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Application of Mn(Ⅱ) as a Mimetic Enzyme of Horseradish Peroxidase

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Abstract: In this study, Mn(Ⅱ) as a mimetic enzyme of horseradish peroxidase (HRP) was applied to the determination of hydrogen peroxide (H_2O_2). The method introduced in this paper is based on Mn(Ⅱ)'s catalytic effect on the oxidation of 4-aminoantipyrine(4-AAP) with modified Trinder's reagent N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3, 5-dimethoxyaniline(DAOS) by H_2O_2. By coupling this mimetic catalytic reaction with the catalytic reaction of glucose oxidase (GOD), glucose can be detected. Under optimum conditions, the calibration graphs for the determination of H_2O_2 and glucose are in the range of 1.0×10^{-3}–1.0×10^{-1} mol/L and 1.0×10^{-3}–14×10^{-3} mol/L respectively. The detection limit is 5.9×10^{-4} mol/L for H_2O_2 and is 9.2×10^{-4} mol/L for glucose. The feasibility of Mn (II) as a HRP mimetic enzyme in practical clinical analysis has been proven in the determination of glucose in human serum. So far, Mn (II) is the simplest and the most inexpensive mimetic enzyme.

Keywords: Mn (II), mimetic enzyme, spectrophotometry, hydrogen peroxide, glucose.

HRP is a most commonly used enzyme in H_2O_2 and glucose detection[1, 2] owing to its strong activity and quite good selectivity. However, natural HRP has many shortcomings, such as its expensiveness and instability; the experimental conditions and storage environment are inconvenient. Therefore, the search for a replacement[3-6] for HRP has become an important and interesting work.

This paper describes a catalytic spectrophotometry method for the determination of H_2O_2 based on Mn(Ⅱ) as an HRP mimetic enzyme. Mn(Ⅱ) catalyzes oxidative coupling of 4-AAP with modified Trinder's reagent DAOS[7] by H_2O_2. By coupling this mimetic catalytic reaction with the catalytic reaction of GOD, glucose can be detected. Mn(Ⅱ) is the simplest and the most inexpensive mimetic enzyme which has been reported. In addition, due to the use of DAOS, the maximum absorbance wavelength of the reaction product of the system is 592 nm, and the most frequent interfering substances in blood, like bilirubin, absorb at 592 nm slightly, meaning that this method has less interference.

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Experimental

Apparatus and Reagents

An HP 8452 UV-Vis spectrophotometer and an HH thermostat were used. All chemicals used were in analytical grade. Deionized water was employed throughout. Standard solutions of 4-AAP solution (1.0×10^{-2} mol/L), DAOS (1.0×10^{-2} mol/L), $MnCl_2$ (1.0×10^{-2} mol/L), glucose (0.2 mol/L), GOD (25.6 U/mL), and H_2O_2 (0.1064 mol/L) were used. Glucose and H_2O_2 working solutions were obtained by diluting standard solutions with distilled water just prior to use. An HAc-NaAc buffer solution with a pH 5.0 and $C_6H_8O_7$-Na_2HPO_4 buffer solution with a pH 6.0 were used.

Procedure 1: Determination of hydrogen peroxide

A 10 mL volumetric tube was filled with a certain volume of pH 5.0 buffer solution, standard solutions 4-AAP, DAOS, $MnCl_2$ and 5.0×10^{-3} mol/L H_2O_2 working solution. The mixture was quickly diluted to 10 mL with water, and allowed to stand at 60℃ for 20 min. The absorbance of solution was measured at 592 nm.

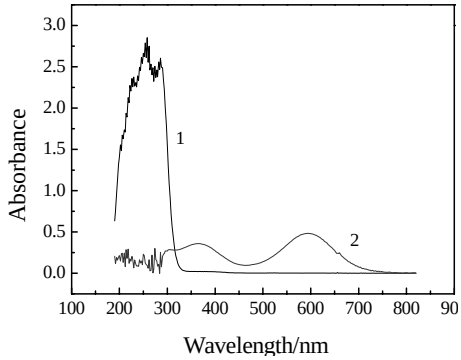
Procedure 2: Determination of glucose in water solution and human serum

A 10 mL volumetric tube was filled with 1.0 mL pH 6.0 buffer solution, 1.0 mL GOD solution, and a certain volume of glucose working solution or human serum. The mixture was incubated at 37℃ for 4 min, then measured as described above.

Results and Discussion

UV/Vis absorption spectra

Figure 1 Absorption spectra of reaction product of the system: H_2O_2-4-AAP-DAOS-Mn(Ⅱ)



(1) Reagent blank (without H_2O_2) (2) With H_2O_2 added
$[H_2O_2]=5.0\times10^{-3}$ mol/L, [4-AAP]= 1.0×10^{-3} mol/L,
[DAOS]=5.0×10^{-3} mol/L, [Mn(Ⅱ)]= 1.0×10^{-4} mol/L, pH=5.0

Figure 1 shows the UV-Vis absorption spectra of the reaction product of the system: H_2O_2-4-AAP-DAOS-Mn(Ⅱ), which has two absorption bands with maxima at 364 nm and 592 nm. Its absorption curve is similar in sharpness to the system of H_2O_2-DAOS-4-AAP-HRP[8]. The products of oxidation were identified and the catalyzed characteristic of Mn(Ⅱ) proved to be similar to that of HRP; in other words, Mn(Ⅱ) can be used as a mimetic enzyme of HRP. Because the substances that interfere most frequently in blood (products of chemolysis like bilirubin) absorb slightly at 592 nm, 592 nm was chosen as the wavelength for monitoring the product of the oxidation reaction.

In this work, only the HAc-NaAc buffer solution could activate absorbance of the product, so it was chosen for the control of the final pH. **Figure 2** shows the effect of the pH on the color development with the addition of 5.0×10^{-3} mol/L H_2O_2 solution. According to **Figure 2**, the reaction was carried out at pH 5.0.

The absorbance increased with increasing Mn(Ⅱ), 4-AAP and DAOS concentrations, then were almost constant at Mn(Ⅱ), 4-AAP and DAOS concentration above 1.0×10^{-4} mol/L, 1.0×10^{-3} mol/L, 5.0×10^{-3} mol/L respectively. So these concentrations were selected for subsequent procedures.

The reaction proceeded faster with elevating temperature and with increasing reaction time. **Figure 3** shows the influence of temperature on the rate of reaction within a range of 20-70℃ at 20 min. For this **Figure**, absorbance measurements were made at 60℃ at a reaction time 20 min after the reaction was initiated.

The calibration graphs for the determination of H_2O_2 and glucose were obtained under the optimum experimental conditions. **Table 1** shows the regression equations and correlation coefficients fitted by the least-equation method. The relative standard deviation (n=7) is 1.6% for 1.0×10^{-3} mol/L H_2O_2.

Figure 2 Influence of pH on absorbance of oxidation product.

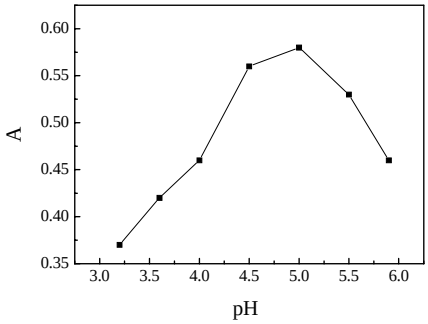
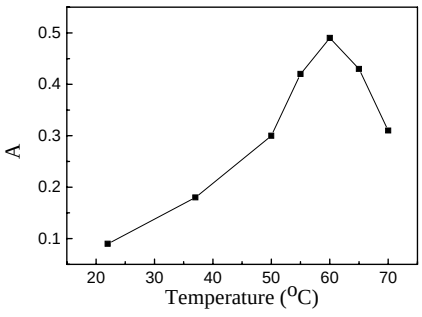


Figure 3 Influence of temperature on color development at 20 min.



Conditions were the same as described in **Figure 1**

Table 1 Analytical characteristics of hydrogen peroxide and glucose

	Linear range (mol/L)	Regression equation (mol/L)	Detection limit (3σ,mol/L)	r (n)
H_2O_2	1.0×10^{-3} – 1.0×10^{-1}	$0.2043 + 0.3301(C/10^{-3})$	5.9×10^{-4}	0.9943 (5)
Glucose	1.0×10^{-3} – 14×10^{-3}	$0.1024 + 0.0165(C/10^{-3})$	9.2×10^{-4}	0.9909 (7)

Table 2 Analytical results of glucose in human serum[a] (mmol glucose / L serum)

Samples	This method	Phenol–4-AAP method[b]
1	3.59	3.64
2	6.77	6.72
3	8.60	8.53
4	10.05	10.12

[a]The value is the average of three results of the analysis of the sample;
[b]The certified values were offered by the Tsinghua University Hospital

When the permitted relative deviation was less than ±5%, common ions (Na^+, K^+, NH_4^+, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, NO_3^-, SO_4^-, PO_4^{3-}, Cl^-) scarcely interfered with the 1×10^{-3} mol/L glucose determination. 50 fold bilirubin did not interfere with the determination.

The glucose content in four samples of human serum was determined. **Table 2** shows that these analytical results coincided fairly well with those certified values that were obtained by the standard phenol–4-AAP method[2].

Acknowledgments

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