# Anew colorimetric method for the determination of Levo-dopa in pharmaceutical preparation via oxidative coupling organic reaction

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## Abstract :

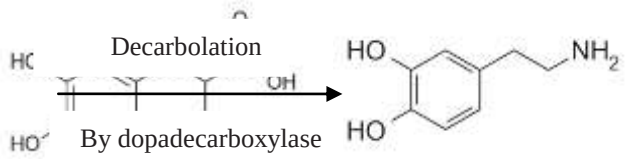
A simple, accurate and sensitive colorimetric method for the determination of L-Dopa in pure and pharmaceutical preparations has been developed .The proposed method uses ortho–Tolidine as a chromogenic reagent . The method is based on the oxidative coupling reaction of L-Dopa with ortho–Tolidine with potassium periodate in neutral media to form a green–bluish water soluble dye product , that has a maximum absorption at 629 nm . Linear calibration graph was in the range of (0.10 - 28) $\mu g.ml^{-1}$ with molar absorptivity of ($2.76 \times 10^4$ $L.mol^{-1}.cm^{-1}$) ,a sandall sensitivity of ($7.14 \times 10^{-6}$ $\mu g.cm^{-2}$) , correlation coefficient of 0.9997 , detection limit ($0.05$ $\mu g.ml^{-1}$) and the relative standard deviation of RSD% (1.87) . The method was applied successfully for the determination of L-Dopa in pharmaceutical preparations and the value of recovery % was from (99.6%) .

**الخلاصة :**

تم تطوير طريقة لونية بسيطة ومضبوطة وحساسة لتقدير الليفو دوبا في صيغته النقية وفي مستحضراته الصيدلانية . الطريقة المقترحة تعتمد على ازدواج الليفو دوبا مع الكاشف اللوني الاورثو تولدين وبوجود بيرايودات البوتاسيوم حيث يتكون ناتج اخضر مزرق يعطي امتصاص اعظم عند الطول الموجي (629)نانو ميتر . اظهرت النتائج ان مدى الخطية بين (0.10 - 28.00) مكغم .مل$^{-1}$ وبمعامل امتصاص مولاري مقداره( $2.76 \times 10^4$) لتر .مول $^{-1}$.سم$^{-1}$ ودلالة ساندل مقدارها ($7.14 \times 10^{-6}$) مكغم .سم-2 وبمعامل ارتباط (0.9997) وحد كشف للطريقة (0.05) مكغم .مل$^{-1}$ وبمعدل انحراف قياسي نسبي ( 1.87 %) .
طبقت الطريقة بنجاح لتقدير الليفو دوبا في مستحضراته الصيدلانية وكانت حدود الاسترداد المؤي افضل من (99.6 %) .

## INTRODUCTION

Levodopa [3-(3,4-dihydroxylphenyl)-L-alanine] is an important neurotransmitter , which has been used for the treatment of neural disorders such as Parkinsons disease[(1)].Parkinson's disease is one of the most difficult problems in the medical field. The cause of this disease is a significant depletion of dopamine due to the death of neurons which can produce dopamine in brain. It leads to tremor, muscle stiffness, bradykinesia, and so on[(2)]. Levodopa a precursor of dopamine, is an important neurotransmitter which is used for the medication of neural disorders such as Parkinson's disease. After administration, levodopa is converted into dopamine through enzymatic reaction catalyzed by dopadecarboxylase[(3)].




Levodopa dopamine

However, some side effects of systemic dopamine can appear if levodopa is taken at high dosages because of the metabolism of levodopa being extracerebral[4]. Therefore, a simple, highly sensitive and selective method that can be established for the determination of levodopa is significant in the medical and life sciences.

At present, different methods to determine levodopa have been employed: high-performance liquid chromatography[5], fluorescence spectrometry[6], electrochemistry method[7], chemiluminescence[8] , flow injection analysis[9,10], and several Others[11,12]. Oxidative coupling organic reactions seem to be one of the most suitable spectrophotometric determination of drugs such as Sulphonamides[13], paracetmole[14],folic acid[15] and phenylphrine.HCl[16] . The proposed method is based on the reaction of the L-dopa drag with ortho-Tolidine in the presence of potassium periodate in neutral medium to form a green- bluish water soluble dye product which shows an absorption maximum at 629 nm .

## Experimental

## Apparatus:

- All spectral and absorbance measurement were carried out in a Double beam UV-Vis spectrophotometer-1800 . Equipped with a 1 cm quarts cell .
- Water bath ( Lab. Companion , BS - 11) .
- Electronic balance (Sartorius AG GÖTTINGEN B2 2105 Gerrmany ) .

## Reagents:

All chemicals used were of analytical-reagent grade .-stock solutions from drag (100 $\mu g.ml^{-1}$) of L-dopa (Fluka) were prepared by dissolving 0.01gm of L-dopa in distilled water and diluting to the mark in 100 ml volumetric flask . Working solutions were prepared by diluting the solution in distilled water .- ortho-Tolidine (0.001M) stock solution was prepared by dissolving 0.0106 gm of ortho-Tolidine in 50 ml of isopropanol and the solution made up to the mark in 100 ml volumetric flaskwithdistalwater .- potassium periodate (0.05M) stock solution was prepared by dissolving 0.57gm of $KIO_4$ in distilled water and diluting to the mark in 100 ml volumetric flask

## Recommended procedure :

In to a series of 25 ml volumetric flask , transfer increasing volume of L-dopa solution (100 $\mu g.ml^{-1}$) to cover the range of calibration curve (0.1 - 28) $\mu g.ml^{-1}$ ,
added 2 ml from ($1 * 10^{-3}$ M) of ortho-Tolidine and shake well . Added 3ml from ($5 * 10^{-2}$ M) of $KIO_4$ , dilute the solution to the mark with distilled water , and allow the reaction to stand for 10 min at room temperature (25 $^{o}$c) .
measure the absorption at( 629 nm) against a reagent blank prepared in the same way but containing no L-dopa .

## procedure for pharmaceutical preparations :

DOPAL FORTE TABLET AND SIENAMAT TABLET :

10 tablets were grinded well and acertain portion of the final powder was accurately weighted to give an equivalent to about 10 mg of L-dopa was dissolved in distilled water . The prepared solution transferred to 100 ml volumetric flask and made up to the mark with distilled water forming a solution of 100 $\mu g.ml^{-1}$ concentration . The solution was filtered by using a Whatmann filter paper No. 42 to avoid any suspended particles .These solution were diluted quantitatively to produce a concentrations in the range of calibration curve .

## Results and Discussion :

### Absorption spectra :

It was found preliminary that the reaction of L-dopa with ortho-tolidine and potassium periodate in neutral media forming an green-bluish water soluble dye product , that has a maximum absorption at ( 629 nm ) Fig (1) . The above reaction can be utilized for the determination of L-dopa using spectrophotometric method . Initial studies were directed toward optimization of the experimental conditions ,in order to establish the most favorable parameters for the determination of L-dopa .

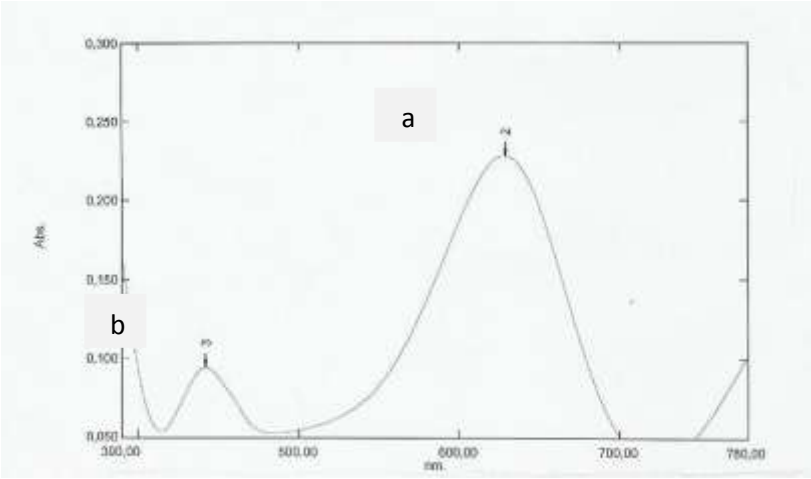



Fig (1) : (a)Absorption spectra of (4 µg.ml$^{-1}$) of L-dopa with ortho-Tolidin ($0.8*10^{-4}$) M , and $KIO_4$($6*10^{-3}$)M at room temperature and measured against blank solution .

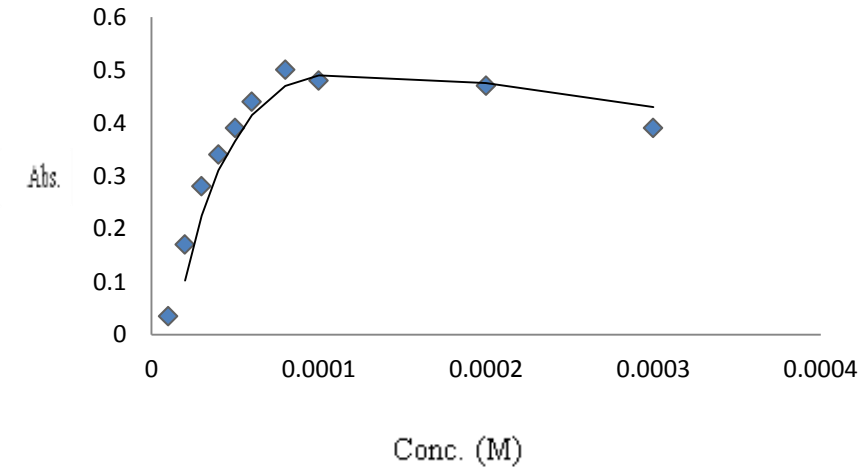
(b) blank solution prepared in the same way but containing no L-dopa . Measured against distilled water .

## Optimization of the Experimental Condition :

The influence of various reaction variables such as concentration of reactants , order of addition ,time and temperature were investigated .

## Effect of ortho-Tolidine Concentration :

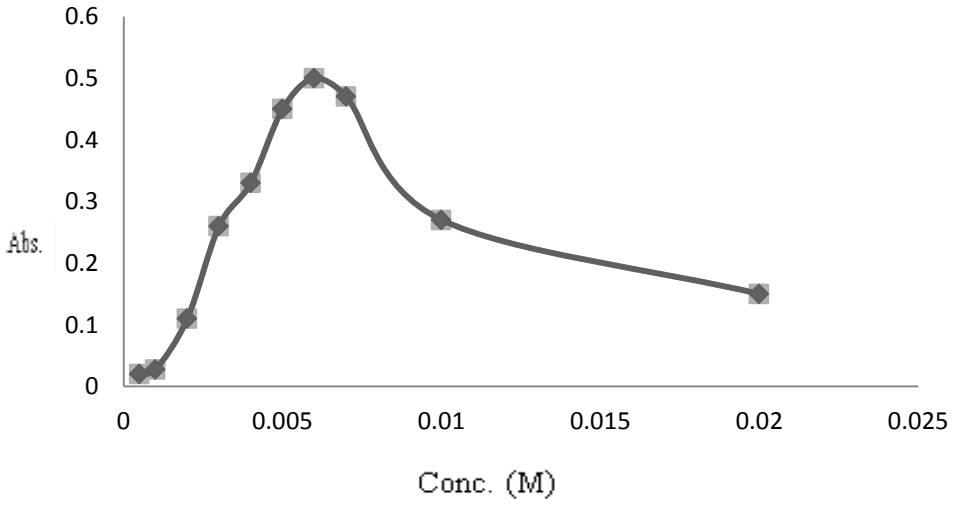
The effects of different concentration of ortho-Tolidine in the range of ($0.1*10^{-4}$ – $0.3*10^{-3}$) M were investigated . A Concentration of ($0.8*10^{-4}$)M give the higher absorption intensity at 629nm for 12 µg . ml$^{-1}$ of L-dopa and ($0.6*10^{-2}$)M of $KIO_4$ Fig (2) and thus was chosen for further use .




Fig(2) : Effect of ortho-Tolidine Concentration on Absorption spectra of (12 µg.ml$^{-1}$) of L-dopa .

## Effect of Potassium periodate $KIO_4$ Concentration :

The effect of $KIO_4$ Concentration in the range of ($0.5* 10^{-3}$ - $0.2 *10^{-1}$)M was similarly studied . A Concentration of ($0.6 * 10^{-2}$)M of $KIO_4$ give the higher absorption intensity at 629 nm for 12 µg . ml$^{-1}$ of L-dopa and ($0.8*10^{-4}$) M of o-Tolidine .Fig (3) and thus was chosen for further use .




Fig(3) :Effect of potassium periodate $KIO_4$ Concentration on Absorption spectra of (12 µg.ml$^{-1}$) of L-dopa .

## Order of addition :

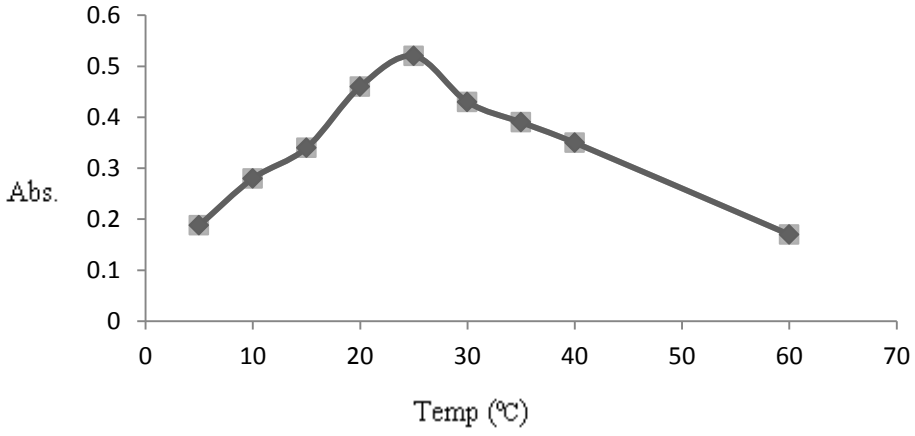
The effect of order of addition on the absorption of green-bluish water soluble day was studied . Table (1) , shows the order of addition could be followed , Drug : ortho-Tolidine : KIO4 . Due to gave the higher absorption .

Table (1) Effect of order of addition

| Order of addition | Absorbance at $\lambda_{max}$ (629)nm |
|---|---|
| Drug : o-Tolidine : KIO4 | 0.49 |
| Drug: KIO4 : o-Tolidine | 0.43 |
| KIO4 : o-Tolidine: Drug | 0.31 |
| KIO4: Drug : o-Tolidine | 0.37 |
| o-Tolidine : Drug : KIO4 | 0.36 |
| o-Tolidine: KIO4 : Drug | 0.34 |

## Effect of Temperature :

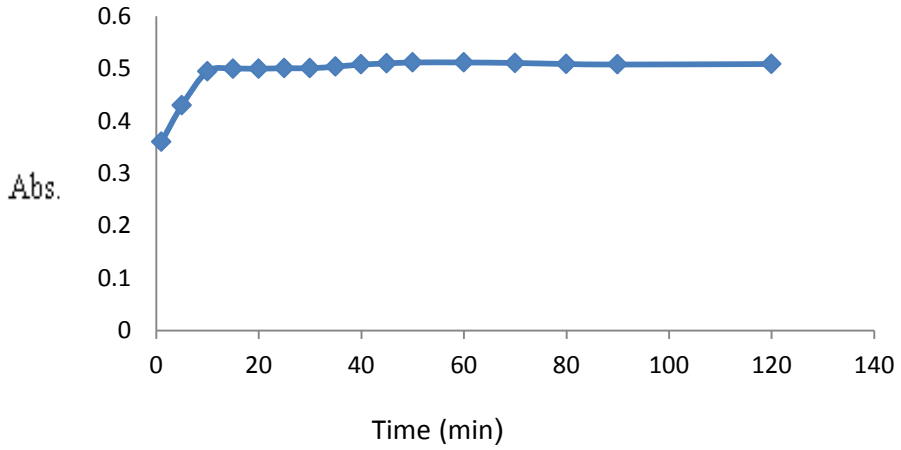
The effect of Temperature on the color intensity of the product was studied in practice the highest absorption was obtained when the colored product was developed at room temperature (25ºc) . as shown in Fig (4)




Fig(4) :Effect of Temperature on Absorption spectra of (12 µg.ml$^{-1}$) of L-dopa .

## Effect of Time :

The color intensity reached a maximum absorption after L-dopa (12 µg . $ml^{-1}$) has been reacted with o-Tolidine and $KIO_4$ at 10 min . Therefore 10min development time was chosen for further use . The results obtained are shown in Fig (5)




Fig(5) :Effect of Time on Absorption spectra of (12 µg.$ml^{-1}$) of L-dopa .

## Calibration Graph :

Under the optimum conditions , a linear calibration graph for the determination of L-dopa was obtained over the concentration range of (0.1 - 28)µg.$ml^{-1}$ . The linear regression equation for the range of (0.1 - 28)µg.$ml^{-1}$ L-dopa is Y=0.032 X + 0.0847 and correlation coefficient of0.9997 the linear calibration graph is shown in Fig (6) .

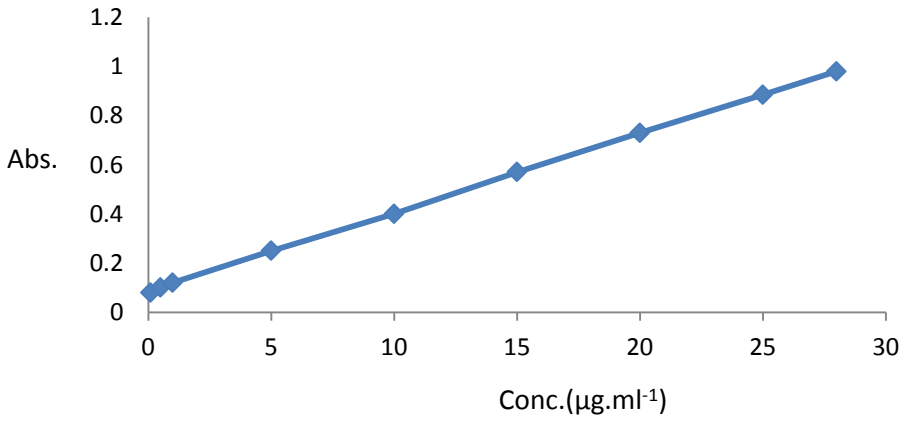



Fig (6) : calibration graph for the determination of L-dopa

## Nature of the dye product :

The stoichiometry of the reaction between L-dopa and ortho-Toidine was investigated using the molar ratio method[17] under the optimized conditions . The results obtained Fig (7) , show a 1:1 drugs to reagent product was formed .

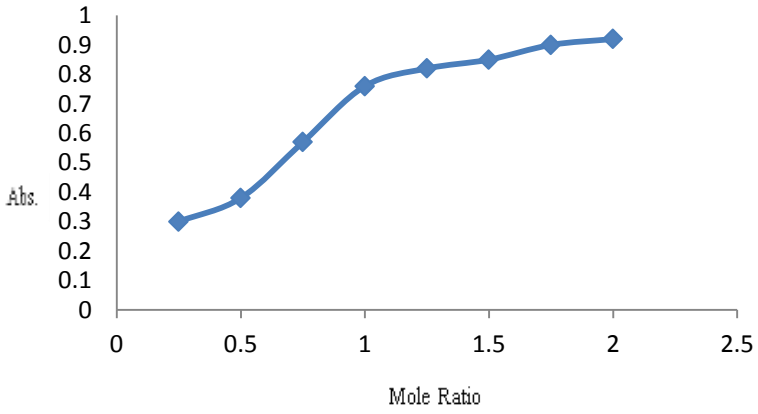



Fig (7) : Molar ratio of drug to reagent

## Interference

Several pharmaceutical preparations are associated with flavoring agents, diluents and excipients. Table (2) shows the effect of organic interfering materials that may be present in pharmaceutical preparations

Table (2) : Influence of excipients and additives as interfering species in the determination of L-dopa .

| Foreign compound | Recovary (%) of 500µg L-dopa per µg compound added | | | | |
|---|---|---|---|---|---|
| | 100 | 500 | 1000 | 2000 | 5000 |
| Glucose | 100.20 | 100.48 | 99.63 | 99.24 | 101.20 |
| Lactose | 99.28 | 101.1 | 100.19 | 101.31 | 102.13 |
| Starch | 101.77 | 100.95 | 101.17 | 99.41 | 100.40 |
| Sucrose | 101.21 | 98.87 | 102.15 | 101.12 | 99.60 |
| Sodium chloride | 100.50 | 102.38 | 102.50 | 101.54 | 102.20 |
| EDTA | 100.38 | 101.31 | 102.13 | 102.13 | 102.60 |
| Citric acid | 100.06 | 101.36 | 100.51 | 98.29 | 98.99 |
| Magnesium setarate | 101.23 | 100.10 | 102.46 | 101.02 | 98.97 |

## Analytical Application :

The proposed method was applied for the determination of L-dopa drag in pharmaceutical preparations. Good accuracy and precision were obtained for the studied drugs . The results obtained were given in Tabel 1 which confirm Finally, the proposed method was compared successfully with the standard method Table(3).

Table (3) : Application of the proposed method for the determination of L-dopa in pharmaceutical preparations .

| Drug sample | Amount of L-dopa µg.ml$^{-1}$ | | Proposed Method | | | Standard Method |
|---|---|---|---|---|---|---|
| | Taken | Found | RSD %* | Error * | Recovery* | Recovery %[18] |
| Pure Levodopa (Fluka) | 5.00 | 4.80 | 2.56 | 0.20 | 99.80 | 100.00 |
| DOPAL FORTE tablets | 5.00 | 4.92 | 2.40 | 0.08 | 99.92 | |
| | 15.00 | 15.50 | 1.50 | 0.50 | 100.50 | |
| | 20.00 | 20.20 | 1.30 | 0.20 | 100.20 | |
| Sienamt tablets | 5.00 | 5.40 | 2.54 | 0.40 | 100.40 | |
| | 15.00 | 14.80 | 1.40 | 0.20 | 99.80 | |
| | 20.00 | 20.10 | 1.42 | 0.10 | 100.10 | |

*Average of five determinations .

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