Spectrophotometric Determination of H₂O₂ in Disinfectant Drug Water by Dibromo-*p*-methyl-arsenazo Oxidation

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By using the property that hydrogen peroxide oxidizes dibromo-*p*-methyl-arsenazo to produce a decolouring reaction and hemoglobin has catalytic action, this paper proposed a new method for decolouring spectrophotometric determination of trace hydrogen peroxide. In pH 5.2 CH₃COOH-CH₃COONa buffer medium, the maximum absorption wavelength is at 400 nm. Beer's law is obeyed over the range of 2.47×10^{-5} - 1.23×10^{-4} mol L⁻¹ for the concentration of H₂O₂. The method was successfully used in the determination of hydrogen peroxide in disinfectant drug water sample. The relative standard deviation of 13 determinations was 3.01 % and the addition standard recovery of method was 103.90 %.

Key Words: Hydrogen peroxide, Decolouring spectrophotometry, Dibromo-*p*-methyl-arsenazo.

INTRODUCTION

Hydrogen peroxide is an important strong oxidant, can be used as a bleaching agent, disinfectant, deoxidizing agent, liquid propellant, *etc.* and has weak acidity. There is a close relationship between the hydrogen peroxide in the atmosphere and rainwater and acid rain¹. The content of hydrogen peroxide in the air shall not exceed 1.4 mg/m³, otherwise, excessive inhalation of hydrogen peroxide can be poisonous to the human. Many substances inside biological organism can produce hydrogen peroxide through some reaction. Therefore, it is of great significance in the analysis of environmental chemistry, biochemistry analysis and clinical diagnosis for determination of hydrogen peroxide². Determination of microamount hydrogen peroxide with conventional titration method has been unable to meet the requirements, needing to establish some sensitive, rapid, simple method for determination of hydrogen peroxide. Monitoring method of hydrogen peroxide caused the great attention of analysts. Although chemiluminescence method, atomic absorption spectrometry *etc.* are proposed for the determination of hydrogen peroxide^{3,4}, the price of instruments used is more expensive than that of spectrophotometer. The enzyme has high specific catalytic properties and the use of some enzymes as peroxidase substitute catalytic system for the determination of H₂O₂ aroused people's interest⁵. In this paper, hemoglobin is used as catalyst and in pH 5.2 CH₃COOH-CH₃COONa buffer solution catalyzes the oxidation of dibromo-

p-methyl-arsenazo (DBM-ASA) by H₂O₂ to fade and between its fading degree and H₂O₂ concentration. Beer's law is obeyed in this process, so as to establish a new method for the determination of H₂O₂. The method is simple, rapid and has been successfully used for the determination of hydrogen peroxide content in disinfectant drug water sample.

EXPERIMENTAL

All the reagents were of analytically pure grade and all the solutions were prepared with doubly distilled water. Dibromo-*p*-methyl arsenazo (DBM-ASA, Jinsheng Chemical Co. Ltd., China): 4.0×10^{-4} mol L⁻¹ aqueous solution was prepared. Hemoglobin solution (bovine hemoglobin, Hb, with a molecular weight of 66, 400, Shanghai Lizhu Dongfeng Biological Technology Co., Ltd., China): 2.0×10^{-5} mol L⁻¹ aqueous solution was prepared and placed in a refrigerator at 4 °C to avoid light for preservation. pH 5.2 CH₃COOH-CH₃COONa buffer solution was used for acidity control of reaction medium. H₂O₂ standard solution: concentrated H₂O₂ was calibrated by KMnO₄ standard solution and a 2.0×10^{-2} mol L⁻¹ of stock solution was prepared. The stock solution was placed in a 4 °C refrigerator, stored away from light. The use valid period is 6 days. 5 mL of the stock solution was placed in a 50 mL volumetric flask, diluted to mark with water, shaken well and a concentration of 2×10^{-3} mol L⁻¹ was prepared for working solution. When the working solution was in use, the preparation was made. A 722 type spectrophotometer

(Shanghai Spectrum Instrument Co., Ltd, China) with matched 1 cm cells was used for the absorbance determination.

Procedure: 0.5 mL of pH 5.2 $\text{CH}_3\text{COOH}-\text{CH}_3\text{COONa}$ buffer solution, 1.0 mL of dibromo-*p*-methyl-arsenazo solution, 2.0 mL of bovine hemoglobin (Hb) solution, 0.5 mL of H_2O_2 solution or not, was, respectively taken, placed in 10 mL comparison tube, diluted to the mark with water, shaken well. At room temperature after 35 min, using 1 cm cell at wavelength 400 nm with water as reference the absorbance (A) of solution was measured. According to the same method, the absorbance (A_0) without adding H_2O_2 system was determined. Absorbance difference $\Delta A = A_0 - A$ was calculated.

RESULTS AND DISCUSSION

Absorption spectra: Fig. 1 shows absorption spectra of DBM-ASA (a), (DBM-ASA) + H_2O_2 (b), (DBM-ASA) + H_2O_2 + Hb (c) system against water. From curve (a) and (b), it can be seen that the addition of H_2O_2 made DBM-ASA absorbance decrease, indicating that H_2O_2 could oxidize DBM-ASA and made it fade. By comparison of b, c it can be seen that the peak of curve (c) decreased significantly, illustrating that Hb played a catalytic role for the fading reaction of DBM-ASA by H_2O_2 oxidation. Fig. 2, respectively shows absorption curves of (DBM-ASA) + Hb and (DBM-ASA) + Hb + H_2O_2 system *versus* water. It can be seen that both presented maximum absorption at 400 nm and ΔA exhibits maximum absorption peak at 400 nm and determination sensitivity was maximum. The experiment selected 400 nm as the measuring wavelength.

Fig. 1. Absorption spectra: (a) DBM-ASA (against water); (b) (DBM-ASA) + H_2O_2 (against water); (c) (DBM-ASA) + H_2O_2 + Hb (against water); [H_2O_2] = $1.0 \times 10^{-4} \text{ mol L}^{-1}$; [Hb] = $4.0 \times 10^{-6} \text{ mol L}^{-1}$; [DBM-ASA] = $4.0 \times 10^{-5} \text{ mol L}^{-1}$; pH 5.2

Effect of pH: According to the standard procedure, at pH 3.2, 4.2, 4.8, 5.2, 5.6, 5.8, 6.2, the absorbance difference of the system, ΔA , was, respectively determined. Results show that (Fig. 3), with the increase of pH, ΔA constantly increased over the range of pH 3.2-4.8. When pH was 4.8-5.6, ΔA is larger and fluctuation was smaller and the experiment sensitivity was higher. When pH was among 5.6-6.2, ΔA dropped, so the method chose pH 5.2. The experimental results showed that the amount of $\text{CH}_3\text{COOH}-\text{CH}_3\text{COONa}$ buffer solution was selected to be 0.5 mL, determination sensitivity was maximum.

Effect of DBM-ASA chromogenic agent, hemoglobin and surface active agent: When the amount of DBM-ASA

Fig. 2. Absorption spectra: (a) (DBM-ASA) + Hb (against water) - A_0 ; (b) (DBM-ASA) + Hb + H_2O_2 (against water) - A ; (c) $\Delta A = A_0 - A$. [H_2O_2] = $1.0 \times 10^{-4} \text{ mol L}^{-1}$; [Hb] = $4.0 \times 10^{-6} \text{ mol L}^{-1}$; [DBM-ASA] = $4.0 \times 10^{-5} \text{ mol L}^{-1}$; pH 5.2

Fig. 3. Effect of pH: (a) (DBM-ASA) + Hb (against water) - A_0 ; (b) (DBM-ASA) + Hb + H_2O_2 (against water) - A ; (c) $\Delta A = A_0 - A$. [H_2O_2] = $1.0 \times 10^{-4} \text{ mol L}^{-1}$; [Hb] = $4.0 \times 10^{-6} \text{ mol L}^{-1}$; [DBM-ASA] = $4.0 \times 10^{-5} \text{ mol L}^{-1}$

was 0-0.9 mL, ΔA constantly increased as dosage of the chromogenic agent increased. When the chromogenic agent amount was 0.9-1.1 mL, ΔA was maximum. When the amount of DBM-ASA was increased to be 1.1-1.5 mL, ΔA decreased. The DBM-ASA dosage was selected to be 1 mL.

When the hemoglobin amount was among 0-1.8 mL, ΔA continuously increased as the amount of Hb increased. At 1.8-2.2 mL, ΔA was maximum. After it, with increase of hemoglobin amount ΔA decreased, the selected hemoglobin amount was 2 mL.

The work, respectively studied effect of OP-100 emulsifier, Tween-80, cetyltrimethylammonium bromide and sodium dodecylsulphate four surfactants on the system, showing that all these surfactants inhibited the system.

System stability: After all the reactants were added for 0.5 h, ΔA was stable. ΔA change was no more than 5 % within 1 h.

Effect of reaction temperature: According to the standard procedure, the absorbance difference ΔA of system was, respectively determined at temperature of 4, 10, 15, 20, 25, 30, 40, 50 $^{\circ}\text{C}$. With the increase of temperature, ΔA continuously increased between 4-20 $^{\circ}\text{C}$. When temperature was 20 $^{\circ}\text{C}$,

TABLE-1
 ANALYTICAL RESULTS OF SAMPLES (n = 13)

Sample	Found (10^{-5} mol L $^{-1}$)	Average (10^{-5} mol L $^{-1}$)	Relative standard deviation (%)	Added (10^{-5} mol L $^{-1}$)	Recovered (10^{-5} mol L $^{-1}$)	Recovery (%)	Crystal violet spectrophotometric control method
Disinfectant drug water	5.03, 4.97, 5.15, 5.03, 4.79, 4.85, 5.21, 5.21, 4.97, 4.91, 5.15, 4.79, 4.91	5.01	3.01	5.00	4.98	99.6	5.01

the ΔA was a maximum and the sensitivity was the highest. Between 20-50 °C, the sensitivity reduced. Thus, this method selected a room temperature of 20 ± 2 °C for the determination.

Effect of coexisting ions: When 1.0×10^{-4} mol L $^{-1}$ H₂O₂ was measured and the relative error was less than ± 5 %, the following amount (in quantity multiple) coexisting ion was allowed to exist: Li⁺, Zn²⁺, Pb²⁺(50); Mg²⁺, Ca²⁺, Ba²⁺ (15); Ag⁺, Bi³⁺ (10); Fe³⁺ (5); Ni²⁺, Fe²⁺, Co²⁺ (2); Cu²⁺ (1); Mn²⁺, (0.5); Cr³⁺, Cr(VI) (0.2); Al³⁺ (0.1); F⁻, Cl⁻ (800); PO₄³⁻ (400); NO₂⁻ (200); BrO₃⁻ (15); Br⁻, C₂O₄²⁻ (10); VO₃⁻, I⁻ (5); MnO₄⁻, SO₄²⁻, MnO₄⁻, S₂O₇²⁻ (2); glucose (50); citric acid, glutamic acid, cysteine, leucine (20); ascorbic acid, lysine (10); bovine serum albumin, phenylalanine (5).

Working curve: The experimental results displayed that Beer's law was obeyed for H₂O₂ in 2.47×10^{-5} - 1.23×10^{-4} mol L $^{-1}$. Its linear regression equation was $\Delta A = 1639.1C$ (C: mol L $^{-1}$) + 0.0079 and the correlation coefficient was 0.9990 and the apparent molar absorption coefficient of method was $\epsilon = 1.81 \times 10^3$ L mol $^{-1}$ cm $^{-1}$. According to the 3S/K method, the detection limit of method calculated was 1.35×10^{-5} mol L $^{-1}$ (S is the standard deviation of eleven parallel blank test, K is regression equation slope).

Sample analysis: 1 mL of disinfection water sample was taken and diluted 200 times. 1 mL of the diluted solution was taken and determined according to the standard procedure. Meanwhile, the addition standard recovery rate experiment was carried out and the crystal violet photometric method⁶

control experiment was made and the measured results are shown in Table-1. It can be seen that when this method was applied to the determination of H₂O₂ in disinfectant drug water sample, satisfactory results were obtained.

Conclusion

This paper reports the optimum reaction conditions of discoloring reaction of dibromo-*p*-methyl-arsenazo by hydrogen peroxide catalyzed by hemoglobin, finding that the maximum absorption wavelength was at 400 nm. Beer's law was obeyed for the content of H₂O₂ in 2.47×10^{-5} - 1.23×10^{-4} mol L $^{-1}$, its linear regression equation was $\Delta A = 1639.1C$ (C: mol L $^{-1}$) + 0.0079 with a correlation coefficient of 0.9990, the apparent molar absorption coefficient of method was $\epsilon = 1.81 \times 10^3$ L mol $^{-1}$ cm $^{-1}$ and the detection limit of method was 1.35×10^{-5} mol L $^{-1}$. By the present method, the content of hydrogen peroxide in the disinfectant drug water sample has been satisfactorily measured.

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