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Novel Spectrophotometric Method for the Assay of Hydrogen Peroxide Using Sulfosalicylic Acid as a Chromogenic Probe

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ABSTRACT

A simple, rapid, and sensitive spectrophotometric method are described for the determination of hydrogen peroxide using ammonium ferrous sulfate (FAS) and sulfosalicylic acid (SSA) are presented. This model is based on the oxidation of FAS by H_2O_2 in acidic condition to form ferric ion, in aqueous medium sulfonic group of SSA is completely ionized to give RH_2^- and RH^{2-}. In acidic medium, this molecule binds to Fe (III) to form a purple-colored FeSSA chelate compound, this has λ_{max} at 510 nm. The optical density is directly proportional to hydrogen peroxide concentration and obeys Beer's law in the range 4.0-24.0 µM. The molar absorptivity, Sandell's sensitivity, detection limit, and quantification limit of the method were found to be 2.2×10^4 L mol^{-1} cm^{-1}, 0. 2 µM/cm^2, 0.073 µmol L^{-1}, and 0.224 µmol L^{-1}, respectively. The results of analysis of the proposed method are compared favorably with those from a reference method. The method is successfully applied to water and plant samples.

Key words: *Sulfosalicylic acid, Ferrous sulfate, Chelate.*

1. INTRODUCTION

Hydrogen peroxide, H_2O_2, is a key species in the atmosphere, over the past two decades; H_2O_2 has received increasing scientific attention because of its central role as the catalyzed and uncatalyzed aqueous phase oxidation of SO_2 to sulfuric acid in clouds [1]. The existence of H_2O_2, at extensively varying levels, has been reported in human and other animal aqueous and vitreous humors. H_2O_2 is present in human body in kidney, urinary tract, and bladder. In exhaled air of humans and of rats contains hydrogen peroxide [2].

The existence of H_2O_2 in environment is one of world serious problem is the death of aquatic animals and aquatic plants, by the high acidity of the rain results from the oxidation reaction of H_2O_2. Several literatures have explained high levels of H_2O_2 as being cytotoxic to a wide range of animal, plant, and bacterial cells in culture. Due to the severe effects of H_2O_2, it is inevitable to quantify the level of H_2O_2 in environmental and biological samples. H_2O_2 is a substance highly used in food, pharmaceutical, clinical, environmental, and other industries. Because of broad applicability and lack of simple methods for hydrogen peroxide, accurate analysis is needed.

Determination of hydrogen peroxide is usually based on the production of colored peroxy compounds or on its oxidizing and reducing properties. The detailed literature survey shows that many analytical methods including titrimetric, fluorometric [3], chemiluminescence [4], and electrochemical [5] these methods are either very expensive or less versatile. Although chemilumine scence method, atomic absorption spectrometry, etc., are proposed for the determination of hydrogen peroxide the price of instruments used is more expensive than that of spectrophotometer.

An exemplary method to quantify the H_2O_2 was made by spectrophotometry due to the rapidity, facile, and inexpensive properties of it. Several spectrophotometric methods are available for the assay of H_2O_2 but these methods include the use of toxic reagents. To overcome all this we developed a novel, spectrophotometric method for the assay of H_2O_2. The developed method is successfully applied

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to different water samples and also the results obtained by the proposed method are compared with standard existing method shows the agreeing values.

The use of nanoparticles in recent years has been much interest in many branches of science and technology [6] in chemical studies, the role of these species is to catalyze reactions between reactants. It was observed that Fe_3O_4 magnetic nanoparticles possessed an intrinsic peroxidase-like activity [7]. Based on this, Fe_3O_4 MNPs is used as a new catalyst which as a potential application in the determination of hydrogen peroxide in water sample and in vegetable samples. Our data showed that this new method is efficient to determine H_2O_2 in water sample and vegetable sample.

2. EXPERIMENTAL

2.1. Materials and Methods

Systronics spectrophotometer model 106 with 1 cm matched glass cuvvets was used for measuring the absorbance. A pH-meter, EQUIP-TRONICS Model EQ-614 was employed for measuring pH.

2.1. Water Sampling

Water samples were done by composite sampling method, totally 15 samples were collected from 15 different places in and around Mysore including lakes, river, and underground water. All samples are collected in high-density polythene bottles of capacity 500 mL and stored in 20°C.

2.2. Soil Samples

The soil samples collected represented the upper layer of soil with a thickness of 20 cm. The samples were dried and ground in a silica crucible.

25 ml of 0.1 M sodium carbonate solution was added to accurately weighed 0.25 g of soil sample taken in a glass beaker. The contents were filtered through Whatman No. 1 filter paper. The precipitates were washed several times with 0.1 M Na_2CO_3 with distilled water. The final volumes of sample solutions were made to 25 ml with distilled water.

2.3. Chemicals and Reagents

All the chemicals used in the analysis were of analytical reagent grade. Double-distilled water was used. Glass vessels were cleaned by washing with acidified solution of $K_2Cr_2O_7$, followed by washing with concentrated HNO_3 and rinsing several times with distilled water.

2.4. General Procedure

To a series of 5 mL standard flasks which contained 1.3 ml of 0.085 mM ferrous sulfate (FAS) solution, and 0.8 ml of 0.5 N potassium dihydrogen and dipotassium hydrogen orthophosphate and varying concentration of H_2O_2 solution was added (0.1 mL to 1.6 mL of 0.04 mM) and 1.3 mL of 3.9 mM sulfosalicylic acid (SSA) reagents were added. The mixture was shaken thoroughly and kept aside for 2 min. The mixture was make it up to the mark with double-distilled water. The red-colored Fe (III) - SSA complex is developed which shows maximum absorbance at 500 nm. The absorbance was measured against the corresponding reagent blank, and the calibration graph was constructed. The linearity was absorbed from 4 to 24 μM.

3. RESULTS AND DISCUSSION

3.1. Absorption Spectra

The formed reddish-colored Fe (III) - SSA complex were scanned in the wavelength range of 360-460 nm against a corresponding reagent blank. The optimum wavelength of maximum absorption of the red-colored product was obtained at 510 nm. Optical density for the different concentration of H_2O_2 is shown in Figures 1 and 2.

3.2. Optimization of the Experimental Conditions

3.2.1. Effect of pH

The pH of the medium had an important factor on the stability of the colored product formed. We compared the stability of the product with different buffer of pH ranging from 4.0 to 10.5. The reaction shows maximum color development in 100 mM KH_2PO_4/ K_2HPO_4 buffer of pH 7.0. Hence, all studies were carried out at this pH.

3.2.2. Effect of FAS and SSA solution

The effect of varying concentrations of FAS and SSA showed that the rate increased on increasing the concentration up to 0.68 and 1.04 mM, respectively, beyond this concentration there was no considerable increase in the rate. Hence, 0.68 and 1.04 mM of FAS and SSA was selected as the optimized concentration. The effect of FAS and SSA are shown in Figure 3.

3.2.3. Effect of temperature

The stability and activity of the reaction are influenced by the temperature. Fe-SSA complex activity increases with temperature but stability decreases. Temperature sensitivity was determined by preincubating the optimized concentration of reaction mixture for 5 min at varying temperatures. The maximum activity was observed at 30°C.

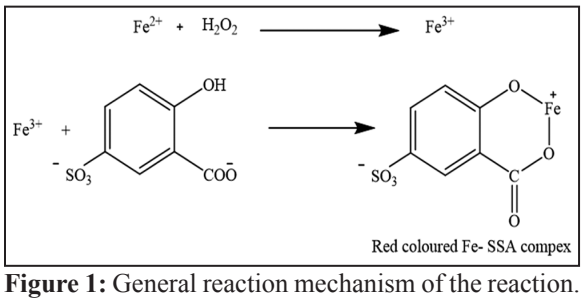

Figure 1: General reaction mechanism of the reaction.

3.3. Effect of Non-Targeting Species

The effect of various non-target species in the determination of H_2O_2 was investigated. The result indicated that there was no considerable interference by any of the ions tested. It can be seen that the method is highly selective. The tolerance ratios are tabulated in Table 1.

3.4. General Reaction Mechanism of the Reaction

Hydrogen peroxide is an oxidizing agent; it oxidizes ferrous to ferric ion. In aqueous medium, sulfonic group of SSA is completely ionized to give RH_2^- and RH^{2-} [8]. In acidic medium, this molecule binds to Fe (III) to form a red-colored Fe-SSA chelate compound, this has λ_{max} at 510 nm. The intensity of the red formed is proportional to the concentration of H_2O_2. The probable reaction is shown in Figure 1.

3.5. Calibration Graphs for the Assay

The calibration curve for H_2O_2 assay was carried out under the optimized experimental condition. The results showed that the linearity for the calibration graph was observed in the range of 4.0-24.0 µM. The molar absorptivity, Sandell's sensitivity, detection limit, and quantification limit of the method were found to be 2.2×10^4 L mol^{-1} cm^{-1}, 0.2 µM/cm^2, 0.073 µmol L^{-1}, and 0.224 µmol L^{-1}, respectively. The calibration graph for the quantification of H_2O_2 is shown in Figure 4.

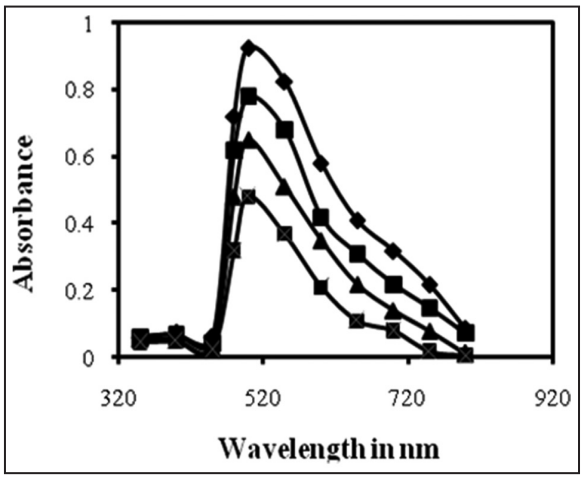

Figure 2: The absorption spectra of Fe-SSS complex for different concentration of H_2O_2.

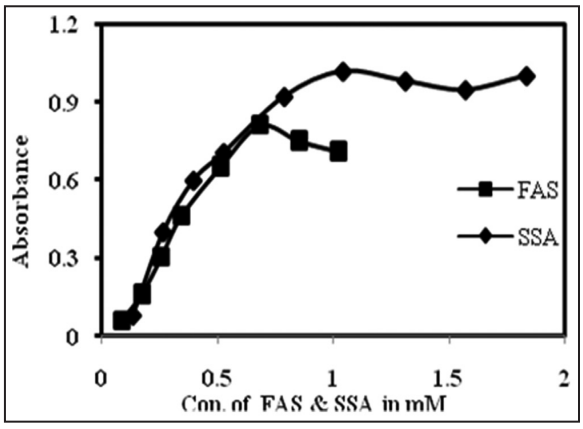

Figure 3: The effect of ferrous sulfate and sulfosalicylic acid.

3.6. Application of the Proposed Method

The developed method is successfully applied to the different water samples of in and around Mysore, Chamarajanagar and Mandya district, which includes both surface and underground water samples and also it is applied to soil samples. The developed method is validated by comparing the obtained results with reference method which is tabulated in the Tables 2 and 3.

Table 1: Possible interfering substances along with their concentrations tested for H_2O_2.

Interference	Interference added as	Stock solution (1000 mg/L)
Cat ions		
Calcium	$CaCl_3$	0.280
Magnesium	$MgSO_4$	0.580
Aluminium	$AlCl_3.6H_2O$	0.650
Copper	$CuCl_2.2H_2O$	0.215
Anions		
Chloride	KCl	0.180
Nitrate	$NaNO_3$	0.150
Sulfate	Na_2SO_4	0.210
Phosphate	$NaH_2PO_4.6H_2O$	0.250

Table 2: Application of the recommended method for determination of H_2O_2 in various water samples.

Water sample	H_2O_2 (µg/L)	
	Proposed method	Reference method
Bannur lake	6	6
Mudukuthore	7.6	7.0
Hassan	7.7	8.0
Hassan river	7.8	7.3
Kalpura	7.8	7.8
Kalpura	7.1	7.0
Udigala	7.3	7.5
Bisalavadi	7.7	7.5
Varuna lake	8.0	7.8
Dalvoy lake	8.2	8.4
Kukkarahalli lake	7.8	7.8
Shetty kere	5.8	6.0

Table 3: Application of the recommended method for determination of H_2O_2 in various soil samples.

Soil sample	H_2O_2 (µg/L)	
	Proposed method	Reference method
Kalpura (Ch. Nagar)	6.7	5.2
Udigala (Ch. Nagar)	5.7	5.4
Hassan town	5.2	5.0
Surface soil Udigala	4.1	4.8
Hassan town	4.2	5.0

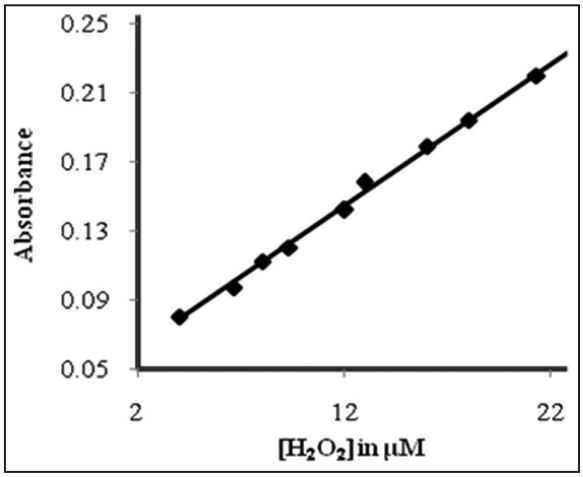

Figure 4: Beer's law graph for fluoride with Fe (III) and SSA.

4. CONCLUSION

The developed method involves the usage of simple economical, less toxic, and water soluble chromogenic reagents. The proposed method is successfully applied to the water and soil samples. The developed method is validated by comparing the result with reference enzymatic method [9]. Around 15 samples are collected from different places which include lakes and bore wells. The more peroxide content was found to be in Varuna Lake water (Mysore Dalvoy lake) and Hassan (bore well) samples. In soil samples, Kalpura (Ch. Nagar) having more H_2O_2 content. Apple and Sapota fruits containing considerable more concentration of H_2O_2. The obtained values are tabulated in Tables 2 and 3.

5. ACKNOWLEDGMENTS

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***Bibliographical Sketch**

Dr. N. A. Chamaraja obtained M.Sc degree with specialization in Analytical Chemistry in 2007 from University of Mysore and he obtained Ph.D degree in 2015 from the same university, in the field of Bio- Analytical Chemistry. He published number of research papers in national and international reputed journals. He worked as a research officer in R & D analytical laboratories and as a Asst. Professor in PG and UG Centres. In addition, guided for some PG and UG Projects work. At present, he guides 4 Research students for Ph.D degree. The main research fields are analytical chemistry, environmental and nano fields. Currently, he has been working in Vidya Vikas Institute of Engineering and Technology, Mysore since 2013.