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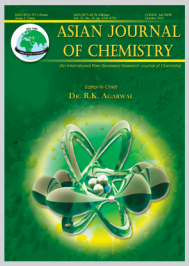

Spectrophotometric Determination of Catecholamines with Hemoglobin as Catalyst

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A high sensitive and simple spectrophotometric method for the determination of catecholamines based on their inhibitory effect on the hemoglobin-catalyzed reaction of H_2O_2 and acid chrome blue K(ACBK) was developed. The concentrations of the catecholamines are in linear relationship with the percentage inhibition (I %) of system under the optimal experimental conditions. The detection limit of the method were 6.1×10^{-8} mol L^{-1} dopamine, 5.2×10^{-8} mol L^{-1} adrenaline and 2.4×10^{-9} mol L^{-1} dobutamine, respectively. This method can be used for the determination of the catecholamines in pharmaceuticals with satisfactory results.

Key Words: Catecholamines, Hemoglobin, Spectrophotometry.

INTRODUCTION

Enzyme-catalyzed analytical kinetic methods have been extensively used for substrate, enzyme, inhibitor and activator analysis in several areas 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. However, natural enzymes have shortcomings in some aspects. For example, they are expensive and unstable in solution and have strict requirements for the experimental conditions and storage environment in order to retain their catalytic activity. Therefore, the search for a replacement for enzymes is significant and interesting work. 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 an active center[2,3]. In a recent paper, hemoglobin was determined based on its similar catalytic function as horseradish peroxidase[4].

Dopamine, adrenaline and dobutamine are known as catecholamines, which play important roles in the central nervous system as neurotransmitters. These catecholamines with a structure of the phenylethyl amine group, are widely used to treat hypertension, bronchial asthma, cardiac arrest, myocardial infarction and cardiac surgery. Therefore, a highly sensitive method is necessary for the determination of the catecholamines in pharmaceutical samples. Several methods have already been reported for the quantitative determination of three catecholamines in pharmaceutical samples and biological fluids, including UV-visible spectrometry[5], fluorimetry[6,7], high-performance liquid chromatography[8] and chemiluminescence[9].

In this paper, a new spectrophotometric method based on the inhibitory effect of hemoglobin (Hb)-catalyzed reaction of H_2O_2 and acid chrome blue K (ACBK) by the catecholamines was proposed. The experimental conditions for the system were optimized and catecholamines were detected by the decreased absorbent intensity. This method is very simple, sensitive and the detection limit were 6.1×10^{-8} mol L^{-1} dopamine, 5.2×10^{-8} mol L^{-1} adrenaline and 2.4×10^{-9} mol L^{-1} dobutamine, respectively. This method can be used for the determination of the catecholaimines in pharmaceuticals with satisfactory results.

EXPERIMENTAL

Hemoglobin (bovine erythrocytes) solution was prepared by dissolving the certain amount of Hb (Shanghai Institute of Biochemistry, Shanghai, China) in distilled water and stored below 4 ºC. 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. Hydrogen peroxide solution was prepared by appropriate 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. Dopamine, adrenaline and dobutamine (Shanghai Aobo Institute of Biochemistry, Shanghai, China) solution was prepared in the concentration of 1.0×10^{-3} mol L^{-1}. Working solution was diluted appropriately before use with distilled water daily. NH_3-NH_4Cl buffer solution had value of pH 9.1-10.7.

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 temperature was controlled by using a TB-85 thermostat bath (Shimadzu) and the pH values were measured with a pHS-3C precision pH meter (Shanghai, China).

Each colour comparison tube was filled with 2 mL of pH 9.8, pH 9.8 and pH 9.5 NH_3-NH_4Cl buffer solution, 1.0, 1.0 and 0.8 mL of 1.0×10^{-3} mol L^{-1} H_2O_2, 2.0, 2.0 and 2.3 mL of 1.0×10^{-4} mol L^{-1} ACBK, a proper amount of dopamine, adrenaline and dobutamine solutions and 0.9, 1.8 and 1.2 mL of 1.0×10^{-5} mol L^{-1} Hb and then diluted with water to 10 mL. After being kept in room temperature for 18, 15 and 15 min, absorbance was monitored at the selected maximum absorption wavelength of 544, 546 and 548 nm. The percentage inhibition (I %) was calculated on the basis of the following equation:

$$I(\%) = 100\left[\frac{(A_s - A_e) - (A_s - A_i)}{(A_s - A_e)}\right] = 100\left[\frac{(A_i - A_e)}{(A_s - A_e)}\right]$$

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

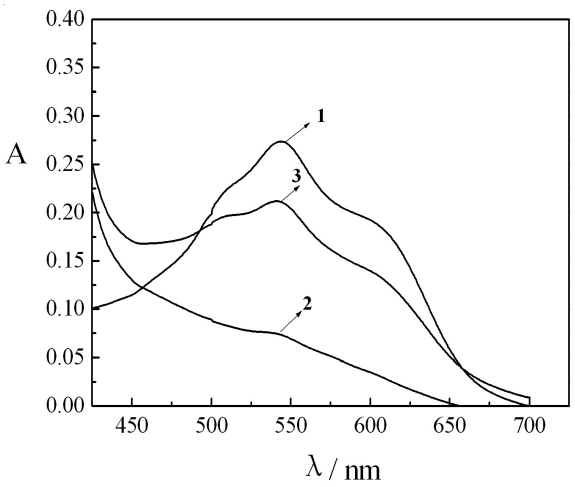
RESULTS AND DISCUSSION

The Hb-catalyzed reaction is shown below:

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

In this redox reaction between H_2O_2 and ACBK, different amounts of dopamine, adrenaline and dobutamine had inhibitory effects on Hb-catalyzed reaction. In addition, there was a good linear relationship between the amounts of the catecholamines and I %, on which a new method was based. The absorption spectra of Hb-catalyzed reaction were obtained (Fig. 1). It is to be noted that both in the absence of the catecholamines and in the presence of the catecholamines, the spectral shapes of the Hb-catalyzed reaction were identical and were consistent with that in the absence of Hb. They were similar in profile but different in size. The addition of the catecholamines resulted in the inhibition effect of the catecholamines on Hb activity.

The variables and ranges were studied. The corresponding recommended values are summarized in Table-1.



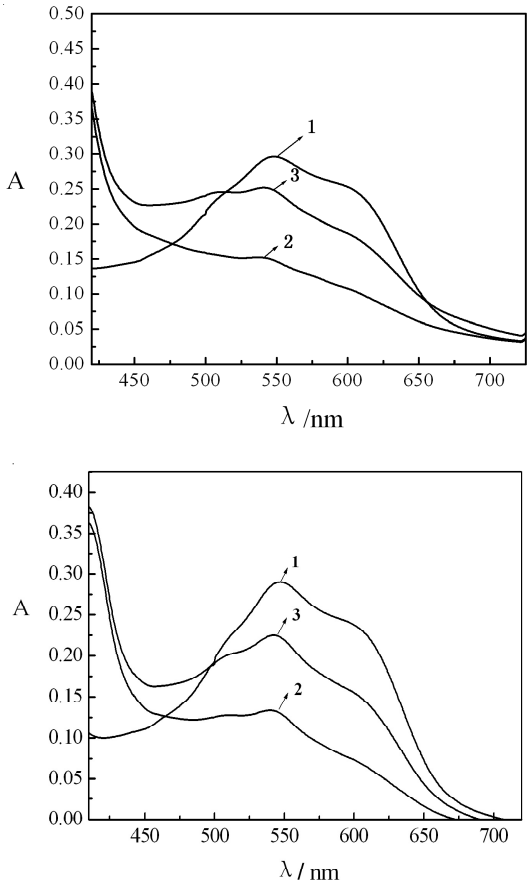


Fig. 1. Absorption spectra of the system. 1:in the absence of Hb and dopamine,adrenaline and dobutamine; 2: in the presence of Hb only; 3: in the presence of Hb and dopamine, adrenaline and dobutamine, respectively

It is noted that the catecholamines has less effect in assay involving higher concentrations of Hb. The per cent inhibition increased with increase in Hb concentration at first, but decreased over 9.0×10^{-7} mol L^{-1} for dopamine, 1.8×10^{-6} mol L^{-1} for adrenadine and 1.2×10^{-6} mol L^{-1} for dobutamine, respectively. It might be due to the loss of substrate inhibition, which occurs at high Hb concentration, which could be due to the inability of the catecholamines to promote conformational changes when Hb is at high concentration. So 9.0×10^{-7} mol L^{-1} for dopamine, 1.8×10^{-6} mol L^{-1} for adrenadine and 1.2×10^{-6} mol L^{-1} for dobutamine of Hb were chosen for further work.

The effect of H_2O_2 concentration on the catecholamines inhibition was studied. The I % increased with increase in H_2O_2 up to 1.0×10^{-4} mol L^{-1} for dopamine, 1.0×10^{-4} mol L^{-1} for adrenadine and 8.0×10^{-5} mol L^{-1} for dobutamine, above which it had little effect. Thus 1.0×10^{-4} mol L^{-1} for dopamine, 1.0×10^{-4} mol L^{-1} for adrenadine and 8.0×10^{-5} mol L^{-1} for dobutamine of H_2O_2 were used in the recommended procedure. The pH-dependence system was investigated over the range of pH 9.1-10.7 using NH_3-NH_4Cl buffer. The I % increases with pH value up to 9.8 for dopamine, 9.8 for adrenadine and 9.5 for dobutamine, respectively. Hence, the NH_3-NH_4Cl buffers with pH 9.8 for dopamine, 9.8 for adrenadine and 9.5 for dobutamine were chosen in the subsequent experiments.

TABLE-1
OPTIMIZATION STUDY FOR THE DETERMINATION OF CATECHOLAMINES BY INHIBITION OF HEMOGLOBIN

Catecholamines	pH	H_2O_2 concentration (mol L^{-1})	ACBK concentration (mol L^{-1})	Hb concentration (mol L^{-1})	Stable time (min)
Dopamine	9.8	1.0×10^{-4}	2.0×10^{-5}	9.0×10^{-7}	18
Adrenaline	9.8	1.0×10^{-4}	2.0×10^{-5}	1.8×10^{-6}	15
Dobutamine	9.5	8.0×10^{-5}	2.3×10^{-5}	1.2×10^{-6}	15

TABLE-2
LINEAR RANGE AND CORRELATION COEFFICIENTS OF THE CALIBRATION GRAPHS AND THEIR LIMIT OF DETECTION

Catecholamines	Linear equation (c: mol L^{-1})	r	Linear range (mol L^{-1})	Limit of detection (mol L^{-1})
Dopamine	$I\ \% = (-1.3136 \pm 1.5512) + (66.7634 \pm 3.1443)\left[\frac{c}{10^{-5}}\right]$	0.9923	$1.1 \times 10^{-7} - 1.1 \times 10^{-5}$	6.1×10^{-8}
Adrenaline	$I\ \% = (0.0999) \pm 0.0160) + (0.6289 \pm 0.0273)\left[\frac{c}{10^{-5}}\right]$	0.9943	$4.5 \times 10^{-7} - 1.4 \times 10^{-5}$	5.2×10^{-8}
Dobutamine	$I\ \% = (17.0732 \pm 1.8013) + (8.2068 \pm 0.4981)\left[\frac{c}{10^{-6}}\right]$	0.9874	$7.2 \times 10^{-8} - 7.2 \times 10^{-6}$	2.4×10^{-9}

TABLE-3
ANALYTICAL RESULTS OF SAMPLE

Samples	Labeled (mg mL^{-1})	Proposed method[a] (mg mL^{-1})	Titration (mg mL^{-1})	t[b]
Injection 1 (091211)	10	9.86 ± 0.08	9.91 ± 0.06	1.69
Injection 2 (110104)	10	10.10 ± 0.07	10.16 ± 0.08	2.27
Injection 3 (100709)	10	10.14 ± 0.07	10.18 ± 0.07	1.36

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

As for the substrate, the I % increased with increase in ACBK up to 2.0×10^{-5} mol L^{-1} for dopamine, 2.0×10^{-5} mol L^{-1} for adrenadine and 2.3×10^{-5} mol L^{-1} for dobutamine, above which it had less effect. Considering of decomposition of H_2O_2 at high temperature, room temperature was chosen for using in subsequent work. Maximum inhibition of the catecholamine appeared after 18 min for dopamine, 15 min for adrenadine and 15 min for dobutamine. Therefore, the measurements were carried out after 18 min for dopamine, 15 min for adrenadine and 15 min for dobutamine, respectively.

From the results obtained under the recommended conditions (Table-1), it was found that the degree of inhibition of the catecholamines on the Hb-catalyzed reaction were linear (Table-2). Table-2 lists the parameters of the calibration curve and relative standard deviations for the detection (n = 11) of dopamaine, adrenaline and dobutamine were 4.5, 3.5 and 4.1 % when the concentrations of the catecholaines were at 6.0×10^{-6} mol L^{-1}, respectively.

Several common ions, reducing compounds and vitamins were investigated for their interference in the determination of 6.0×10^{-6} mol L^{-1} catecholamines. When the permitted relative deviation is larger than ± 5.0 %, the examined species may cause a significant alteration in the results. Most of the compounds at low concentration hardly influence the catalysis activity of Hb, while ions such as copper(II), iron(III) inhibit the catalysis activity of Hb. 0.1 mmol L^{-1} EDTA was added to buffer to elimate the interference.

The proposed method was applied to determine the catecholamines in pharmaceuticals by using the procedure described in the experimental section. The injection solutions of dopamaine, adrenaline and dobutamine (different batch number) were appropriately diluted with water. So, the final concentration was in the working range for further sample analysis. In order to examine the results, the titration was also used for determinations following a procedure described in the literature[10]. The results obtained by the two different methods are statistically compared in Table-3. It can be seen that no significant differences were found between them. This confirms the validity of the method proposed in this work. This method can be used for the determination of catecholamines in pharmaceuticals with satisfactory results.

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