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Research Article

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Spectrophotometric determination of Tiopronin based inhibitory effect on hemoglobin

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ABSTRACT

A highly simple and sensitive catalytic spectrophotometric method for the determination of tiopronin based on its inhibitory effect on the hemoglobin-catalyzed the reaction of H_2O_2 and acid chrome blue K(ACBK) was developed. The concentration of tiopronin is linear with the percentage inhibition (I%) of system under the optimal experimental conditions. The calibration graph is linear in range 0.05 to 2 mg/L with the detection limit of 8.5μg/L. The relative standard deviation was 4.1 % for 11 determinations of 1.5 mg/L. This method can be used for the determination of tiopronin in pharmaceutical preparations with satisfactory results.

Keywords: Tiopronin**,** Catalytic spectrophotometry, Hemoglobin

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INTRODUCTION

Tiopronin(N-(2-mercaptopropionyl), TP) is a synthetic thiol compound that has been used as an antidote to heavy metal poisoning and a radioprotective agent. It is effective in the treatment of cystinuria, rheumatoid arthritis, as well as hepatic disorders [1,2]. Additionally, it can increase intracellular concentration of glutathione and other non-protein sulphydryl groups and thereby increase intracellular defense against oxygen free radicals [3]. Several methods have already been reported for the quantitative determination of tiopronin, including spectrophotometry[4], fluorimetry [5,6], chemiluminescence [7], reversed-phase high-performance liquid chromatography [8] and capillary electrophoresis [9]. However, some methods lack sensitivity and selectivity, others are laborious and time-consuming.

Enzyme-catalyzed analytical kinetic methods have been extensively used for substrate, enzyme, inhibitor and activator analysis in several areas of analytical chemistry such as in clinical, pharmaceutical, agricultural, industrial applications and process monitoring [10]. Horseradish peroxidase (HRP; EC 1.11.1.7) is one of the most important oxidases in biology. However, natural enzymes do have shortcomings in some aspects, for example, it is expensive and unstable in solution and has strict requirements for the experimental conditions and storage environment in order to retain its catalytic activity. Hemoglobin(Hb), a necessary vehicle for oxygen carriage in body, has the natural quaternary structure as enzymes. In a recent paper Hb was used based on its similar catalytic function as HRP [11].

In this paper, a new spectrophotometric method based on inhibitory effect of tiopronin on the hemoglobin-catalyzed the reaction of H_2O_2 and ACBK was proposed. The experimental conditions for the system were optimized, and tiopronin was detected by the decreased absorbance. This method is very simple, sensitive and the detection limit is 8.5 μg/L. The method has been applied to the determination of tiopronin in pharmaceutical preparations with satisfactory results.

EXPERIMENTAL SECTION

Hemoglobin (bovine erythrocytes) solution was prepared by dissolving certain amount of Hb (Shanghai Institute of Biochemistry, Shanghai, China) in distilled water and stored below 4□. ACBK (Beijing Chemical Plant, Beijing, China) stock solution was prepared by dissolving 0.0586 g of ACBK in 100 mL of water, which was 10^{-3} mol/L in ACBK and diluted appropriately before use. H_2O_2 solution was prepared by appropriately diluting 0.01 mL of 30% H_2O_2 (standardized by titration with $KMnO_4$) to 100 mL. It was stored in a brown bottle in a refrigerator. Tiopronin (Shanghai Institute of Biochemistry, Shanghai, China) solution was prepared in the concentration of 1.0 g/L. Working solution was diluted appropriately before use with distilled water daily.

Doubly distilled water was used throughout. All other chemicals were of analytical-reagent grade.

The spectrophotometric detection was carried out on a V-530 UV-VIS spectrophotometer (Jasco). The pH values were measured with a pHS-3C precision pH meter (Shanghai, China).

Each color comparison tube was filled with 2.00 mL of pH 9.5 NH_3–NH_4Cl buffer solutions, 1.50 mL of 1.0×10^{-4} mol/L ACBK, 0.50 mL of 1.0×10^{-3} mol/L H_2O_2, a proper amount of tiopronin solutions, and 1.00 mL of 5.0×10^{-6} mol/L hemoglobin, and then diluted with water to 10 mL. After 18 min, absorbance was monitored at the selected maximum absorption wavelength of 563nm. The percentage inhibition (%I) was calculated on the base of the following equation:

$$\%I=100[(A_s-A_e)-(A_s-A_i)]/(A_s-A_e)=100[(A_i-A_e)/(A_s-A_e)]$$

where A_s, substrate absorbance alone; A_i, substrate absorbance in presence of hemoglobin and inhibitor and A_e, substrate absorbance in the presence hemoglobin only.

RESULTS AND DISCUSSION

The hemoglobin-catalyzed reaction is shown below:

$$H_2O_2 + ACBK \xrightarrow{hemoglobin[O]} ACBK^+ + H_2O$$

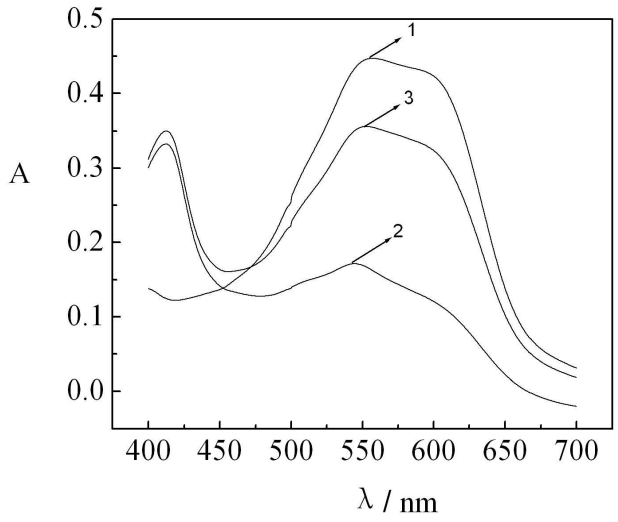

Fig.1. Absorbent spectra of the system

In this redox reaction between H_2O_2 and ACBK, different amounts of tiopronin had inhibitory effects on hemoglobin-catalyzed reaction. In addition, there was a good linearity between the amounts of tiopronin and I%, on which a new method was based. The absorbent spectra of hemoglobin-catalyzed reaction were obtained and are shown in Fig.1. It is noted that both in the absence of tiopronin and in the presence of tiopronin, the spectral shapes of the hemoglobin-catalyzed reaction were identical, and were consistent with that of the case in the absence of hemoglobin. They were similar in profile but different in size. The addition of tiopronin resulted in the inhibition of tiopronin on hemoglobin activity.

The variable and ranges studied and the consequent recommended values are summarized in Table 1.

Table 1 Optimization study for tiopronin determination by inhibition of hemoglobin

Variable	Range studied	Recommended value
pH	8.9-10.1	9.5
Hemoglobin (mol/L)	3.00-9.00× 10^{-7}	5.00× 10^{-7}
H_2O_2 (mol/L)	3.00-8.00× 10^{-5}	5.00× 10^{-5}
ACBK (mol/L)	0.50-3.00× 10^{-5}	1.50× 10^{-5}
Time (min)	1-25	18

It is noted that tiopronin has less effect in assay involving higher concentrations of hemoglobin. The percent inhibition increased with increase in hemoglobin concentration at first, but decreased over 5.00× 10^{-7} mol/L. So 5.00× 10^{-7} mol/L of hemoglobin was selected for further work.

The effect of H_2O_2 concentration on inhibition was studied. The I% increased with the increase in H_2O_2 up to 5.00× 10^{-5} mol/L, above which it had little effect. Thus 5.00× 10^{-5} mol/L H_2O_2 was selected for further study. The I% was greatest at pH 9.5. Considering the absorbance intensity getting too weak at very low ACBK concentration, 1.50× 10^{-5} mol/L ACBK was chosen for further study.

The effect of temperature on the system was investigated in a range from room up to 50□. The time needed to reach equilibrium, no more than 18 min, was prolonged with the decreasing temperature. Given decomposition of H_2O_2 at high temperature, temperature was kept at room temperature and the measurements were carried out after 18 min.

From the results obtained under the recommended conditions (Table 1), it was found that the degree of inhibition of tiopronin on the hemoglobin-catalyzed reaction was linear in the range 0.05 to 2 mg/L. The linear response can be fitted to an equation as follows:

$$I\% = (16.9031 \pm 2.3586) + (38.1377 \pm 2.5758)\,c \quad (r = 0.9866, n = 8)$$

"*c*" is the concentration of tiopronin in mg/L. "*r*" and "*n*" are the linear correlation coefficient and the number of experiments, respectively. The detection limit, calculated according to the $3S_b/k$ criterion (in which "*k*" is the slope over the range of linear used and "S_b" is the standard deviation (n=11) of the signal from the blank), was found to be 8.5μg/L. The relative standard deviation for 11 replicate determination of 1.5 mg/L tiopronin was 4.08%. The existing methods for the determination of tiopronin are summarized in Table 2. It can be seen that the proposed method is simple and sensitive.

Table 2 Comparison of existing method for the determination of tiopronin **with proposed method[a]**

Methods of determination	Detection limit (μg/L)	Linear range (mg/L)	References
SP(EI)	8.5	0.05 ～ 2	Proposed method in this paper
SP	200	0.39 ～ 15.7	[4]
FL	26.9	0.1 ～ 1	[5]
FL	150	0.15 ～ 20	[6]
CL	32.6	0.08 ～ 490	[7]
HPLC	12	0.04 ～ 4	[8]
LC-ESI-MS	12	0.03～8.2	[9]

[a]*SP: spectrophotometry; EI: enzymatic inhibition; FL: fluorimetry; CL: chemiluminescence; HPLC: high-performance liquid chromatography; LC-ESI-MS: high performance liquid chromatography-telectrospray ionization-mass spectrometry.*

Several common amino, reducing compounds and vitamins were investigated for their interference for the determination of 1.5 mg/L tiopronin. When the permitted relative deviation is larger than ±5.0%, the examined species may cause a significant alteration in the results. The results are shown in Table 3. Results show that the proposed method has good selectivity.

Table 3 The effect of various species on hemoglobin activity

Species	Tolerance ratio
K^+, Na^+, Cl^-, Ca^{2+}, Zn^{2+}, Mg^{2+}, glucose, fructose, BSA, methanol,	1000
Glycine, alanine, serine, threonine	500
Cystine, Cu^{2+}	100
Cysteine, tyrosine, Fe^{2+}	10
Fe^{3+}	2

The study carried out in this work was applied to determine tiopronin in tablets and injections. For analysis of tablets, accurate amount of powered tablets were dissolved in soluble distilled water and then the solution was filtered into a 100-mL calibrated flask. The injection solutions of tiopronin were appropriately diluted with distilled water. So, the final concentration was in the working range for further sample analysis. In order to examine the results, HPLC method was also used for determinations following a procedure described in literature [12]. The results obtained by the two different methods are statistically compared in Table 4. It can be seen that no significant differences were found between them. This confirms the validity of the method proposed in this work.

Table 4 Determination of tiopronin in pharmaceutical preparations

Samples	Label	Proposed method[a]	HPLC[12]	t[b]
Tablets (mg)	100	98.6 ± 1.8	99.1 ± 1.6	1.96
Injection (mg)	100	100.1± 0.7	99.6 ± 0.8	2.57

[a] Mean ±standard deviation of five determinations. [b] Theoretical value is 2.78, n=5, with 95% confidence level.

CONCLUSION

A new spectrophotometric method for trace amount of tiopronin determination was developed based on inhibitory effect of tiopronin on Hb-catalyzed reaction. The current method is very simple, sensitive and the detection limit is 8.5μg/L. The current method can be used for the determination of tiopronin in pharmaceuticals with satisfactory results.

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