Journal of Pharmaceutical & Biomedical Analysis
Vol. 9, Nos 10–12, pp. 1157–1160, 1991
Printed in Great Britain

0731–7085/91 $3.00 + 0.00

Short Communication

Quantitative spectrophotometric assay of levodopa as its Pd(II) complex in water and dosage forms*

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Keywords: *Levodopa; palladium(II) chloride; complexiметry; colorimetry.*

Introduction

Levodopa [(−)-3-(3,4-dihydroxyphenyl)-L-alanine] is used in the treatment of Parkinsonism. It is mainly used in the form of tablets and capsules. Various analytical methods have been described for the quantitative determination of levodopa in dosage forms, extensively in tablets and capsules. Of great importance are the electrochemical methods which are based on amperometric measurements [1, 2], potentiometric determination with copper-selective electrodes [3] and anodic voltmetry at rotating disc electrodes [4]. A difference spectrophotometric assay was applied to determination of levodopa besides the other components [5]. Reversed-phase HPLC systems with a UV detector [6, 7] or electrochemical detector [8] and chiral-mobile-phase HPLC [9] have also been used for the determination levodopa in pharmaceutical dosage forms.

The present paper reports the results during the study of levodopa–Pd(II) complex as the basis for the determination of levodopa, besides the other components, in tablets and capsules.

Experimental

Materials

Levodopa (Roche), palladium(II) chloride (Merck, Darmstadt, Germany), potassium chloride (Merck) were obtained from various sources and used as received. All other chemicals were of analytical-grade purity (Merck). "Nakom" tablets (Frosst–Pharma, Germany) containing 250 mg of levodopa and "Madopar" capsules (Roche) containing 200 and 100 mg of levodopa were used as received.

Solutions

The following solutions were prepared in water: 5×10^{-3} M of lidocain, 10^{-2} M of palladium(II) chloride and 2 M potassium chloride. A calibration curve was prepared with 10 standard solutions having concentrations from 0.19 to 1.9 mg ml^{-1} (10^{-3}–10^{-2} M). Britton–Robinson buffer solutions covering the pH region 4.0–8.0 were made by mixing 0.03 M phosphoric acid, boric acid and acetic acid with the appropriate volume of 0.2 M sodium hydroxide.

Equipment

Spectrophotometric analysis was performed with Specord M 40 Carl Zeiss (Jena) spectrophotometer. The pH was adjusted with a Zeromatic SS-3 Beckman (Germany) pH-meter.

Procedure

A 1 ml volume of levodopa solution and 2 ml of palladium(II) chloride solution were placed in a 10-ml volumetric flask. The pH was adjusted by adding 4 ml of pH 6.5 Britton–

* Presented at the "Third International Symposium on Pharmaceutical and Biomedical Analysis", April 1991, Boston, MA, USA.

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Robinson buffer. The ionic strength was kept constant by the addition of 2 ml potassium chloride, and then the solution diluted to volume with water. The absorbance of solution was measured after 10 min at 392 nm against the reagent blank. The same procedure was applied with each standard solutions of levodopa for calibration curve.

The method was then applied to the assay of levodopa from tablets and capsules. The levodopa was extracted with water, and diluted to 5×10^{-3} M. Subsequent steps were the same as described for the levodopa bulk drug. Ten analyses were performed with both dosage forms.

Results and Discussion

The properties of the complex

This work presents a new method for the spectrophotometric determination of levodopa with palladium(II) chloride as a colour-forming reagent. Levodopa reacts with Pd(II) chloride to produce an orange complex that is soluble in Britton–Robinson buffer in the pH range 4–8. Absorption spectra were recorded over the range 350–600 nm. The levodopa–Pd(II) complex shows two absorbance maxima at 392 and 525 nm (Fig. 1, curve 2). The Pd(II) chloride has a maximum absorbance at 480 nm (Fig. 1, curve 1) under the same conditions, which was the reason why all measurements of the complex were performed at 392 nm against the reagent blank.

The reaction rate and the amount of levodopa–Pd(II) complex produced are influenced considerably by the pH of reaction mixture (Fig. 2).

The absorbance increases gradually from 4 to 6.3 to reach a plateau at pH values between

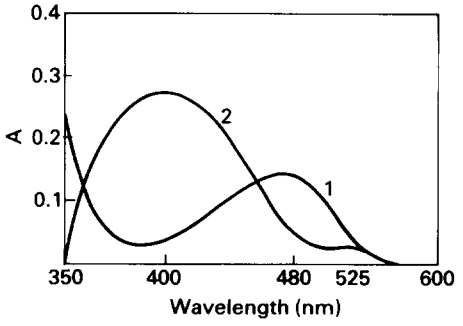

Figure 1
Absorption spectra of levodopa–Pd(II) complex (curve 2) and Pd(II) chloride (curve 1). [Levodopa] = 3×10^{-4} M; [Pd(II)] = 2×10^{-3} M; μ = 0.4; pH = 6.5.

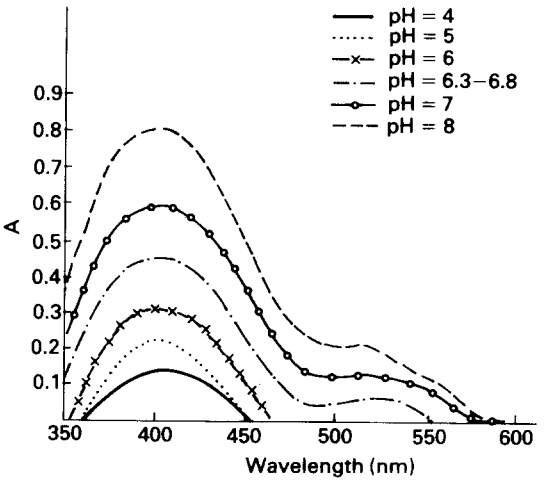

Figure 2
The effect of pH on complex formation. [Levodopa] = 5×10^{-4} M; [Pd(II)] = 2×10^{-3} M; μ = 0.4 M.

6.3 and 6.8. Thus, pH 6.5 was used as the working pH. The shape of the absorption spectrum and the position of absorption maximum of the complex did not vary with pH which indicates that only one type of complex is formed.

Pd(II) chloride solution is added in excess. Investigation on the effect of reagent concentration on the formation of the complex showed that the absorbance increases for molar ratios up to 4:1 of Pd(II)–levodopa. The absorbance did not increase when a further excess of Pd(II) chloride was used.

The reaction time was 5 min and the absorbance then remained unchanged for at least 24 h. The ionic strength was investigated at range 0.2–0.8 M, and there was found that it had little influence on the complex formation. The best spectrum was obtained at ionic strength of 0.4 M.

Composition of the complex and conditional stability constant

The stoichiometric ratio of levodopa to Pd(II) chloride in the complex was determined by Job's method of equimolar solutions and molar ratio method. Job's curve of equimolar solutions (Fig. 3) reached a maximum value at a mole fraction x_{max} of 0.5 which indicated the formation of a 1:1 complex. This result was achieved using the molar-ratio method, where the obtained curve shows a break point at Pd(II)–levodopa 1:1.

Conditional stability constant of the complex was calculated according to the method of

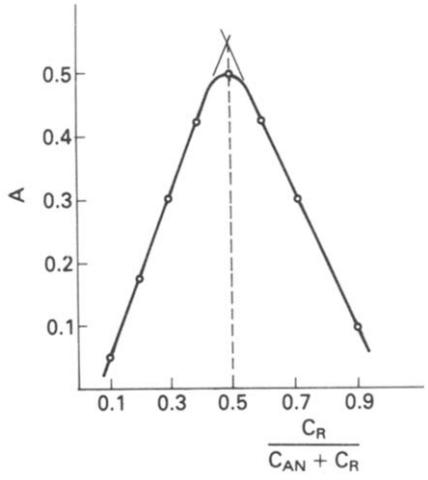

Figure 3
Job's curve of equimolar solution at 392 nm. [Levodopa] + [Pd(II)] = 3×10^{-3} M; pH = 6.5; μ = 0.4; $C_r = 3 \times 10^{-3}$ M; $C_{AN} = 3 \times 10^{-3}$ M.

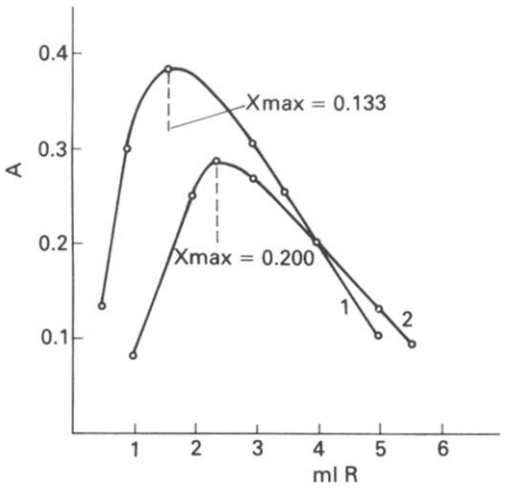

Figure 4
Job's curve of non-equimolar solutions. [Levodopa] = $2.5\ 10^{-4}$ M; p = 5 (curve 1); p = 10 (curve 2); pH = 6.5; μ = 0.4; R = $PdCl_2$.

Table 1
Conditional stability constant of the levodopa–Pd(II) complex calculated according to Sommer's method*

log K'	log K'_{min}	log K'_{max}	SD	RSD (%)
4.75	4.45	5.05	0.08	1.69

*Conditoins: pH = 6.3; μ = 0.4 M; t = 25 ± 0.5°C.

Table 2
Conditional stability constant of the levodopa–Pd(II) complex calculated according to Job's method of non-equimolar solutions*

Pd(II)	p	x_{max}	log K'
1.25×10^{-3}	5	0.200	4.68
2.5×10^{-3}	10	1.333	4.38
		Mean:	4.53

*Conditions: pH = 6.3; μ = 0.4 M; t = 25 ± 0.5°C; n = 12; p = five or 10-fold of reagent.

Sommer by using Job's method of equimolar solutions. The results are presented in Table 1.

By Job's method of non-equimolar solutions the curves for five-fold (Fig. 4, curve 1) and 10-fold (Fig. 4, curve 2) excess of reagent were obtained.

The conditional stability constant was calculated according to the equation:

$$K' = \frac{(p - 1)(1 - 2x_{max})}{c_{levodopa}[(1 - p)x_{max} - 1]^2}. \quad (1)$$

The values of log K' are presented in Table 2.

The values of log K' obtained with both methods indicate the good stability of the Pd(II)–levodopa complex.

Quantification and precision of the method

The linear dependence of the absorbance at

Table 3
Spectrophotometric determination of levodopa in the pure form and in pharmaceutical dosage forms

	Taken (μg ml^{-1})	Found (μg ml^{-1})	SD (μg)	RSD (%)	R	S$\bar{x}$
Levodopa	59.16	58.79	1.115	1.897	99.37	0.25
	78.88	78.62	1.240	1.577	99.67	0.28
	98.60	98.55	1.019	1.035	99.94	0.23
"Nakom" tablets (250 mg of levadopa)	98.60	98.40	1.162	1.180	99.80	0.26
"Madopar" caps (100 mg of levodopa)	98.60	98.39	1.116	1.182	99.79	0.25
"Madopar" caps (200 mg of levodopa)	98.60	98.37	1.156	1.175	99.76	0.26

R = Recovery.
S$\bar{x}$ = Relative error.

392 nm and levodopa concentration was investigated. Beer's law was verified for levodopa concentrations from 0.1 to 1 mM, as seen from the regression equation $y = 0.0090 - 0.3922x$, and obtained values of correlation coefficient, $r = 0.997$. The lower limit of sensitivity of the method was found to be 3 μg ml^{-1} (0.014 mmol l^{-1}).

The precision of the method was checked at three different concentrations (Table 3). The RSD varied from 1.0 to 1.8% for concentration of levodopa from 0.3 to 0.5 mM. After these investigations, the method was then applied to determination of the levodopa in tablets and capsules. The results given in Table 3 shows low values of RSD and good recovery, which indicates the applicability of the method for the assay of simple dosage forms.

The proposed method is simple, rapid and accurate and suitable for the routine analysis of pharmaceutical preparations.

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[Received for review 29 April 1991]