Use of integral and differential methods for the determination of L-dopa in pure form and pharmaceutical preparations

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Abstract

A new simple and selective kinetic method for the determination of L-dopa, using differential and integral methods, is described. The spectrophotometric measurements were recorded by measuring the increase in absorbance at 300 nm. The concentration range was valid from 5–35 ppm. The complex ratio showed a (1:2) of L-dopa with respect to sodium hydroxide, with formation constant 5.06×10^6 and molar absorptivity of $3.85 \times 10^3 \, \text{l mol}^{-1} \, \text{cm}^{-1}$. © 1997 Elsevier Science B.V.

Keywords: L-Dopa; Determination; Kinetic method

1. Introduction

Levodopa 3 (3,4-dihydroxyphenyl)-L-alanine is used in combination with carbidopa to treat Parkinson's disease [1]. The US Pharmacopeia (USPXX) [2] specifies maximum acceptable limits of two impurities in each of the two separate bulk powders of levodopa and carbidopa. For levodopa, it requires that the major component be assayed by non-aqueous titration. The current (USPXX II) [3] has used a potentiometric titration for determining the end point for the pure levodopa and for the assay of capsules and tablets using the ultraviolet spectrophotometric method. The British Pharmacopeia [4] also required a non-aqueous titration for the assay of levodopa and the impurities are measured by thin layer chromatography (TLC).

The spectrophotometric determination of this drug offers advantages of both sensitivity and simplicity, thus, various spectrophotometric methods have been described [5–15]. The literature has also recorded some techniques which have been utilized for the analysis of levodopa, such as amperometric oxidation [16], HPLC with electrochemical detection [17], coulometric titration [18] and flow injection analysis (FIA) [19,20].

Recently, titrimetric method [20] using bromine chloride, have been used, and voltammetric oxidation at platinum and glassy carbon electrodes [21]. However, less attention has been given to the kinetic study of such reaction. The development of a kinetic method which could display high selectivity, accuracy and simplicity has always been of interest, therefore, the aim of this study is

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to (i) develop an accurate and sensitive kinetic method for the analysis of levodopa, (ii) to perform the analysis of levodopa in pharmaceutical preparations either when present alone or in combination with carbidopa without further process of separation, and (iii) to propose simple method and fast determination of the drug.

2. Experimental

2.1. Apparatus

A Bausch and Lomb Spectronic-1001 spectrophotometer was used to record the absorbance. A controlled temperature water bath NSW-133 was used.

2.2. Reagents

All chemicals used were of analytical or pharmaceutical grade. The stock solution of sodium hydroxide (0.05 M) was freshly prepared. Double distilled water was used throughout.

Pure levodopa 5 mg ml^{-1} (Wallace, India), or 2.535×10^{-2} mol l^{-1} was dissolved in distilled water, and the apparent purity was checked by 1, 10 phenanthroline method [14]. the stock solution of levodopa should be kept in a well-closed dark container to avoid direct contact with light.

Levodopa was also prepared from pharmaceutical preparations. Tablets to be analysed were powdered and dissolved in distilled water and filtered through a Whatman No. 1 filter paper. The filtrate was adjusted to the final concentration of 5 mg ml^{-1}. The recommended procedure was followed.

2.3. Procedure for the determination of levodopa

Aliquots of 0.01–0.06 ml of the standard solution of levodopa (2.535×10^{-2} mol l^{-1}) were pipetted into a series of 10 ml volumetric flasks. Then, 1.0 ml of 0.05 mol l^{-1} sodium hydroxide was added, dilute to volume with double distilled water at 303 K. After mixing, immediately transfer it to the spectrophotometric cell and after 1 min record the absorbance-time curve at 300 nm,

prepare a blank in the same way but omit levodopa sample. Obtain the intercept and slope from the initial straight line portions of these curves. The calibration graph can be obtained by plotting $\log v$ vs. $\log c$ or plotting intercept (K_t) vs. levodopa concentration.

3. Results and discussion

The interaction of levodopa (1,2-diphenolic) with hydroxide ion (OH$^-$) gives rise to the formation of a yellow colour product, having an absorption maximum at 300 nm (Fig. 1). The intensity of the yellow colour increases with time, then starts decreasing and finally changes to deep brown product. From the absorption spectra, it is observed that pure levodopa in distilled water having a maxima at 280 nm (Fig. 1a), and a shift in maxima is attained after adding sodium hydroxide (Fig. 1b). This type of reaction may ac-
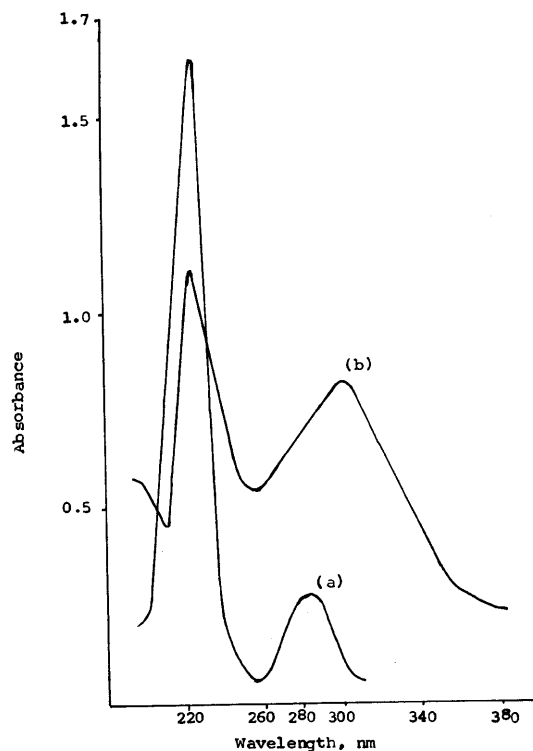

Fig. 1. Absorption spectra: (a) 15 ppm of pure levodopa in water, (b) 15 ppm L-dopa with 0.1M NaOH.
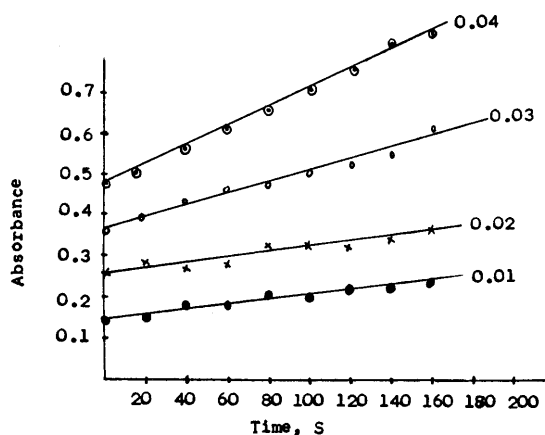
Fig. 2. Kinetic graph for the reaction of L-dopa (2.54×10^{-2} mol L^{-1}) with 0.05 mol L^{-1} sodium hydroxide, at room temperature (303 K), $\lambda_{max} = 300$ nm.

count for the formation of anionic sigma complex or aryloxy free radical, where the product formed is highly unstable. The slope of the absorbance-time curve (Fig. 2) is used as a measure of the reaction rate, for solutions containing different concentrations of levodopa. The differential as well as the integral methods have been used to calculate the true order. According to the rate equation, the reactions are made pseudo-first order by taking one reagent in a small amount than the other. The plot of $\log A_\infty / A_\infty - A_t$ vs. time has also been drawn, showing a linear relationship and can be utilized to calculate the order of the reaction and rate constant, which is equally the same as obtained by the differential method. Beer's law valid over a narrow concentration range from 5–35 pm. The molar absorptivity is equal to 3.85×10^3 1 mol^{-1} cm^{-1}. Continuous variation method has been used (Fig. 3) to establish the stoichiometry of levodopa with hydroxide ion. The ratio shows (1:2) of levodopa with respect to hydroxide ion. This ratio indicates that 1 mol of levodopa reacts with 2 mol of hydroxide ion to form an anionic sigma complex or aryloxy free radical.

3.1. Features of the kinetic method

Calibration graphs Figs. 4–6 constructed by plotting $\log V$ vs. $\log C$ (Fig. 4), K_t vs. C (Fig. 5), and $\log K_t$ vs. $\log C$ (Fig. 6) show a linear rela-

tionship with respect to the concentration of levodopa in the range of 5–35 ppm. Where, V is the initial rate constant, C is the concentration of
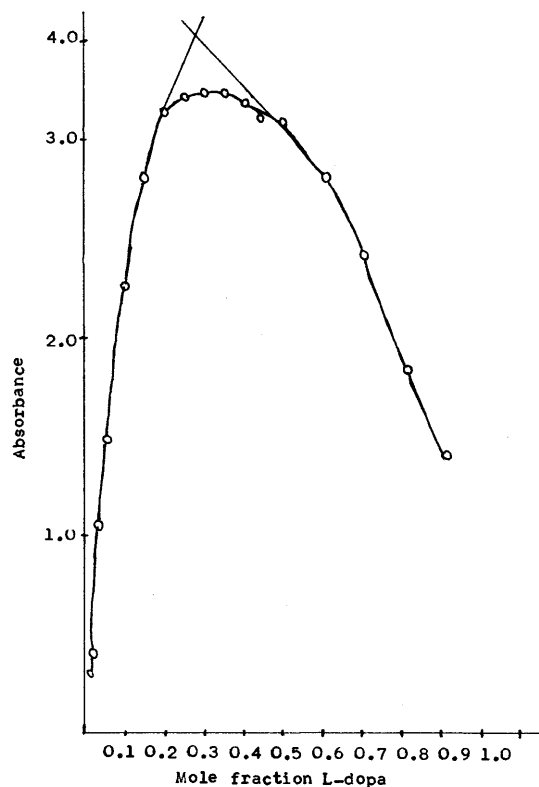

Fig. 3. Jobs plot for the reaction of L-dopa with hydroxide ion at 300 nm, 303 K.
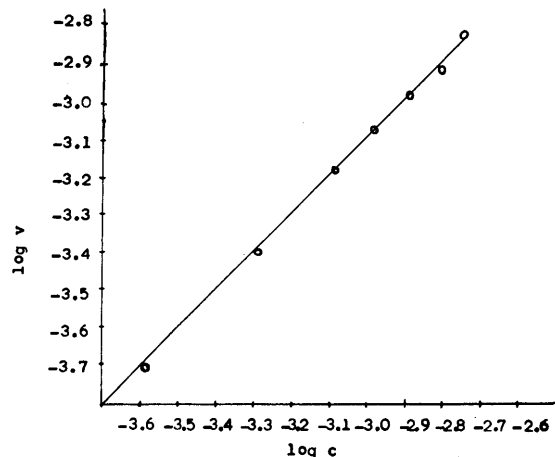

Fig. 4. Calibration graph 1., $\log v$ (initial rate constant) versus $\log c$ (concentration of L-dopa mol L^{-1}) using 0.05M NaOH.
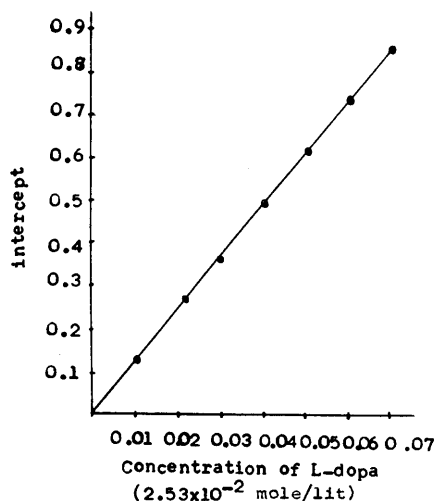
Fig. 5. Calibration graph 2, concentration of L-dopa versus intercept.

Fig. 6. Log (K_t) intercept versus log c (L-dopa concentrations in mol L^{-1})

the drug in mol 1^{-1} and K_t is the intercept with respect to levodopa concentration. Fig. 6 is used to calculate the order of the reaction and found to be equal to $+1.0$. This value matches the order obtained from the graph of log V vs. log C (Fig. 4). The precision and accuracy of the proposed kinetic method was checked by running different concentrations of levodopa. The mean standard deviation and mean relative standard deviation were found to be ± 0.3 ppm and 1.55%, respectively. The mean % recovery is equal to 100.0%.

Therefore, a reproducible results are indicated with high precision and accuracy.

3.2. Standard addition techniques

The validity of the proposed kinetic method was checked by applying the standard addition techniques, and the results obtained are summarized in Table 1.

Table 1
Results obtained by the standard addition technique

Sample[a]	Claimed added (ppm)	Authentic added (ppm)	Total added (ppm)	Total[b] found (ppm)	% Recovery
1. Levopa	10.0	5.0	15.0	15.13	100.83
	5.0	10.0	15.0	15.25	101.67
	10.0	15.0	25.0	24.75	99.00
	10.0	20.0	30.0	29.88	99.58
2. Syndopa-110	10.0	10.0	20.0	20.00	100.00
	10.0	0.50	15.0	14.88	99.17
	10.0	25.0	35.0	35.00	100.00
	10.0	20.0	30.0	30.25	100.83
3. Syndopa-275	10.0	5.0	15.0	14.75	98.33
	20.0	15.0	35.0	35.00	100.00
	20.0	10.0	30.0	30.75	102.50
	5.0	30.0	35.0	35.00	100.00

[a] 1–3, are in tablet form.
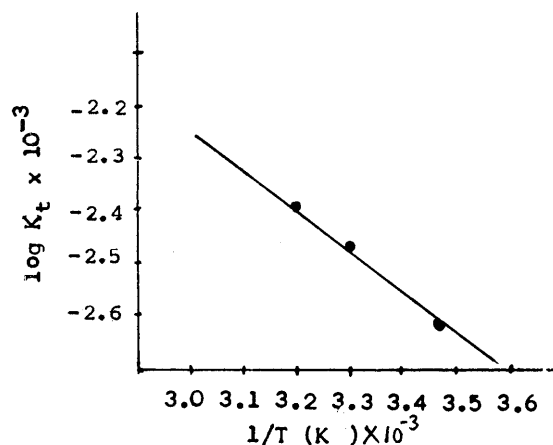[b] Average of three determinations for each sample.

Fig. 7. Dependence of K intercept on temperature.

3.3. Effect of reaction variables

The effect of sodium hydroxide concentration was studied in the range of 0.01–0.3 mol l^{-1}. A logarithmic plot shows that the reaction rate is dependent on sodium hydroxide concentration in the range 0.01–0.05 mol l^{-1}. therefore, 1.0 ml of 0.05 M was used for further studies.

3.4. Effect of temperature

The effect of temperature on the reaction rate was studied in the range 288–323 K. The absorbance-time curves show the temperature dependence and increase in the absorbance with time, it is observed that levodopa reacts faster with hydroxide ion (OH^{-}) to form the product within a short time (3–120 s). The reaction shows

Table 2
Maximum tolerance amount of interferences added in the analysis of levodopa (20 ppm)

Sample no.	Common excipient	Maximum tolerance (mg)
1	Lactose	90.10
2	Fractose	27.04
3	Starch	11.81
4	Glucose	27.04
5	Sucrose	85.6

a linear relation with temperature. The plots of log K_t vs. $1/T$ are linear at 308–313 K (Fig. 7). Therefore, the optimum reaction temperature 303 K was fixed throughout the experiment. At higher temperature (323 K) inaccurate results are observed and a fear of decomposition of the product that may take place.

3.5. Interferences

Some common excipients such as lactose, starch, glucose, sucrose, and fructose, which are usually present in preparation of tablets and capsules, show no influence in the determination and the maximum tolerance amounts were calculated using the procentual change in absorbance of levodopa measured value. The results are recorded in Table 2.

3.6. Application of the proposed kinetic method

The proposed method has been successfully applied to the determination of levodopa in dosage

Table 3
Results obtained for the determination of levodopa in dosage form compared with reference method [14]

Drug name	Composition (mg^{-1})	% Recovery ($\pm$ SD)		t[b]
		Proposed[a] method	Reference method	
1. Levopa (Wallace)	500 mg levodopa	99.4 $\pm$ 0.65	99.96 $\pm$ 0.77	0.52
2. Syndopa-110 (Sun Pharma)	100 mg levodopa/10 mg carbidopa	99.75 $\pm$ 0.31	–	0.18
3. Syndopa-275 (Sun Pharma)	250 mg levodopa/25 mg carbidopa	99.50 $\pm$ 0.14	–	1.59
4. Syndopa Plus (Sun Pharma)	100 mg levodopa/25 mg carbidopa	98.75 $\pm$ 0.13	–	2.15

[a] Average of five determinations, assay as a percentage of label claim.
[b] Theoretical value (2.776) for 95% confidence level.

forms (Table 3) and the presence of carbidopa in combination with levodopa in various dosage forms did not interfere with the results. Furthermore, the results obtained are compared with those obtained by reference method [14], which are listed in Table 3. The calculated t-test values did not exceed the theoretical values which indicate the absence of any significance difference in terms of precision and accuracy.

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