Asian Journal of Chemistry; Vol. 25, No. 7 (2013), 4099-4102

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.14475C

Spectrophotometric Determination of Hydrogen Peroxide Based on Fading of Tribromoarsenazo Oxidation by Hydrogen Peroxide Catalyzed by Hemoglobin

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(*Received*: 24 August 2012; *Accepted*: 29 January 2013) AJC-12766

In pH 3.2 CH_3COOH-CH_3COONa buffer solution, bovine hemoglobin (Hb) catalyzes the decolouring of oxidation tribromoarsenazo (TB-ASA) by hydrogen peroxide and between fading degree ΔA and H_2O_2 concentration a linear relationship exists. Based on this principle, a novel method for the determination of H_2O_2 has been developed. The results showed that the maximum absorption wavelength of the system is 400 nm. At this wavelength, the apparent molar absorptivity of the method is $\varepsilon_{400\,nm} = 1.01 \times 10^3$ L·mol^{-1}·cm^{-1} and the linear range is 4.0×10^{-5}-3.4×10^{-4} mol L^{-1} with a detection limit of 2.1×10^{-5} mol L^{-1}. The present method has been successfully applied to the determination of H_2O_2 in rain waters.

Key Words: Hydrogen peroxide, Tribromoarsenazo, Catalytic oxidation, Bovine hemoglobin, Rain water, Spectrophotometry.

INTRODUCTION

Hydrogen peroxide is an important active intermediate and is widely applied in environmental chemistry, chemical engineering, spin, paper making, food hygiene and clinical medicine, *etc*. Hydrogen peroxide is an important active oxygen intermediate and continues to decompose in body to produce super oxygen anion O^{2-} *etc*. Many kinds of free radicals participating in the aging process of biological cells. Many biological chemical reactions including some of the biological chemical reactions inside body can produce active oxygen free radicals, which is closely linked to the physiological phenomena of senescence, disease, *etc*. Meanwhile the detection of hydrogen peroxide is the base of enzyme coupling analysis of the many biological substances that can produce hydrogen peroxide. The studies in recent years showed that hydrogen peroxide also exists in nature and it is one of important reasons for the formation of acidic rains. The detection of hydrogen peroxide is very important in environmental chemistry and clinical medicine *etc.*[1].

For the determination of macro hydrogen peroxide, titration method can be used. With respect to the determination of trace hydrogen peroxide, although some determination methods have already been proposed and the repored methods have chemical luminescent method, fluorescent method, electrochemical luminescent method, *etc*.[2]. For these methods the operations are trivial, the instrumentations are expensive and the reagent selectivity are poor and thus they are difficult to be used. Compared with the above methods, kinetic analytical method[3-7] has the advantages that the selectivity is better and the sensitivity is high. Enzyme catalytic reaction, in a general case, has higher sensitivity and wholesomeness. At present, enzyme catalytic kinetic spectrophotometry is mostly applied to solve the problems in life science[8]. Enzyme catalytic reaction has extremely high sensitivity because enzyme is a kind of biological catalyst and its catalytic reaction speed is higher 10^8-10^{20} times than that of non-catalytic reaction and higher 10^6-10^{14} times than that of chemical catalytic reaction. As each enzyme can only catalyze a sort of reaction, which has high specificity, thus the selectivity of method is very nice. In addition, enzyme catalytic reaction conditions are mild (20-40 ºC, pH 4-9) and no special reagents are needed. The catalyzed reactive substances can be chemicals and can also be agricultural, forestry industrial, stockbreeding industrial and fishing products and their waste waters and waste garbage. Therefore, the applications are extremely extensive.

Chromotropic acid arsenazo kind reagents are often used as the spectrophotometric analytical chromogenic agent of rare earths elements. Tribromoarsenazo (TB-ASA), 3-[2,4,6-tribromophenylazo]-6-(2-arsenophenylazo)-4,5-dihydroxynaphthalene-2,7-disulfonic acid, was ever used as the rare earth chromogenic agent[9,10]. This paper discovers that in pH 3.2 CH_3COOH-CH_3COONa buffer medium hydrogen peroxide can oxidize tribromoarsenazo to make TB-ASA decolour and hemoglobin

has a catalytic effect on the reaction. Based on this property, the authors established a novel method for the determination of H_2O_2. The present article established the optimum experimental conditions of determination of hydrogen peroxide with the system, observing and studying the sensitivity, detection limit, accuracy, precision, linear range and selectivity of this method. The present method was applied to the determination of H_2O_2 contents in rainwater and satisfactory results were obtained. The advantages of method are simple and convenient, fast, easy to be operated and the determination can be made at room temperature, having higher practical value and applied foreground.

EXPERIMENTAL

A 722 spectrophotometer (Shanghai Prism Light Technology Corporation, Ltd., China) with 1 cm cells was employed for all absorbance measurements. A standard stock solution containing 2.0×10^{-2} mol L^{-1} H_2O_2 was prepared by suitable dilution of concentrated H_2O_2 after the H_2O_2 was standardized by titrimetric method using $KMnO_4$ standard solution. The stock solution was placed in a 4 ºC refrigerator for storage avoiding light. It could be used for 6 days. When it was in use, the stock solution was suitably diluted and 2.0×10^{-3} mol L^{-1} working solution was obtained. The working solution was freshly prepared each time. 2.0×10^{-4} mol L^{-1} of tribromoarsenazo (TB-ASA, Shanghai Jinsheng Chemical Corporation, Ltd., China) solution was prepared as the chromogenic agent solution. 1.0×10^{-3} mol L^{-1} of bovine hemoglobin (Hb, molecular weight 66400, Shanghai Lizhu Dongfeng Biological Technology Corporation, Ltd., China) was prepared and placed in a 4 ºC refrigerator for storage avoiding light. pH 3.2 CH_3COOH-CH_3COONa buffer solution was applied for controlling the acidity of reaction medium. All the reagents used were of analytical pure grade and doubly distilled water was used in the experiment.

In 10 mL comparison tubes, in turn 0.5 mL of pH 3.2 CH_3COOH-CH_3COONa buffer solution, 2.0 mL of 2.0×10^{-4} mol L^{-1} TB-AS solution, 4.0 mL of 1.0×10^{-5} mol L^{-1} Hb solution and 1.5 mL of 2.0×10^{-3} mol L^{-1} H_2O_2 solution were added. Water was used to dilute to the mark and the solution was shaken homogeneously. After the solution was set for 30 min at room temperature,the absorbance (A) of solution was determined at 400 nm with 1 cm cells using water as reference. According to the same procedure, absorbance (A_0) of non-catalytic system (without addition of H_2O_2) was determined. Absorbance difference $\Delta A = A_0 - A$ was calculated.

RESULTS AND DISCUSSION

Absorption spectra: Fig. 1 is the absorption spectra of different reaction systems. Curves a,b in the figure are respectively absorption spectra of TB-ASA (against water) and (TB-ASA) + H_2O_2 system (against water). It can be seen that the addition of H_2O_2 could make the absorbance of ASA-TB decrease, showing that H_2O_2 could oxidize TB-ASA and make it decolour. Curve c is the absorption curve of (TB-ASA) + H_2O_2 + Hb system (against water). Relative to (TB-ASA) + H_2O_2 system, decrease in absorbance peak is larger, showing that Hb has a catalytic action of the decolouring reaction of TB-ASA oxidized by H_2O_2 and plays a part of catalyst in this system. The experimental results showed that the difference of absorbance between non-catalytic reaction and catalytic reaction of the system is located at 400 nm.

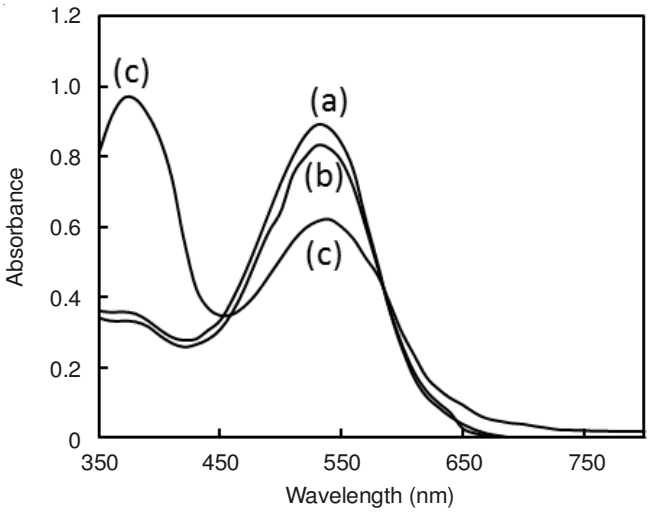

Fig. 1. Absorption spectra: (a) TB-ASA (against water); (b) TB-ASA + H_2O_2 (against water); (c) TB-ASA + H_2O_2 + Hb (against water); $[H_2O_2] = 3.0 \times 10^{-4}$ mol L^{-1}; [TB-ASA] = 4.0×10^{-5} mol L^{-1}; [Hb] = 4.0×10^{-6} mol L^{-1} pH 3.2

Optimization of experimental variables

Effects of acidity: According to the standard procedure under different pH conditions, pH 1.2, 2.2, 3.2, 4.2, 5.2, 6.2, 7.2 buffer solutions were respectively added and the acidity effect experiments were made. The results showed that the absorbance difference increased over the range of 1.2-3.2. When pH was within 3.2-7.2, the absorbance difference gradually decreased. When pH was within 2.2-4.2, the variation of absorbance difference was smaller. Therefore, in this experiment pH = 3.2 was selected for further study. The experimental results of buffer solution amount effect revealed that when buffer solution amount was 0.3-0.7 mL, the absorbance difference was maximum and stable. 0.5 mL of pH 3.2 CH_3COOH-CH_3COONa buffer solution was selected to control the acidity of medium.

Effect of the amount of tribromoarsenazo: The effect of 0, 0.5, 1, 1.5, 2, 2.2, 2.5, 3 mL of 2.0×10^{-4} mol L^{-1} TB-ASA was experimentalized. The results revealed that when TB-ASA amount was 0-1.5 mL, the absorbance difference value ceaselessly increased. Over the range of 1.5-2.2 mL, the absorbance difference value achieved maximum and was basically stable. The chromogenic agent amount was sequentially increased, then the absorbance difference value gradually decreased. Therefore, the amount of TB-ASA selected was 2.0 mL and at this time the TB-ASA concentration of solution was 4.0×10^{-5} mol L^{-1}.

Effect of the amount of hemoglobin: According to the standard procedure, the added amount of Hb was respectively 0, 1.0, 2.0, 3.0, 3.5, 4.0, 4.5, 5.0 6.0 mL. The experimental results revealed that when Hb amount was 0-3.5 mL, the absorbance difference value gradually increased. Over the range of 3.5-4.5 mL, the absorbance difference value was maximum and smooth. After it, as Hb amount increased, the absorbance

TABLE-1
ANALYTICAL RESULTS OF SAMPLE

Sample	Found (10^{-5} mol·L^{-1})	Average (10^{-5} mol·L^{-1})	Relative standard deviation (%)	Added (10^{-4} mol·L^{-1})	Recovered (10^{-4} mol·L^{-1})	Recovery (%)	Contrast method [11]* (10^{-5} mol·L^{-1})
No.1	5.95, 5.76, 5.57, 5.38, 5.76, 5.57, 5.57, 5.38, 5.95, 5.57, 5.57	5.62	3.73	2.00	2.07	103.5	5.62
No.2	1.31, 1.59, 1.31, 1.47, 1.47, 1.59, 1.53,. 1.47, 1.59, 1.59, 1.47	1.58	0.89	2.00	1.99	99.5	1.56

*Crystal violet spectrophotometry

difference value reduced. In the experiment, 4 mL was selected for further study and at this time the Hb concentration in solution was 4×10^{-6} mol L^{-1}.

Effect of surfactant: The effects of cation surfactant cetyltrimethylammonium bromide, anion surfactant sodium dodecylsulphate and nonionic surfactant emulsifier OP-100, Tween-80 on the reaction were respectively experimentalized. The results showed that these surfactants played a reduce role in the sensitivity of system and had inhibitive effects on the catalytic reaction.

Effect of addition order of reagent: The experimental results showed that the addition order of reagent had not great effect on the sensitivity of reaction. The experimentally employed order was:buffer solution, tribromoarsenazo, bovine hemoglobin, H_2O_2. This order could utmost exert the oxidation ability of hydrogen peroxide, overcoming the effect that hydrogen peroxide is easily decomposed when it meets with light.

Effect of temperature: According to the standard procedure,the effect of temperature at 4, 10, 15, 18, 20, 22, 25, 30, 40, 50 ºC was respectively carried out after constant temperature for 30 min. The experimental results showed that when the temperature was between 4-18 ºC, the absorbance difference gradually increased as the temperature increased. Over the range of 18-22 ºC, the absorbance difference attained maximum. Over the range of 22-50 ºC, the complex absorbance difference decreased as the temperature increased. Up to 50 ºC, the absorbance difference had been already zero and at this time the speed of catalytic reaction and non-catalytic reaction was the same. This work was made at room temperature (20 ± 2 ºC).

Reaction time and system stability: Under the optimum experimental conditions, for the determination of 3.0×10^{-4} mol L^{-1} hydrogen peroxide the experimental results revealed that after various reactant solutions were added for 25 min, ΔA attained a maximum and kept stable and the relative error was within 5 %. The system could be stable within 1.5 h, so in the experiment it was chosen that the determination was made after various reactant solutions were added for 30 min.

System characteristics and reaction mechanism

Working curve and analytical characteristics: Under the optimum conditions, working curve was prepared. The results showed that Beer's law is obeyed over the range of 4.0×10^{-5} -3.4×10^{-4} mol L^{-1} for H_2O_2 concentration between absorbance difference and H_2O_2 concentration. The linear regression equation of method is ΔA = 523.94C (mol L^{-1}) + 0.0108, with a correlation coefficient of r = 0.9967. The apparent molar absorptivity of method was calculated to be 1.01×10^3 L mol^{-1} cm^{-1} at 400 nm. For eleven parallel determinations of 2.0×10^{-4} mol L^{-1} hydrogen peroxide, the relative standard deviation of method was calculated to be 2.47 %. For eleven parallel determinations of reagent blank, the detection limit of method was calculated to be 2.1×10^{-5} mol L^{-1} according to 3S/K method (S is the standard deviation of eleven blank experiments, K is the slope of regression equation).

Selectivity of method: Under the optimum experimental conditions, when 3.0×10^{-4} mol L^{-1} H_2O_2 was determined and the relative error was controlled within ±5 %, the allowable amounts (*m/m*) of various existing substances are as follows: cations: Mg^{2+}, Cu^{2+}, Mn^{2+}, Cr^{3+} (0.5); Fe^{2+} (1); Ca^{2+}, Ni^{2+}, Co^{2+}, Fe^{3+}, Al^{3+}, Cr^{6+} (2); Bi^{3+} (10); Ag^{+}, Sn^{2+} (20); Li^{+}, Pb^{2+} (50); NH_4^{+} (100); Zn^{2+} (200). Anions: Br^{-}, MnO_4^{-}, $S_2O_7^{2-}$, $Cr_2O_7^{2-}$ (2); VO_3^{-} (5); BrO_3^{-} (15); I^{-}, MoO_4^{2-}, $C_2O_4^{2-}$ (20); SO_4^{2-}, CO_3^{2-} (400); PO_4^{3-} (500); F^{-}, Cl^{-} (800); NO_2^{-} (1000). Biological substances: glutamic acid, lysine, cysteine, phenylalanine, leucine, citric acid (20); ascorbic acid (30); glucose (100).

Reaction mechanism: This article preliminary deduces the reaction mechanism as follows:

(1) Hb is firstly oxidized by H_2O_2 to lose 2 electrons, producing the middle oxide form of Hb, Hb-I.

(2) Hb-I reacts with the molecules of supply electron, TB-ASA to produce another kind of middle oxidation form of Hb, Hb-II and the free radical of TB-ASA.

(3) The free radical of TB-ASA is not stable and further transforms to be colourless product, producing a decolourization phenomenon.

The involved reaction formula is as follows:

$$Hb + H_2O_2 \rightarrow Hb\text{-}I + H_2O \quad (1)$$

$$Hb\text{-}I + TB\text{-}ASA \rightarrow Hb\text{-}II + TB\text{-}ASA^{*} \quad (2)$$

$$Hb\text{-}II + TB\text{-}ASA \rightarrow Hb + \text{colourless product} \quad (3)$$

Analytical application: To validate the practicability of method, the present system was used in the analysis of rainwater sample. According to the standard procedure, 1 mL of rainwater was taken and the other reagents and procedure were the same as the standard procedure for determination of the content of hydrogen peroxide. According to the working curve linear calibration equation, H_2O_2 content in the sample was calculated. Meanwhile, recovery experiment and contrast experiment were made. The determined results are shown in Table-1. The present article used TB-ASA as the chromogenic agent for the determination of hydrogen peroxide, determined rainwater and the obtained results were in compliance with those by crystal spectrophotometry[11]. The relative standard deviation of eleven determinations was 0.89-3.73 % and the recovery was between 99.5-103.5 %. The rather satisfactory analytical results were achieved.

Conclusion

This paper proposed a novel catalytic system H_2O_2-tribromoarsenazo-hemoglobin for the determination of hydrogen peroxide. At the maximum absorption wavelength 400 nm, the apparent molar absorptivity of the method is 1.01×10^3 L mol^{-1} cm^{-1}, the linear range is 4.0×10^{-5}-3.4×10^{-4} mol L^{-1}, the regression equation is: $\Delta A = 523.94C$ (mol L^{-1}) + 0.0108, with a correlation coefficient of r = 0.9967 and detection limit is 2.1×10^{-5} mol L^{-1}. The present method has been successfully used in the determination of hydrogen peroxide in rain waters and the satisfactory results were obtained. The operation of present method is simple, fast. The determination can be made at room temperature.

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