

## Kinetic-Spectrophotometric Determination of L-Dopa, Methyl-dopa, Dopamine and Adrenaline

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A simple, sensitive and reliable method is proposed for the determination of trace quantities of L-dopa, methyl-dopa, dopamine and adrenaline. The method is based on the inhibition effect of these catecholamines on the reaction of nitrite with neutral red in acidic media. The reaction is monitored spectrophotometrically by measuring the decrease in the absorbance of neutral red solution at 530 nm with a fixed time method of 3 min. The calibration graphs were linear in the ranges of  $3.05 \times 10^{-6}$ – $1.62 \times 10^{-5}$  M for L-dopa,  $1.8 \times 10^{-6}$ – $1.33 \times 10^{-5}$  M for methyl-dopa,  $2.61 \times 10^{-6}$ – $2.67 \times 10^{-5}$  M for dopamine and  $1.59 \times 10^{-6}$ – $3.27 \times 10^{-6}$  M for adrenaline. This method was applied for the determination of the catecholamines in pharmaceutical formulations with satisfactory results.

**Key words:** kinetic, spectrophotometric determination, L-dopa, methyl-dopa, dopamine, adrenaline

### INTRODUCTION

Catecholamines are widely used in pharmaceutical preparations. Therefore their determination is of special interest. Although several methods have been reported for the determination of such compounds<sup>1–6</sup>, but the need for a rapid, sensitive, simple and reliable method for determination of these compounds is still recognized. Kinetic methods of chemical analyses which have several advantages, including high sensitivity, low detection limit, good selectivity, rapid analysis and inexpensive instruments such as a spectrophotometer or spectrofluorimeter are widely used for the determination of different inorganic and organic species<sup>7–10</sup>.

In this paper a kinetic spectrophotometric method based on the inhibitory effect of L-dopa, methyl-dopa, dopamine and adrenaline in the reaction of neutral red with nitrite ion has been introduced for the determination of trace amounts of these catecholamines. The proposed method is rapid, simple, reliable and sensitive and is suitable for the determination of trace quantities of L-dopa, methyl-dopa, dopamine and adrenaline both in pure form and pharmaceutical preparations.

### EXPERIMENTAL

All solutions were prepared using analytical-reagent grade and triply-distilled water. A 1000 mg/L nitrite stock solution was prepared by dissolving 1.5000 g

NaNO<sub>2</sub> (Merck), previously dried for 2 h at 110°C, in water and diluting to the mark in a 100-mL volumetric flask. This solution was standardized<sup>11</sup>. A 100 mg/L neutral red solution was prepared by dissolving 0.0100 g neutral red (Merck) in water and diluting to 100 mL in a volumetric flask. L-Dopa, methyl-dopa, dopamine and adrenaline stock solutions ( $5 \times 10^{-3}$  M) were prepared from Sigma products in water and stored in dark bottles in a refrigerator. Working solutions were prepared by diluting the stock solutions with water. Sulfuric acid solution was prepared by appropriate dilution of concentrated sulfuric acid (Merck).

A Cecil model CE 1020 spectrophotometer with a 1-cm glass cell was used for absorbance measurements.

### Procedure

The reaction was followed spectrophotometrically by monitoring the change in absorbance at 530 nm. All the solutions were equilibrated at  $25 \pm 0.1^\circ\text{C}$  before beginning of the reaction.

A suitable aliquot of sample solution containing suitable amount of L-dopa, methyl-dopa, dopamine or adrenaline was transferred into a 10-mL volumetric flask and then 1.0 mL of 5.0 M sulfuric acid solution was added, followed by 1.0 mL of 25.0 mg/mL nitrite solution. The solution was diluted to *ca.* 7.5 mL with water. To initiate the reaction, 2.5 mL of 100  $\mu\text{g/mL}$  neutral red solution was added. The solution was diluted to the mark with water and a portion was transferred into a glass cell within 30 s. The decrease in absorbance at 530 nm was measured for the first 3 min after initiation of reaction.

## RESULTS AND DISCUSSION

Neutral red is a nitrosable aromatic amine. Its reaction with nitrite in acidic media could be monitored spectrophotometrically by measuring the decrease in the absorbance of reaction mixture<sup>12</sup> at 530 nm ( $\lambda_{\text{max}}$  for the absorption spectra of neutral red). We observed that the presence of trace quantities of L-dopa, methyl-dopa, dopamine or adrenaline inhibits the reaction and the decrease in the reaction rate is proportional to the catecholamine quantity in a concentration range. Therefore the reaction was used for the determination of trace amounts of L-dopa, methyl-dopa, dopamine and adrenaline.

**Effect of variables:** Various experimental parameters, including reagent concentrations, ionic strength and temperature, were studied in order to obtain optimized systems. These parameters were optimized by setting all parameters to be constant and optimizing one each time; L-dopa was used as model catecholamine.

The effect of temperature was studied in the range of 5–45°C. An increase in temperature caused an increase in the absorbance change of both the uninhibited (the reaction in the absence of L-dopa) and inhibited (the reaction in the presence of L-dopa) reactions up to 30°C and decreased at higher temperatures. Fig. 1 shows the difference between the absorbance change of uninhibited reaction and inhibited reactions as a function of temperature. As Fig. 1 shows, this difference increases up to 25°C and decreases at higher temperatures. Therefore, 25°C was selected as optimum temperature.

The effect of nitrite ion concentration was studied in the range of 0.0–4.0  $\mu\text{g/mL}$ . An increase in nitrite ion concentration caused an increase in the absorbance change of both the inhibited and uninhibited reactions up to 2.5  $\mu\text{g/mL}$  and decreased at higher concentrations. Fig. 2 shows the difference between the absorbance change of uninhibited reaction and inhibited reactions as a function of nitrite ion concentration. As Fig. 2 shows, this difference increases up to 2  $\mu\text{g/mL}$  and remains nearly constant at higher concentrations. Therefore, 2.5  $\mu\text{g/mL}$  of nitrite was selected as optimum concentration.

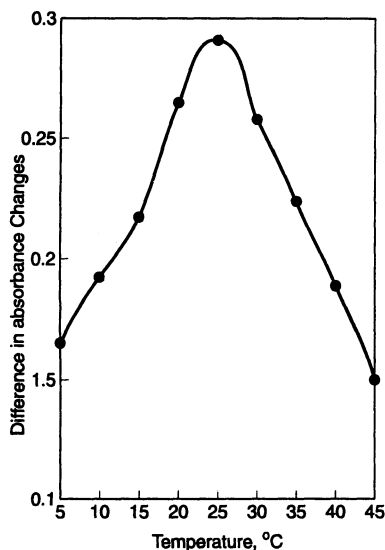


Fig. 1. The difference between inhibited and uninhibited reaction rates as a function of temperature.

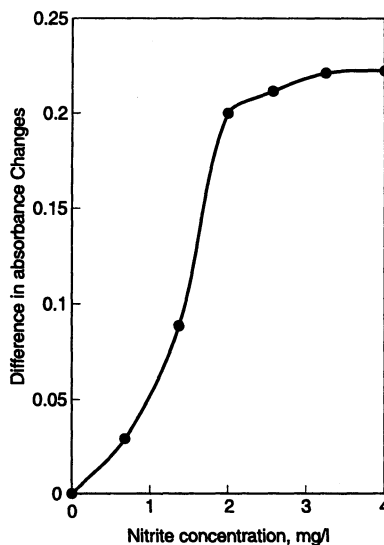


Fig. 2. The difference between inhibited and uninhibited reaction rates as a function of nitrite concentration.

The reaction proceeds in acidic media. Different acids were tested and sulfuric acid was found as the best. The effect of sulfuric acid concentration was also investigated. As Fig. 3 shows, a final concentration of 0.5 M sulfuric acid was the optimum.

The effect of neutral red concentration was studied in the range of 0.0–40.0  $\mu\text{g/mL}$ . Fig. 4 shows the difference between the absorbance change of uninhibited reaction and inhibited reactions as a function of neutral red concentration. As Fig. 4 shows this difference increases with increasing neutral red concentration up to 25  $\mu\text{g/mL}$  and decreases at higher concentrations. Therefore, a concentration of 25  $\mu\text{g/mL}$  neutral red was selected as optimum value.

Ionic strength had no considerable effect up to 1.0 M.

**Analytical Parameters:** The calibration graph was obtained under the optimum conditions described above. Table-1 shows a linear calibration data for the

investigated catecholamines. For determination of the accuracy and precision of the method, a series of independent synthetic samples were analyzed by the proposed methods. The results are given in Table-2.

TABLE-1  
LINEAR REGRESSION OF CALIBRATION DATA FOR INVESTIGATED  
CATECHOLAMINES

Catecholamine	Slope ( $M^{-1}$ )	Intercept	Correlation coefficient	Calibration range ( $\mu M$ )	Limit of detection <sup>a</sup> ( $\mu M$ )
L-Dopa	$-3.31 \times 10^4$	0.493	0.9985	3.05–16.2	1.18
Methyldopa	$-1.54 \times 10^4$	0.528	0.9991	1.89–13.3	1.02
Dopamine	$-9.7 \times 10^3$	0.501	0.9978	2.61–26.7	1.52
Adrenaline	$-6.6 \times 10^3$	0.507	0.9995	6.8–33.3	3.21

<sup>a</sup> Defined as  $Y_{LOD} = Y_B - 3S_B$ <sup>13</sup> where  $Y_{LOD}$ ,  $Y_B$  and  $3S_B$  are signal of detection limit, signal of blank and standard deviation of blank respectively.

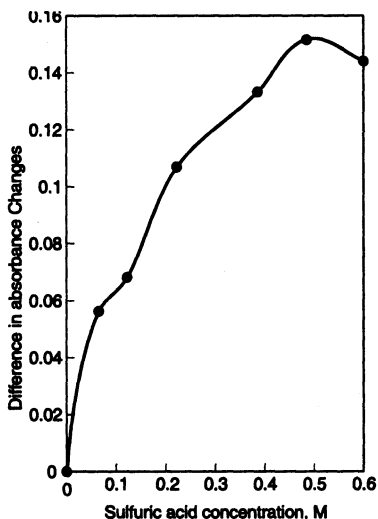


Fig. 3. The difference between inhibited and uninhibited reaction rates as a function of sulfuric acid concentration.

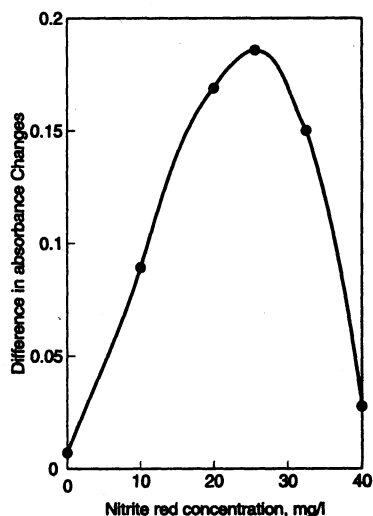


Fig. 4. The difference between inhibited and uninhibited reaction rates as a function of neutral red concentration.

**Application:** To evaluate the analytical applicability of the method, the recommended procedure was applied to the determination of L-dopa, methyldopa, dopamine and adrenaline in pharmaceutical preparations. For the analysis of tablets, 20 tablets were weighed carefully and powdered. An accurately weighed

quantity of the powder was shaken with water. After filtration and proper dilution of the solution the recommended procedure was followed. The results are given in Table-3. As Table-3 shows, there is a good agreement between the obtained values and manufacturer's values.

TABLE-2  
ACCURACY AND PRECISION OF THE METHOD

Catecholamine	Taken ( $\mu\text{M}$ )	Found ( $\mu\text{M}$ )	Relative error (%)	Relative standard deviation (n = 10) (%)
L-Dopa	3.28	3.35	+2.13	1.94
	4.37	4.32	-0.68	1.62
	5.46	5.53	+1.28	1.15
	10.93	11.02	+0.82	0.54
Methyldopa	2.84	2.91	+2.46	1.84
	3.79	3.75	+1.05	1.53
	4.74	4.82	+1.69	1.12
	9.48	9.40	-0.85	0.97
Dopamine	3.92	3.86	-1.45	1.95
	5.23	5.27	+0.76	1.43
	6.54	6.61	+1.12	1.35
	13.07	12.94	-0.98	1.08
Adrenaline	8.25	8.03	-2.67	2.17
	15.32	15.63	+2.02	1.86
	22.63	22.63	0.00	1.54
	28.05	27.95	-0.36	1.15

TABLE 3  
ASSAY OF SOME DRUG DOSAGE FORMS  
BY THE PROPOSED METHOD

Analyte	Nominal value	Found by proposed method <sup>a</sup>
L-Dopa	500 mg/tablet	493 mg/tablet
Methyldopa	250 mg/tablet	252 mg/tablet
Dopamine	10.0 mg mL <sup>-1</sup>	9.81 mg mL <sup>-1</sup>
Adrenaline	1.8 mg mL <sup>-1</sup>	1.85 mg mL <sup>-1</sup>

<sup>a</sup> average of seven determinations

## Conclusion

The proposed kinetic method provides a simple and sensitive approach for the determination of catecholamines in pharmaceutical preparations. No sample pretreatment is necessary and the procedure is rapid. The proposed method compares well with the others in regard to simplicity, speed and detectability.

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(Received: 24 August 2001; Accepted: 24 October 2001)

AJC-2498