

Spectrophotometric Determination of Hydrogen Peroxide in Water and Cream Samples

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Abstract A simple, rapid and sensitive spectrophotometric method is described for the determination of hydrogen peroxide using toluidine blue as a reagent. The proposed method is based on the liberation of iodine in acidic medium equivalent to the amount of hydrogen peroxide present. The liberated iodine bleaches the blue color of toluidine blue and is measured at 628 nm. The decrease in absorbance is directly proportional to hydrogen peroxide concentration and obeys Beer's law in the range 0.2×10^{-6} – 14×10^{-6} mol L⁻¹. The molar absorptivity, sandel's sensitivity, detection limit and quantitation limit of the method were found to be 1.82×10^4 L mol⁻¹ cm⁻¹, 2.01×10^{-9} mol L⁻¹, 1.41×10^{-6} mol L⁻¹ and 4.28×10^{-6} mol L⁻¹ respectively. The results of analysis of the proposed method are compared favorably with those from a reference method.

Keywords Hydrogen peroxide · Spectrophotometry · Toluidine blue

Hydrogen peroxide is a chemically liable oxidant which plays key role in variety of important redox processes occurring within the troposphere, including the conversion of a number of highly reactive free radicals and trace metals. The developments of analytical technique for the determination of hydrogen peroxide are significant because of the following reasons: (a) hydrogen peroxide and peroxy acetic acid, in particular are widely used as disinfectants in food, beverages and pharmaceutical industries, (b) hydrogen

peroxide in the troposphere is an important oxidant, producing sulphuric acid in the aqueous phase and are also thought to have some toxic effect on plants, (c) they are used as textile, paper and pulp bleaching agents, (d) exposure to high concentration levels causes damage of the eyes, skin, lungs and respiratory tract.

Hydrogen peroxide is used widely as an oxidant, a disinfectant and a bleaching agent in various industrial and household products (Li 1996). Micro and trace determination of H₂O₂ is considerably important in clinical chemistry, analytical biochemistry and environmental science since H₂O₂ is produced in stoichiometric amounts during the oxidation of biological analytes (e.g. glucose) by dissolved oxygen in the presence of corresponding oxidase (Bowers 1986). It is also used as an intermediate in the synthesis of plasticizers, organic peroxy compounds (e.g., methyl ethyl ketone peroxide, benzoyl peroxide), peroxy carbonates, amine oxides and used as a polymerization promoter, a foaming agent, to age wines and liquors, to disinfect water and hides, to refine oils and fats and for gilding and silvering of metal surfaces (Bethesda 1995).

Sophisticated instrumental techniques, such as tunable diode laser spectrophotometry (Newman 1993) and fiber-array spectrometry have been employed to determine gaseous hydrogen peroxide, where as simpler methods utilize chemiluminescence reactions or the production of a fluorescent species by an enzyme catalyzed reactions. Earlier determination of hydrogen peroxide employed colorimetric technique using titanium salt (Wolfe 1962). The aqueous concentration of hydrogen peroxide is highly overestimated by the Ti (IV) method in the presence of peroxy acetic acid. It is having advantage of simple analytical instrumentation and sampling, which facilitates field measurements. Very sensitive enzymatic methods based on the dimerization with p-hydroxyphenyl acetic acid, catalyzed by horseradish

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Table 1 Analytical parameters of reported reagents

Reagents Used	Detection limit	Linear range
Iron-tetrasulfonatophthalocyanine (with γ -CD) (Yuan et al. 2003)	$7.4 \times 10^{-8} \text{ mol L}^{-1}$	5.0×10^{-6} – $8.0 \times 10^{-5} \text{ mol L}^{-1}$
Metavanadate (Nogueira et al. 2005)	$1.43 \times 10^{-4} \text{ mol L}^{-1}$	–
Hemoglobin as catalyst (Zhang et al. 2000)	$9.2 \times 10^{-9} \text{ mol L}^{-1}$	5.0×10^{-8} – $3.5 \times 10^{-6} \text{ mol L}^{-1}$
Immobilized peroxidase (Pappas et al. 2002)	–	2.0 – $70.0 \times 10^{-8} \text{ mol L}^{-1}$
Cation exchange (Almuaibed and Townshend, 1994)	$1.0 \times 10^{-6} \text{ M}$	4.0×10^{-6} – $6 \times 10^{-5} \text{ M}$
Leuco Patent Blue Violet (Clapp and Evans 1991)	–	1.0×10^{-7} – $10^{-5} \text{ mol dm}^{-3}$

peroxidase, followed by fluorescence detection (Mayer and Karst 1999) are limited due to high cost and low stability of the reagents. Other fluorescence method has included the peroxidase mediated oxidation of scopoletin by hydrogen peroxide in a buffered solution (Willey et al. 1996). The oxidation of acetaminophen (Jie et al. 1995) by hydrogen peroxide. Other approaches have utilized UV-Visible absorption spectrometry, following the reactions of hydrogen peroxide to give intensely coloured products make use of reagents like pyridine-2,6-dicarboxylic acid and vanadate(V) in acidic solution (Tanner and Wong 1998). It is clear that there is still a need to improve the measurement methods, especially accurate and practicable instruments for the nanogram measurements of hydrogen peroxide. Few of the recent reagents used for the determination of hydrogen peroxide and its analytical parameters are listed in Table 1.

In the present study a spectrophotometric method is described making use of the reactions of hydrogen peroxide with potassium iodide in acidic medium, liberating iodine which intern bleaches the blue colour of toluidine blue which is having absorption maximum at 628 nm.

Materials and Methods

Apparatus and Reagents

A Secomam Anthelie NUA 022 UV-Visible spectrophotometer with 1 cm matching quartz cells were used for the absorbance measurement and a WTW pH 330, pH meter was used. All reagents used were of analytical pure grade. Standard stock solution of hydrogen peroxide was prepared by suitable dilution of 30% hydrogen peroxide (FISCHER, India). Working solutions were prepared by the suitable dilution of the stock and was standardized by titrimetric method (Jeffery et al. 1995). Two percentage of potassium iodide solution, 0.01% toluidine blue indicator, 2 M Hydrochloric acid and 2 M sodium acetate solution were used.

Procedure

Aliquots of stock solution containing 0.2×10^{-6} – $14 \times 10^{-6} \text{ mol L}^{-1}$ of hydrogen peroxide was pipetted out in to

a series of 10 mL standard flasks. To each of this was added 1 mL potassium iodide followed by 1 mL of 2 M hydrochloric acid. The mixture was gently shaken until the appearance of yellow color, indicating the liberation of iodine. Then 0.5 mL of 0.01% toluidine blue was added, followed by 2 mL of 2 M sodium acetate solution. The contents were made up to the mark by using distilled water and mixed well. Absorbance was measured at 628 nm against reagent blank.

Determination of Hydrogen Peroxide in Water Samples

Rainwater samples were collected in 25 dm³ polyethylene bottles using 15 cm diameter polyethylene funnels. In order to prevent the escape hydrogen peroxide samples were kept below 4°C until the final analysis.

Known amounts of hydrogen peroxide were taken in 10 mL standard flasks, to each of this added 3 mL of water samples and 1 mL of potassium iodide followed by 1 mL of 2 M hydrochloric acid. The mixture was shaken well until the appearance of yellow color and then added 0.5 mL of 0.01% toluidine blue indicator solution followed by 2 mL of 2 M Sodium acetate solution. The contents were made up to the mark and mixed well. Absorbance was measured at 628 nm against reagent blank.

Determination of Hydrogen Peroxide in Bleach Cream

Dissolved 0.1 g of bleach cream in 20 mL of ethyl alcohol and clear solution was made up to 100 mL. Known amount of made up solution was taken and analyzed for hydrogen peroxide content following the procedure described for the analysis of water sample.

Results and Discussion

Absorption Spectrum

Hydrogen peroxide reacts with potassium iodide in acid medium liberating iodine. The liberated iodine bleaches the blue color of toluidine blue in the presence of sodium acetate to a colourless species and is measured at 628 nm (Fig. 1).

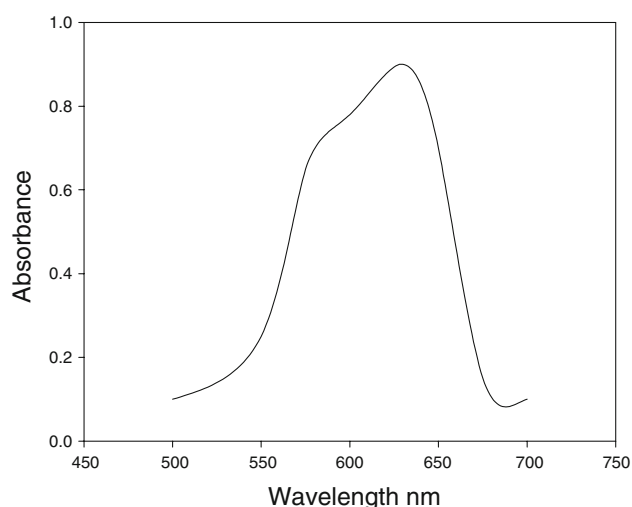
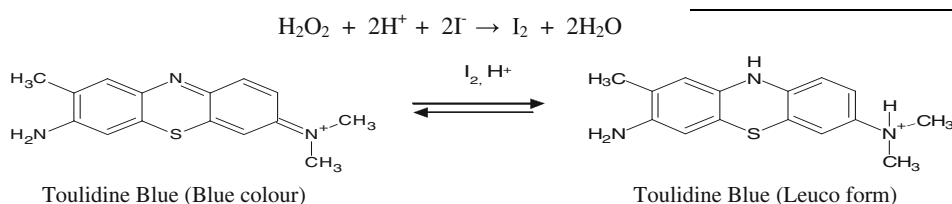


Fig. 1 Absorption spectrum of toluidine blue

Effect of Iodide Concentration and Acidity

The liberation of iodine by hydrogen peroxide is effective in the pH range 2.0–3.0, which could be maintained by adding 1 mL of 2 M hydrochloric acid in a final volume of 10 mL. The liberation of iodine from KI in any acid medium is quantitative. The appearance of yellow colour indicates the liberation of iodine. Any excess of iodide in the solution will not interfere. However, the concentration of the acid should be maintained in the 1.5 to 2.5 M range. It was found that 1 mL each of potassium iodide (2% solution) and 2 M hydrochloric acid was sufficient for the liberation of iodine.

Reaction scheme:



Effect of pH

When toluidine blue indicator is used as the coloring agent, constant and maximum absorbance values were obtained in the pH range 2.0–3.0. This could be achieved by the addition of 1 mL of 2 M hydrochloric acid in a total volume of 10 mL.

Effect of Diverse Ions

The effect of diverse ions in the determination of $10 \times 10^{-6} \text{ mol L}^{-1}$ of hydrogen peroxide is examined. An error of $\pm 2\%$ in the reading of absorbance is considered tolerable. The tolerance limits of various ions are summarized in Table 2. Various oxidants such as Fe^{3+} , Ce^{4+} and Cu^{2+} were found to interfere and these ions could be masked by the addition of an appropriate amount of EDTA solution. Fe^{3+} ion is masked by sodium fluoride.

Analytical Data

The adherence to Beer's law is studied by measuring the absorbance values of solutions by varying hydrogen peroxide concentration. A straight line graph is obtained by plotting absorbance against concentration of hydrogen peroxide. Beer's law is obeyed in the range of 0.2×10^{-6} – $14 \times 10^{-6} \text{ mol L}^{-1}$. Adherence to Beer's law graph for the determination hydrogen peroxide presented in the Fig. 2. The decrease in absorbance is directly proportional to the concentration of hydrogen peroxide. The molar absorptivity, sandel's sensitivity, detection limit and quantitation limit of the method were found to be $1.82 \times 10^4 \text{ L}$

Table 2 Effect of diverse ions (Hydrogen peroxide $10 \times 10^{-6} \text{ mol L}^{-1}$)

Diverse ion	Tolerance limit (mol L^{-1})	Diverse ion	Tolerance limit (mol L^{-1})	Diverse ion	Tolerance limit (mol L^{-1})
Pb(II)	50	Nitrate	200	Zn(II)	500
U(VI)	25	Phosphate	200	Ca^{2+}	2000
Ba(II)	25	Chloride	200	Na^+	2000
Ce(IV)*	25	Bromide	200	K^+	2000
Fe(III)*	25	Acetate	200	Al(III)	150
Cu(II)*	25	Sulphate	200	Cr(III)	150
Cd(II)	100	Borate	100		

* Masked by masking agents

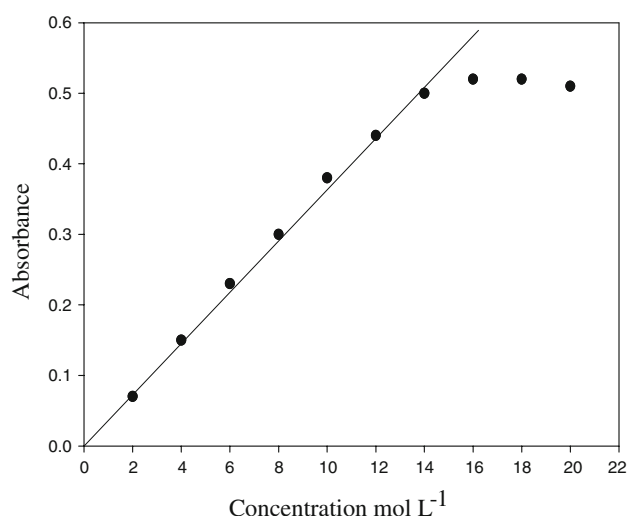


Fig. 2 Adherence to Beer's Law

$\text{mol}^{-1} \text{cm}^{-1}$, $2.01 \times 10^{-9} \text{ mol L}^{-1}$, $1.41 \times 10^{-6} \text{ mol L}^{-1}$ and $4.28 \times 10^{-6} \text{ mol L}^{-1}$ respectively.

Applications

The developed method is applied to the determination of hydrogen peroxide in different water samples and also

some pharmaceutical samples. The results of analysis of the samples of proposed method are compared favorably with those from a reference method. Statistical analysis of the results by *t* and *F*-tests show that there is no significant difference in accuracy and precision of the proposed and reference method (Baga et al. 1988) and results were summarized in Tables 3 and 4. The precision of the proposed method is evaluated by a replicate analysis of samples containing hydrogen peroxide at four different concentrations.

Conclusions

A comparison of the results obtained for the determination of hydrogen peroxide using the proposed method with the published methods is given in Table 5. The modification of the spectrophotometric determination of hydrogen peroxide method is useful for low concentrations of hydrogen peroxide. Excellent reproducibility and high sensitivity are the hallmarks of the method. The method is useful for the determination of hydrogen peroxide at low concentrations in water samples with satisfactory results.

Table 3 Analysis of hydrogen peroxide in water samples

Sample	H_2O_2 added ($\times 10^{-6} \text{ mol L}^{-1}$)	Proposed method		Reference method		<i>t</i> -test ^b	<i>F</i> -test ^c
		H_2O_2 found ($\times 10^{-6} \text{ mol L}^{-1}$) ^a	Recovery (%)	H_2O_2 found ($\times 10^{-6} \text{ mol L}^{-1}$) ^a	Recovery (%)		
Natural water	2	1.97 ± 0.02	98.00	1.96 ± 0.02	98.00	0.89	1.63
	6	5.95 ± 0.03	99.16	5.95 ± 0.03	99.16	0.29	1.21
	10	9.96 ± 0.03	99.60	9.95 ± 0.02	99.50	0.86	1.69
Polluted water	2	1.98 ± 0.01	99.00	1.98 ± 0.02	99.00	0.44	1.62
	6	5.98 ± 0.02	99.66	5.96 ± 0.02	99.33	1.12	1.23
	10	9.96 ± 0.03	99.60	9.95 ± 0.03	99.50	0.31	1.02
Rain water	2	1.95 ± 0.03	97.50	1.94 ± 0.02	97.00	0.79	2.42
	6	5.95 ± 0.03	99.16	5.94 ± 0.02	99.16	0.67	1.70
	10	9.95 ± 0.04	99.50	9.94 ± 0.03	99.40	0.47	1.25

^a mean \pm standard deviation ($n = 5$); ^b Tabulated *t*-value for 5 degree of freedom at 95% probability is 2.776; ^c Tabulated *F*-value for (5, 5) degree of freedom at 95% probability level is 6.39

Table 4 Analysis of hydrogen peroxide in bleach cream

Sample	H_2O_2 content ($\times 10^{-6} \text{ mol L}^{-1}$)	Proposed method		Reference method		<i>t</i> -test ^b	<i>F</i> -test ^c
		H_2O_2 found ($\times 10^{-6} \text{ mol L}^{-1}$) ^a	Recovery (%)	H_2O_2 found ($\times 10^{-6} \text{ mol L}^{-1}$) ^a	Recovery (%)		
OXY	2	1.94 ± 0.03	97.00	1.94 ± 0.04	97.00	1.67	1.77
Bleach	6	5.98 ± 0.02	99.66	5.97 ± 0.01	99.50	2.23	1.27
Cream ^d	10	9.98 ± 0.02	99.80	9.96 ± 0.03	99.60	2.24	1.67

^a mean \pm standard deviation ($n = 5$); ^b Tabulated *t*-value for 5 degree of freedom at 95% probability is 2.776; ^c Tabulated *F*-value for (5, 5) degree of freedom at 95% probability level is 6.39. Sample: ^d FEM CARE PHARMA LTD., Plot No.3, Survey No. 283–285 & 287, At-Manakpur, Tehsil-Nalagarh, Dist-Solan, H.P.174101Mkt. By: MIRASU MARKETING LTD

Table 5 Comparison of the proposed method with the existing ones

Reagent/method	Remarks
Tunable Diode laser Spectrometry (Newman 1993)	Sophisticated instrumentation
Spectrophotometry, Titanium salt (Wolfe 1962)	High cost, low stability of the colored complex
Fluorescence detection, p-Hydroxyphenylacetic acid + horseradish peroxidase (Tanner and Wong 1998)	High cost and low stability of the reagents, interference from other peroxides
Chemiluminescence-Hemin Cu(II) Catalyst, based on luminol (Ibusuki 1983).	Presence of SO ₂ interferes severely
Amperometry – electrochemical method (Cosogrove et al. 1988).	Interference by other oxidizing species
Proposed method: potassium iodide-Toluidine blue	Simple method, low detection limit, majority of the species are non interfering

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