some interfering species in the hydroxylamine determination (NH_2OH = 3 μg)

Species	Amount tolerated (μg)
Phosphate, oxalate, citrate tartrate, borate, ethanol chloride, carbonate	1000
Glucose, acetone	25
TeO_3^{2-}	2000
Hydrazine	0.1
Hydrazine [a]	1000
Ba(II), Pb(II), Mg(II), Co(II), Cd(II), Bi(III), Ni(II), Li(I), Mn(II), Sr(II), Cr(III)	1000
Fe(II)	1.5
Cu(II), Hg(II)	50
NO_2^-	100
NO_2^{-b}	1000
NO_3^-	10
NO_3^{-c}	75

[a]Treated with 1 mL of 0.2% sodium tellurite heated and centrifuged to remove the precipitated tellurium before the addition of bromine solution.

[b]Treated with 1 mL of 0.5% sulphamic acid before treating with bromine solution.

[c]The interference was overcome by nitrating toluene and extracting the nitrotoluene with unreacted toluene prior to the addition of bromine solution.

Nitrite greater than 100 μg and nitrate greater than 10 μg interfere in the determination hydroxylamine. The interference of nitrite upto 1000 μg was overcome by the addition of 1 mL of 0.5% sulphamic acid. The interference of nitrate was overcome by nitrating toluene in the presence of 3:1 sulphuric acid: water and extracting the nitro toluene formed with unreacted toluene. This method has been used for the determination of nitrate in aqueous samples[16]. Ten mL of sample solution containing 30 μg of hydroxylamine and 75 μg of nitrate was treated with 5 mL of toluene and 15 mL of 3:1 sulphuric acid:water

mixture. After 5 min the nitrotoluene formed was extracted with unreacted toluene. The aqueous layer was separated and the volume was made up to 100 mL. 10 mL portion was subjected to analysis as before. By this procedure the interference of nitrate upto 75 μg was eliminated.

Application

Hydroxamic acids, oximes and pharmaceutical preparations like PAM injection (pyridine-2-aldoxime methyl iodide) and oxyurea capsules (hydroxy urea) are first hydrolyzed to hydroxylamine by heating with 7 M HCl for 90 min in a boiling water bath. During boiling operation the sample volume was maintained by periodic addition of distilled water. The generated hydroxylamine was determined by the present method. The results were compared with that obtained by Verma and Gupta's method[8] and by standard addition of hydroxylamine and recovery studies. The results obtained in the determination of derivatives of hydroxylamine and the pharmaceutical formulations are tabulated in Tables 2 and 3, respectively.

Table 2 — Determination of hydroxylamine in derivatives (oximes and hydroxamic acids)

Derivative of hydroxylamine	Amount of the sample taken (μg)	Added NH_2OH (μg)	Amount of NH_2OH found (μg)[a] (Proposed method)	% Recovery of added NH_2OH	Verma & Gupta's method[8]		Amount as derivatives (μg)*	
					NH_2OH present[b] (μg)	NH_2OH present (μg)	Proposed method	Verma & Gupta's method[8]
Benzohydroxamic acid	100	-	2.81	-	1.40	2.80	98.30 ±0.70	98.30 ±0.80
		3	5.80	99.66	-	-		
		-	2.83	-	1.42	2.84		
		3	5.83	100.00	-	-		
		-	2.85	-	1.42	2.84		
		3	5.84	99.66	-	-		
			2.83*			2.83*		
α-Benzoin oxime	100	-	1.75	-	0.89	1.78	100.34 ±0.87	100.34 ±1.32
		3	4.75	100.00	-	-		
		-	1.74	-	0.87	1.74		
		3	4.72	99.33	-	-		
		-	1.77	-	0.87	1.74		
		3	4.74	99.00	-	-		
			1.75*			1.75*		
Dimethyl glyoxime	100	-	2.30	-	1.15	2.30	99.76 ±1.11	99.76 ±1.34
		1	3.29	99.00	-	-		
		-	2.25	-	1.12	2.24		
		1	3.25	100.00	-	-		
		-	2.27	-	1.14	2.28		
		1	3.24	97.00	-	-		
			2.27*			2.27*		

[a]Volume of sample used: Benzohydroxamic acid (3 mL), α -benzoin oxime (3 mL), DMG (1 mL)

[b]Value corresponding to 1:1 diluted solution

*Average of three values

Table 3 — Determination of hydroxylamine in drug formulations

Derivative of hydroxylamine	Label Claim (mg)	Amount of the sample taken (μg)	Added NH_2OH (μg)	Amount of NH_2OH found (μg)[a] (Proposed method)	% Recovery of added NH_2OH	Verma & Gupta's method[8]		Amount as derivatives (μg)*	
						NH_2OH Present (μg)[b]	NH_2OH present (μg)	Proposed method	Verma & Gupta's method[8]
PAM injection[c]	500/20 mL	100	-	2.53	-	1.24	2.48	100.40 ±0.83	98.40 ±2.11
			3	5.50	99.00	-	-		
			-	2.49	-	1.20	2.40		
			3	5.48	99.66	-	-		
			-	2.50	-	1.25	2.50		
			3	5.47	99.00	-	-		
				2.51*			2.46*		
Oxyrea capsules[d]	500	100	-	3.47	-	1.74	3.48	99.76 ±0.97	99.76 ±1.46
			2	5.49	101.00	-	-		
			-	3.41	-	1.72	3.44		
			2	5.40	99.5	-	-		
			-	3.45	-	1.69	3.38		
			2	5.43	99.00	-	-		
				3.44*			3.43*		

[a]Volume of sample used: PAM injection (5 mL), Oxyrea capsules (2 mL)
[b]Value corresponding to 1:1 diluted solution
[c]Pyridine-2-aldoxime methyl iodide, Courtesy from M/S SPM Pharma, Bhavani, Tamil Nadu, India.
[d]Hydroxy urea, Courtesy from M/S Cadila Pharma, Ahmedabad, India
*Average of 3 values

Conclusion

The developed method is more sensitive ($\varepsilon = 9.8\times10^4$ $Lmol^{-1}cm^{-1}$) compared to Verma and Gupta's method[8] ($\varepsilon = 4.1\times10^4$ $Lmol^{-1}cm^{-1}$) and to that of Deepa *et al.*[12] method ($\varepsilon = 6.7\times10^4$ $Lmol^{-1}cm^{-1}$). The developed method is practically free from interferences for the determination of hydroxylamine and its derivatives in pharmaceutical preparations. This method will serve as a good alternative for the existing methods.

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