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Spectrophotometric Determination of Promethazine Hydrochloride Based on Inhibition of Hemoglobin

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A highly sensitive and simple catalytic spectrophotometric method for the determination of promethazine hydrochloride based on inhibitory effect of promethazine hydrochloride on the hemoglobin-catalyzed the reaction of H_2O_2 and acid chrome blue K (ACBK) was developed. The concentration of promethazine hydrochloride is linear with the percentage inhibition (I %) of system under the optimal experimental conditions. The calibration graph is linear in range 6.23×10^{-8} to 1.56×10^{-5} mol L^{-1} with the detection limit of 9.32×10^{-10} mol L^{-1}. This method can be used for the determination of promethazine hydrochloride in tablets and injection solution of promethazine hydrochloride with satisfactory results.

Key Words: Catalytic spectrophotometry, Promethazine hydrochloride, Hemoglobin.

INTRODUCTION

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[1]. Horseradish peroxidase (HRP; EC 1.11.1.7) is one of the most important oxidases in biology. Having the function of active molecular oxygen, HRP can enhance the oxidation of H_2O_2 directly into H_2O. 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. Therefore, the search for a replacement for enzyme has been significant and interesting work. The mimicking of peroxidase is one of the interesting trends in enzymatic analysis[2-4]. Hemin has been used as a substitute for peroxidase[5]. The complex of ironporphyrin with β-cyclodextrin (β-CD) was proposed as a better substitute for native peroxide proteinase due to its three dimensions structure[6]. However, their catalytic activity was still much less than that of peroxidase. Hemoglobin (Hb), a necessary vehicle for oxygen carriage in body has the natural quaternary structure as enzymes. It contains four subunits of polypeptide and each polypeptide chain contains a heme group that may be able to serve as the active center[7,8]. In a recent paper hemoglobin was used based on its similar catalytic function as horseradish peroxidase[9].

Promethazine hydrochloride, a prominent compound in the large group of phenothiazine derivatives, is widely used as a therapeutic agent for treating various mental and personality disorder. Therefore, the determination of promethazine hydrochloride is one of the significant work in clinical analysis. Several methods have already been reported for the quantitative determination of promethazine, including UV-visible spectrophotometry[10,11], fluoriometry[12,13], chemiluminence[14], high-performance liquid chromatograph[15] and electrochemical analytical method[16,17]. In this paper, a new sepectrophotometric method based on inhibitory effect of promethazine hydrochloride on the hemoglobin-catalyzed the reaction of H_2O_2 and acid chrome blue K (ACBK) was proposed. The experimental conditions for the system were optimized and promethazine hydrochloride was detected by the decreased absorbance. This method is very simple, sensitive and the detection limit is 9.32×10^{-10} mol L^{-1}. The method has been applied to the determination of promethazine hydrochloride in tablets and injection solution of promethazine hydrochloride with satisfactory results.

EXPERIMENTAL

Hemoglobin (bovine erythrocytes) solution was prepared by dissolving certain amount of hemoglobin (Shanghai Institute of Biochemistry, Shanghai, China) in distilled water and stored below 4 ºC. Acid chrome blue K (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^{-1} 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. Promethazine hydrochloride (Kunming Kexiang Institute of Biochemistry, Kunming, China) solution was prepared in the concentration of 3.10×10^{-3} mol L^{-1}. 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 colour comparison tube was filled with 2.00 mL of pH 9.5 NH_3-NH_4Cl buffer solutions, 3.00 mL of 1.0×10^{-4} mol L^{-1} ACBK, 0.80 mL of 1.0×10^{-3} mol L^{-1} H_2O_2, a proper amount of promethazine hydrochloride solutions and 1.00 mL of 5.0×10^{-6} mol L^{-1} hemoglobin and then diluted with water to 10 mL. After 15 min, absorbance was monitored at the selected maximum absorption wavelength of 555 nm. 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:

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

In this redox reaction between H_2O_2 and ACBK, different amounts of promethazine hydrochloride had inhibitory effects on hemoglobin-catalyzed reaction. In addition, there was a good linearity between the amounts of promethazine hydrochloride 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 promethazine hydrochloride and in the presence of promethazine hydrochloride, 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 promethazine hydrochloride resulted in the inhibition of promethazine hydrochloride on hemoglobin activity.

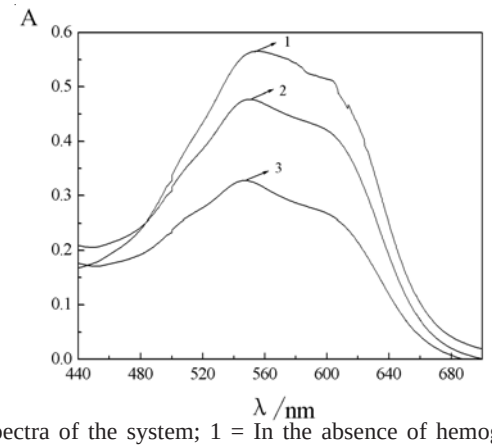

Fig.1. Absorbent spectra of the system; 1 = In the absence of hemoglobin and promethazine hydrochloride; 2 = In the presence of hemoglobin only; 3 = In the presence of hemoglobin and promethazine hydrochloride, 8.00×10^{-5} mol L^{-1} H_2O_2, 3.00×10^{-5} mol L^{-1} ACBK, 5.00×10^{-7} mol L^{-1} hemoglobin and 9.23×10^{-6} mol L^{-1} promethazine hydrochloride

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

TABLE-1
OPTIMIZATION STUDY FOR PROMETHAZINE HYDROCHLORIDE DETERMINATION BY INHIBITION OF HEMOGLOBIN

Variable	Range studied	Recommended value
pH	9.3-11.0	9.5
Hemoglobin (mol L^{-1})	$0.50\text{-}7.50 \times 10^{-7}$	5.00×10^{-7}
H_2O_2 (mol L^{-1})	$0.10\text{-}1.50 \times 10^{-4}$	8.00×10^{-5}
ACBK (mol L^{-1})	$1.50\text{-}3.50 \times 10^{-5}$	3.00×10^{-5}
Time (min)	1-25	15

It is noted that promethazine hydrochloride has less effect in assay involving higher concentrations of hemoglobin. The per cent inhibition increased with increase in hemoglobin concentration at first, but decreased over 5.00×10^{-7} mol L^{-1}. It might be due to the loss of substrate inhibition, which occurs at high hemoglobin concentration, which could be due to the inability of promethazine hydrochloride to promote conformational changes when hemoglobin is at high concentration. So 5.00×10^{-7} mol L^{-1} 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 8.00×10^{-5} mol L^{-1}, above which it had little effect. Thus 8.00×10^{-5} mol L^{-1} 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, 3.00×10^{-5} mol L^{-1} ACBK was chosen for further study.

The effect of temperature on the system was investigated in a range from room temperature up to 50 ºC. The time needed to reach equilibrium, not more than 15 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 15 min.

From the results obtained under the recommended conditions (Table-1), it was found that the degree of inhibition of promethazine hydrochloride on the hemoglobin-catalyzed reaction was linear in the range 6.23×10^{-8} to 1.56×10^{-5} mol L^{-1}. The linear response can be fitted to an equation as follows:

$$I\ \% = (10.7873 \pm 0.9032) + (5.4305 \pm 0.1359) \times \frac{C}{10^{-6}}\ (R = 0.997,\ n = 9)$$

'C' is the concentration of promethazine hydrochloride in mol L^{-1}. '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 9.32×10^{-10} mol L^{-1}. The relative standard deviation for 11 replicate determination of 3.10×10^{-6} mol L^{-1} promethazine hydrochloride was 4.10 %. The existing methods for the determination of promethazine hydrochloride are summarized in Table-2. It can be seen that the proposed method has higher sensitivity.

Several common amino, reducing compounds and vitamins were investigated for their interference for the determination of 9.32×10^{-6} mol L^{-1} promethazine hydrochloride. When the permitted relative deviation is larger than ± 5.0 %, the examined species may cause a significant alteration in the results. Results show that the proposed method has good selectivity.

The proposed method was applied to determine promethazine hydrochloride in pharmaceuticals by using the procedure described in the experimental section. For analysis of tablets, accurate amount of powdered tablets were dissolved in double distilled water and then the solution was filtered into a 100 mL calibrated flask.

TABLE-2
COMPARISON OF EXISTING METHOD FOR THE DETERMINATION OF PROMETHAZINE HYDROCHLORIDE WITH PROPOSED METHOD*

Methods of determination	Detection limit (mol L^{-1})	Linear range (mol L^{-1})	References
UV-Vis (EI)	9.32×10^{-10}	6.23×10^{-8} to 1.56×10^{-5}	Proposed method
UV-Vis	2.96×10^{-6}	1.03×10^{-5}~8.32×10^{-4}	10
UV-Vis	2.97×10^{-6}	7.42×10^{-6}~1.48×10^{-4}	11
FL	3.10×10^{-7}	3.10×10^{-7}~3.1×10^{-5}	12
FL	–	1.60×10^{-6}~2.5×10^{-4}	13
CL	4.40×10^{-6}	6.00×10^{-5}~8.0×10^{-4}	14
ECL	3.00×10^{-10}	4.70×10^{-10}~9.3×10^{-9}	16
ECL	2.20×10^{-4}	1.60×10^{-5}~1.9×10^{-3}	17

*UV-Vis = Ultraviolet spectrophotometry; EI = Enzymatic inhibition; FL = Fluorimetry; CL = Chemiluminescence; ECL = Electrochemical analytical method.

The injection solutions of promethazine hydrochloride (different batch number) were diluted to different concentrations with double distilled water, so the final concentration was in the working range for further sample analysis. In order to evaluate the validity of the proposed method, UV-Vis spectrophotometric method was also used for the determinations by closely following a procedure described in the pharmacopoeia[18]. The results obtained by the two different methods were statistically compared in Table-3. It can be seen that no significant differences were found between them. It is indicated that the method is reliable for the determination of promethazine hydrochloride in pharmaceutical preparations.

TABLE-3
DETERMINATION OF PROMETHAZINE HYDROCHLORIDE IN PHARMACEUTICAL PREPARATIONS

Sample	Label	Proposed method*	UV-Vis spectrophotometric [Ref. 18]	t**
Tablets 1 (mg /tablet)	12.5	12.48 ± 0.05	12.51 ± 0.07	2.06
Tablets 2 (mg /tablet)	25.0	24.96 ± 0.05	12.08 ± 0.07	2.53
Injection (mg mL^{-1})	25.0	25.12 ± 0.03	25.18 ± 0.06	1.97

*Mean ± Standard deviation of five determinations.
**Theoretical value = 2.78, n = 5, with 95 % confidence limits.

Conclusion

A new spectrophotometric method for trace amount of promethazine hydrochloride determination was developed based on inhibitory effect of promethazine hydrochloride on hemoglobin-catalyzed reaction. This method can be used for the determination of promethazine hydrochloride in pharmaceuticals with satisfactory results.

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