A Simple spectrophotometeric Assay for determination of L-Dopa in pharmaceutical preparations via oxidative coupling reaction.

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Abstract:

A sensitive and simple spectrophotometric method for the estimation of levodopa (LDP) in either pure form or in its pharmaceutical formulation is described. The method is based on the reaction of levodopa with thiourea in the presence of ferric chloride as oxidative reagent , to form an intense blue – water solution dye that is stable and has a maximum absorption at 660 nm. A rectilinear relationship between absorbance and concentration range 2-32 $\mu g.ml^{-1}$ with a molar absorptivity of $2.027x10^{3}$ $L.mol^{-1}.cm^{-1}$ sandell sensitivity of $0.97x10^{-4}$ $\mu g.cm^{-2}$, a relative error of ((-0.625)-3.06%) and a relative standard deviation(RSD) of(0.823-1.247%) depending on the concentration of levodopa . The detection limit of the method was found to be 0.349 $\mu g.ml^{-1}$. The stoichiometric ratio was found to be 1:1of Levodopa- Thiourea .The mole ratio was applied for determination of the conditional stability constant of the formed compound and was found to be $9.2x10^{7}L.mol^{-1}$. Finally, the proposed method have been applied successfully for the determination of levodopa in pharmaceutical preparations.

الخلاصة:

يتضمن البحث طريقة حساسة وبسيطة لتقدير اليفو دوبا بصورة مباشرة في الوسط المائي ، تعتمد الطريقة على اقتران الليفادوبا مع كاشف الثايويوريا بوجود كلوريد الحديديك كعامل مؤكسد لتكوين صبغة زرقاء ذائبة ومستقره في الماءتعطي اعلى امتصاص عند طول موجي660 نانوميتر. وكان حدود قانون بيير في مدى التراكيز (2-32)مايكروغرام/لتر وبلغ الامتصاص المولاري للطريقة2.07×10^{3} لتر/مول.سم ودلالة ساندل 0.97×10^{-4} مايكروغرام سم$^{-2}$ كما بلغ الخطأ النسبي في مدى (-0.625-3.06 %) والانحراف النسبي في مدى(0.823-1.247 %)اعتمادا على مستوى التركيز التي تم تقديره وكان مقدار حد الكشف 0.349 مايكروغرام/مل كما درست طبيعة المركب الناتج بطريقة النسب المولية ووجد ان نسبة ارتباط الليفادوبا الى الثايويوريا هي 1:1 وبلغت قيمة ثابت الاستقرار للمركب الناتج هي9.2×10^{7}لتر.مول$^{-1}$. طبقت الطريقة بنجاح لتقدير اليفودوبا في بعض المستحضرات الصيدلانية.

Introduction:

The aminoacid levodopa [3-(3,4-dihydroxyphenyl)-L-alanine] Fig.1 is a precursor of the neurotransmitter dopamine, which easily enters the central nervous system. In Parkinson's disease, dopamine transmitting neurons die in the area of brain due to its deficiency. To treat this disease, levodopa(LDP), a drug, is used, which easily gets converted to dopamine in the brain and readily crosses the blood brain[1-3].

Numerous analytical methods have been developed for the quantitative assay of levodopa in pharmaceutical preparations and in biological fluids, including spectrophotometry[4-9], capillary zone electrophoresis [10], Flow-injection analysis (FIA)[11-12], high performance liquid chromatography (HPLC) [13-16], (TLC) [17-18], radio-immunoassay[19], chemiluminescence [20], voltammetric determination [21], ion-exchange column Chromatography[22] and gas chromatography [23].

The purpose of the present investigation is to develop a simple and sensitive method for the determination of L-dopa in pharmaceutical formulations by oxidative coupling reaction with Thiourea catalyzed by ferric chloride.

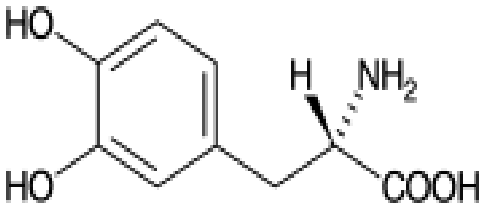


Fig.1 the structure of levodopa

Experimental:

Apparatus:

A shimadzu UV–VIS 1800 Spectrometer (Japan) was used equipped with a quarts cell of 1.0 cm width for the λ_{max} determination and all absorbance measurements**.**

Reagents Materials:

Analytical reagents grade chemicals and distilled water were used throughout. Pure drug(levodopa) samples were provided by BDH.

- Standard solution 100 µg ml^{-1} of levodopa was prepared in distilled water. Working standard solutions were prepared by dilution the stock standard immediately before use.

-Dosage forms containing the studied drug being purchased from commercial sources provided by IBN HAYYAN pharm.syria.

- Iron chloride(III) 0.03M solution:

was prepared by dissolving 0.4866gm of $FeCl_3$. in 1ml concentrated HCl and made up to100 ml of distilled water.

- Thiourea solution(0.2%):

was prepared by dissolving 0.2gm of Thiourea in 100 ml of distilled water.

Recommended procedure:

Into a series of 25ml volumetric flask, transfer increasing volumes of levodopa solution (100 µg.ml^{-1}) to cover the range of calibration curve(2-32µg.ml^{-1}) , and added 0.5ml (0.2%) of Thiourea shake well., added 0.5ml (0.03M) of $FeCl_3$ dilute the solution to the mark with distilled water, and allow the reaction to stand For 5min.Measure the absorbance at 660 nm against a reagent blank prepared in the same way but containing no levodopa. The color of Blue product is stable for more than 60min .

Nature of the form product:

The composition of the yielded compound was established by molar ratio method[24]. The absorbance was measured after 5 min at 660 nm against a reagent blank. Molar ratio method was employed by preparing a series of mixtures by adding a constant volume of levodopa solution(0.0010M) with varying amount of the same concentration of the NH_2CSNH_2 ,with a 0.5ml of a 0.03M of $FeCl_3$ added and keeping the final volume constant. The absorbance was then measured as the molar ratio method and plotted against the molar ratio of NH_2CSNH_2 in the mixture.

Procedure for pharmaceutical preparations:

- levodopa tablets (250mg):

Weight and finally powdered(10 tablets), extract an accurately weight portion of the powder equivalent to about 250mg of levodopa in 100ml distilled water using 100ml volumetric flask to obtained 2500µg.ml^{-1} of levodopa,fliter the solution and 4ml from the above solution was transferred into 100ml volumetric flask and complete to the mark with distilled water to obtained 100µg.ml^{-1}. This solution was suitable to analyze by taking a convenient volumes in the range of calibration curve under a general procedure.

Results and discussion:

Absorption Spectra of the Colored Complex:

The levodopa drug reacts with iron(III) to produce ketoform which is reacted with Thiourea to yeild a blue compound measurable at 660 nm[25] (Fig. 2). Because of their strong absorbance, the absorbance of the blue complex is directly related to the concentration of levodopa and can be used for its spectrophotometric determination. The development of the color depends very much on the reaction conditions. Therefore it is very important to optimize the reaction conditions.

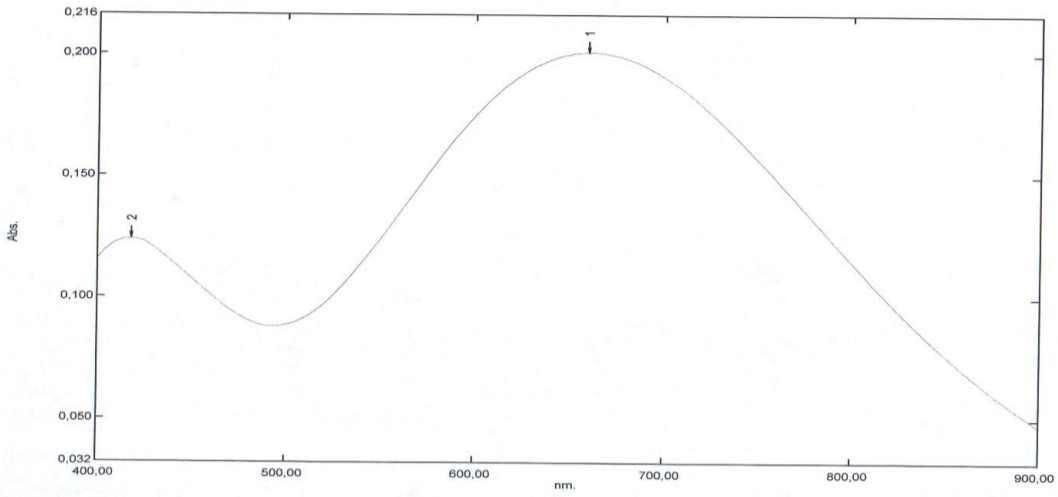


Fig. 2: Spectrum of the blue complex resulted from (20 µg ml^{-1})of levodopa with0.5 ml (0.03 M) $FeCl_3$ and 0.5ml of 0.2% NH_2CSNH_2 measured against a blank solution.

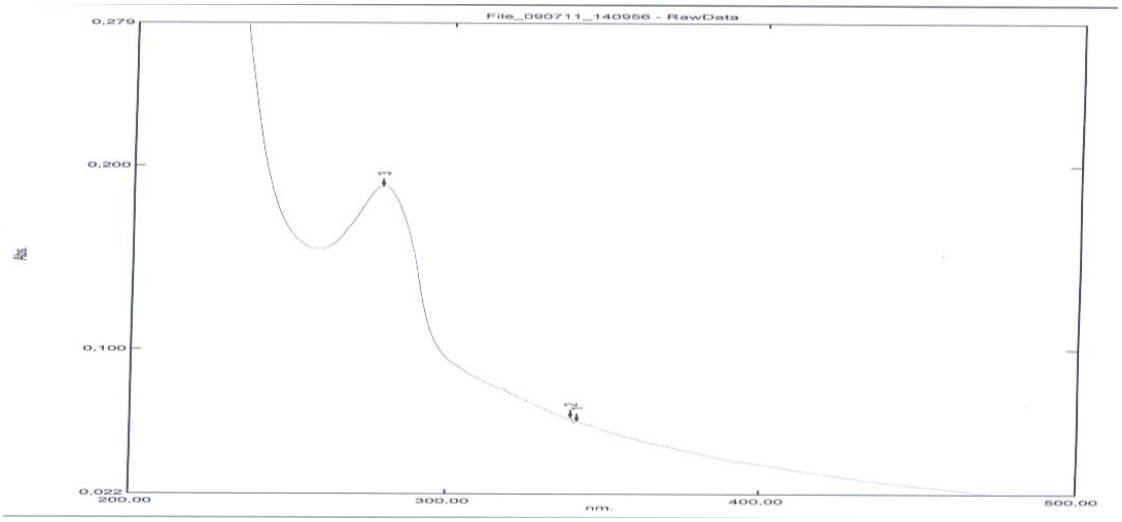


Fig. 3: Spectrum of (12 µg ml^{-1}) of levodopa before oxidation coupling measured against a blank solution.

Effect of Iron(III) Chloride:

The effect of iron(III) chloride concentration on the absorbance of the blue color product was investigate in the range of ($1.2x10^{-4}$ -$3.6x10^{-3}$M) in a final volume 25ml, ($6.0x10^{-4}$M) gave the highest absorbance as shown in Fig.4.

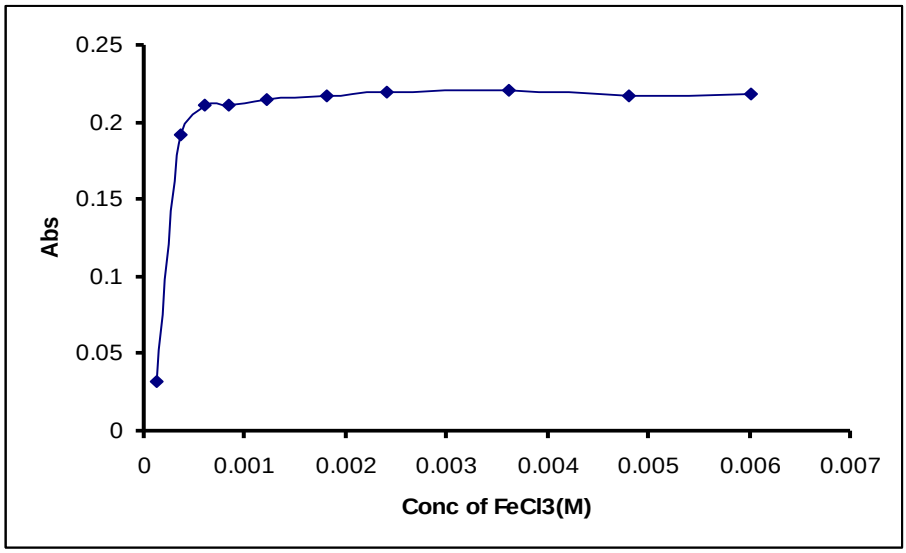


Fig.4: Effect of Iron(III) chloride concentration.

Effect of Thiourea:

he effect of Thiourea concentration was similarly studied in the range($1.05x10^{-4}$-$2.1x10^{-3}$ M) in a final volume of 25ml. The absorbance increased with increasing Thiourea concentration up to $5.2x10^{-4}$ M, above which it stable as shown in Fig. 5. Therefore it is chosen for others uses.

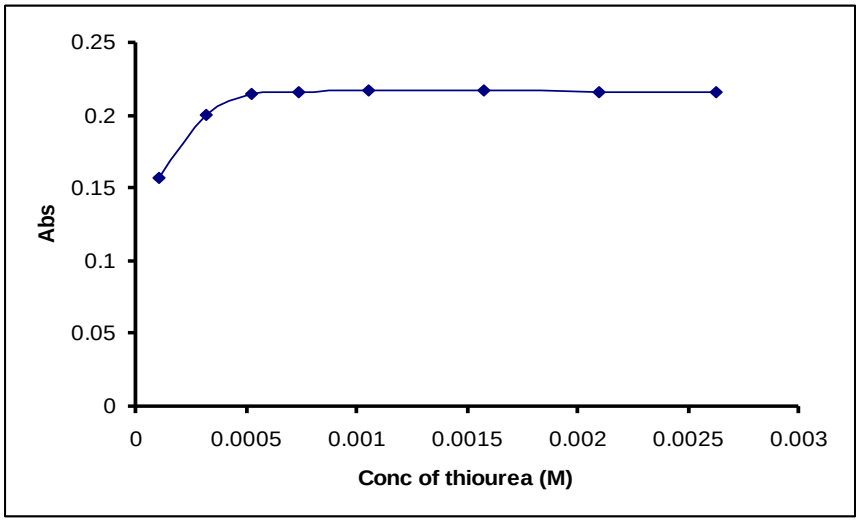


Fig.5: Effect of Thiourea concentration.

Effect of Temperature:

The effect of temperature on the color intensity of the product was studied. In practice the same absorbance or very closed was obtained when the color was developed at room temperature(25^0C) ,or when the calibrated flask was placed in an water bath(50^0C) . or when the calibrated flask was placed in an ice-bath bath(0^0C). Therefore, it is recommended that the reaction should be carried out at room temperature as shown in table .1.

Table.1
Effect of temperature

Temperature (^{0}C)	Absorbance
0 ^{0}C	0.241
25 ^{0}C	0.243
50 ^{0}C	0.245

Effect of Order of Addition:

The effect of order of addition on the absorbance of the blue color product was studied .Table.2, shows there is significant effect for order of addition on the absorbance . Therefore, the order of addition could be followed, drug: NH_2CSNH_2 : $FeCl_3$.

Table.2.
Effect of order of addition

Order of additon	Absorbance
Drug : $FeCl_3$: NH_2CSNH_2	0.202
$FeCl_3$: Drug : NH_2CSNH_2	0.204
NH_2CSNH_2 : $FeCl_3$: Drug	0.245
Drug : NH_2CSNH_2 : $FeCl_3$	0.247

Effect of reaction time :

The color intensity reached a maximum after mixing the levodopa with NH_2CSNH_2 and $FeCl_3$ for 5min. Therefore, 5min development time was selected as optimum in the general procedure. The color obtained was stable for at least one hour as shown in Fig.6.

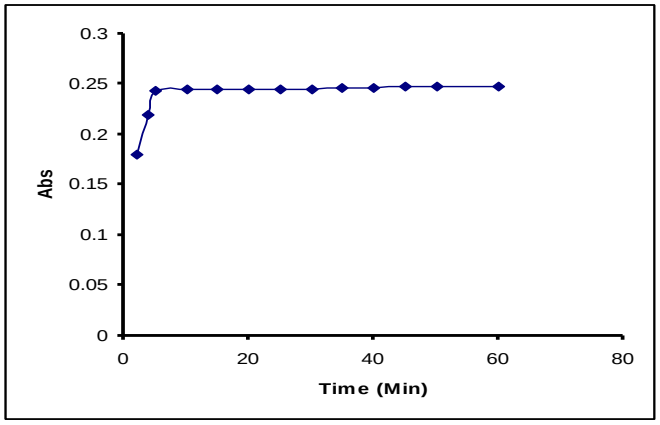
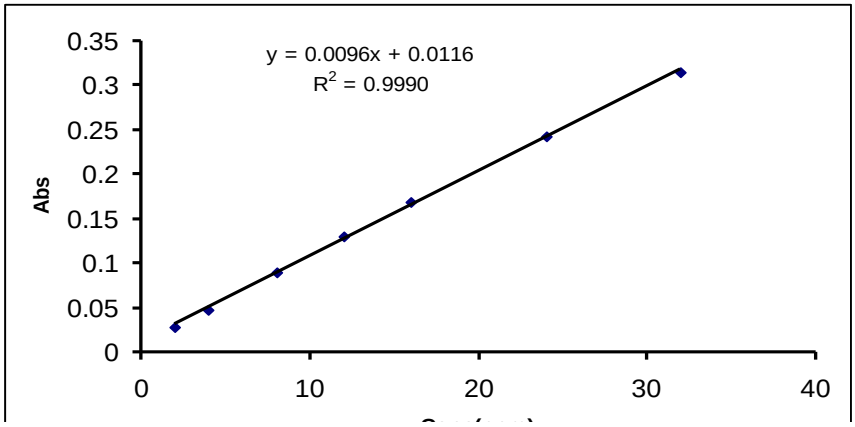


Fig.6: Effect of reaction time (min).

Calibration Graph:

Under the described experimental conditions, standard calibration curve for the studied levodopa were constructed by plotting the absorbance versus concentration. Conformity to Beer's Law was evident over the concentration range of (2-32µg.ml^{-1}) Fig.7, with the mean correlation coefficient of 0.9990 and an intercept of 0.0116. The conditional molar absorptivity of the blue color product was found to be (2.027x10^3 L.mol^{-1} .cm^{-1}) and the sandell sensitivity (0.97x10^{-4} µg.cm^{-2}).



Conc(µg.ml^{-1})

Fig.7: Calibration Graph of levodopa.

Accuracy and Precision:

Levodopa was determined at three different concentrations with five replicated . The results obtained are shown in table(3). A satisfactorily precision and accuracy could be obtained using the proposed method.

Table.3

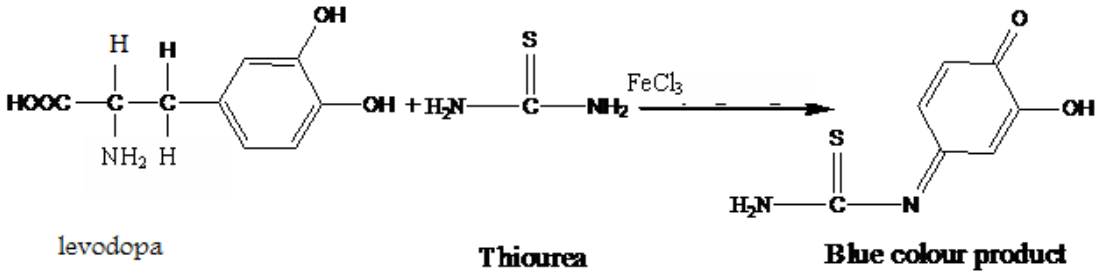
Accuracy and precision of the proposed method.

Concentration of L- Dopa (µg.ml^{-1})		**Recovery* %**	**R.S.D* %**	**Error* %**
Taken	Found			
12	12.368	103.067	1.15	3.06
16	15.90	99.41	1.247	-0.625
24	24.104	100.43	0.823	0.433

* Average of five replicated

Nature of the formed compound:

The reaction stoichiometry between Levodopa and thiourea has been determined spectrophotometrically by applying molar ratio method .The plot obtained by the method confirm the formation of (Levodopa-Thiourea) compound in a mole ratio of 1:1 where a break point at 1.0 was obtained (Fig.8). Therefore the reaction represented by the equation below[25] . The mole ratio was applied for determination of the conditional stability constant of the formed compound and was found to be 9.2x10^{7} L.mol^{-1}.



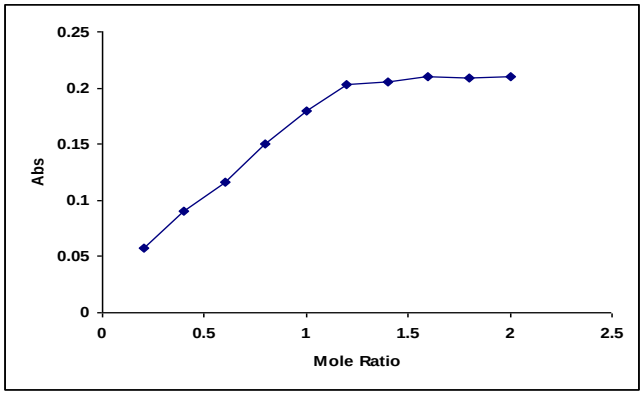


Fig.8 The mole ratio for the reaction of levodopa with thoiurea in the presence of $FeCl_3$.

Pharmaceutical Applications:

When the proposed method was applied to the determination of L-dopa in DOPAL FORT(250mg) tablets, the relative standard deviation varied from 0.6-0.83% which indicates the applicability of the assay to dosage form. The values of the RSD were in almost the same range as for the pure substance showing that excipients in the tablets did not interfere with the absorbance values even if present in the large excess. It's evident that the proposed spectrophotometric method, which uses thiourea as the analytical reagent, is rapid and simple as well as accurate and sensitive, and can be successfully applied to the determination of L-dopa both as the substance and its dosage forms. The results were listed in Table 4.

Table.4

Application of the proposed method for the determination of Levodopa in pharmaceutical tablets.

Drug sample	**Concentration of L-Dopa ($\mu g.ml^{-1}$)**		**Recovery*%**	**R.S.D*%**	**Error*%**
DOPAL FORT(250mg) IBN HAYYAN Pharm	Taken	Found			
	16	16.56	103.51	0.873	3.5
	24	24.08	100. 34	0.60	0.33

*Average of three determination.

Conclusions:

The proposed method is found to be simple, selective and highly sensitive than any reported methods on Levodopa . The statistical parameters and the recovery study data clearly indicate the reproducibility and accuracy of the method. Thus, the method can be adopted as an alternative to the existing methods.

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