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SELECTIVE SPECTROPHOTOMETRIC DETERMINATION OF LEVODOPA

M. E. El-Kommos

Department of Pharmaceutical Chemistry, Faculty of Pharmacy
Assiut University, Assiut, Egypt

ABSTRACT

A selective spectrophotometric method is developed for the quantitative determination of levodopa. The proposed method is based on the interaction of levodopa with thiosemicarbazide in an alkaline medium to form a red coloured product. At the maximum absorption of 500 nm, Beer's law is adhered to over the 0.5-8 μg/ml range. Molar absorptivity of the coloured product is 2.58×10^4. Job's plot indicates 1:2 ratio of levodopa to thiosemicarbazide. Results of analysis of Larodopa tablets by the proposed method agree well with those of U.S.P. XXI. The method is highly selective since a catecholic function with free adjacent positions is required.

INTRODUCTION

Levodopa, (-)-3-(3,4-dihydroxyphenyl)-L-alanine, is widely used in the treatment of arteriosclerotic, idiopathic and post-encephalitic parkinsonism and in the control of the neurological symptoms of chronic manganese poisoning[1].

Existing analytical methods for the determination of levodopa include non-aqueous titration[2,3], potentiometry[4], UV-spectrophotometry[3,5,6], colorimetry[7], fluorimetry[8], GLC[9] and HPLC[10]. The official B.P. 1980[2] and U.S.P. XXI[3] procedures involve non-aqueous titrimetric and UV-spectrophotometric methods.

In the present paper, a simple, rapid and sensitive spectrophotometric assay of levodopa in pure and tablet forms is reported. The method is based on the interaction of levodopa with thiosemicarbazide to form a highly absorbing red coloured triazine derivative.

EXPERIMENTAL

Materials:

Levodopa, obtained as a gift from Roche Products Ltd-UK, was used as a working standard.

Thiosemicarbazide (BDH,UK), Hydrochloric acid AR (Prolabo, France). Propan-2-ol (Merck, F.R.Germany). Other solvents used were of analytical grade.

Larodopa tablete(each tablet contains 500 mg of levodopa) were obtained as a sample from Roche Products Ltd - UK.

Reagents :

1- Thiosemicarbazide solution, 0.3 % w/v in distilled water. This solution is stable for 1 week at $\sim 4^{\circ}C$.

2- Sodium hydroxide solution, 0.3 N.

3- Hydrochloric acid, 0.01 N.

Apparatus :

1- Uvidec-320 spectrophotometer, JASCO, Tokyo, Japan.

2- Z-230 centrifuge, Hermle Gmbh & Co., F.R. Germany.

Selective Spectrophotometric Determination of Levodopa

Preparation of Samples :

Powder : Accurately weigh about 25 mg of levodopa, dissolve in 20 ml of 0.01 N HCl in 50-ml volumetric flask and complete to volume with the same solvent. Dilute 5 ml of this solution to 50 ml in a volumetric flask with 0.01 N HCl.

Tablets : Weigh and finely powder 20 tablets. Accurately weigh a portion of the powder, equivalent to 25 mg of levodopa and extract with 0.01 N HCl by centrifungation for 5 min at a speed of 5,000 rpm (twice each with 5 ml). Dilute the mixed extracts with the same solvent to contain 50 mc of levodopa per ml.

Procedure :

Pipet 1.0 ml of the sample solution into a 10-ml volumetric flask. Add 1 ml of thiosemicarbazide solution and 1 ml of 0.3 N sodium hydroxide solution and mix thoroughly. Heat the mixture for 3 min in a water bath at 60±5°C, cool and dilute to volume with propan-2-ol. Measure the absorbance of the solution in 1 cm cell at 500 nm against a blank prepared under the same conditions using 1 ml of 0.01 N HCl instead of the sample solution. Calculate the concentration of levodopa from a calibration graph covering the range 5-80 mcg/ml (points taken in this investigation are : 5,10,20,30,40,50,60,70 and 80 mcg/ml) or from the following linear regression equation:

$$A = 0.1313\ C - 0.0058$$

Where A = Recorded absorbabce

C = Concentration of levodopa in the final assay solution in mc/ml

RESULTS AND DISCUSSION

Thiosemicarbazide has been used as a chromogenic reagent for the spectrophotometric determination of epinephrine[11], isoprenaline sulphate[12], methyldopa[13], dobutamine hydrochloride[14] and dopamine[15]. This reagent reacts specifically with a catecholic function with free adjacent positions[16].

Reaction Involved :

The resulting coloured product has an absorption maximum of 500 nm and apparent molar absorptivity of 2.58×10^4. Fig.1 shows the absorption spectra of levodopa, reagent and chromogen formed in both acidic and alkaline media. It seems apparent that changing pH of the medium to the alkaline side induces a type of tautomerism leading to a bathochromic shift of 95 nm.

The continuous molar variation of levodopa and thiosemicarbazide was performed using Job's plot. Standard solutions of levodopa (10^{-3}M) and aqueous solutions of thiosemicarbazide (10^{-3}M) were used. A series of mixtures of the two standard solutions in 11 different complementary proportions totalling 2 ml (from 0+2 to 2+0 inclusive, Fig. 2) were prepared, treated each with 1 ml of 0.3 N NaOH and subjected to the general assay procedure. Fig. 2 shows that the interaction between these two compounds occurs in the ratio 1:2.

Attempts to separate the chromogen in a pure form were unsuccessful owing to the formation of a resinous coloured mass. The possibility of formation of the thiosemicarbazone of the o-quinone, formed in situ, is unlikely as thiosemicarbazone formation is generally an acid catalysed reaction[17]. In addition, no colour was produced when levodopa was allowed to react with semicarbazide under the same

conditions. Addition of thiosemicarbazide through the sulphur atom to the o-quinone (newly formed) in analogy to the addition of thiourea to catechol[18,19] is also unlikely, as levodopa was found to react with thiourea under the same conditions giving only a faint yellow colour. A nucleophilic attack of the o-quinone, formed in situ by the amino groups of the hydrazide moieties of 2 molecules of thiosemicarbazide, may occur[18,20] according to Scheme 1, in anology to the interaction of dopamine with thiosemicarbazide[15].

Optimization of Variables :

a) Effect of thiosemicarbazide concentration. The optimum concentration of thiosemicarbazide leading to maximum colour intensity was found to be 0.03 % in the final solution, corresponding to 1 ml of 0.3 % thiosemicarbazide reagent per 10 ml of the reaction mixture.

b) Effect of alkali concentration. The optimum concentration of sodium hydroxide leading to maximum intensity was found to be 0.03 N in the final solution, corresponding to 1 ml of 0.3 N NaOH per 10 ml of the reaction mixture. Alkali concentrations higher than 0.03 N may lead to partial decomposition of the coloured product.

c) Effect of heating time. Maximum colour intensity was obtained after heating the original reaction mixture at 60±5°C for 3 min. After cooling to room temperature and dilution with propan-2-ol, the colour is stable for at least 3 hours.

d) Effect of solvent. The solvent affects both the wavelength and intensity of maximum absorption. The solvents investigated were water, methanol, ethanol, propan-1-ol,

propan-2-ol and dimethylsulphoxide (DMSO). Fig. 3 shows that DMSO gives the highest absorption intensity and the longest λ_{max}. However, it was found that DMSO renders the colour unstable and, consequently, unsuitable for spectrophotometric measurements. Therefore, propan-2-ol was used as a diluting solvent in all experiments.

Quantification, Accuracy and Precision :

A linear correlation (r=0.9996) was found between absorbance at 500 nm and the concentration of levodopa in the range 0.5-8 mc^2 /ml in the final assay solution.

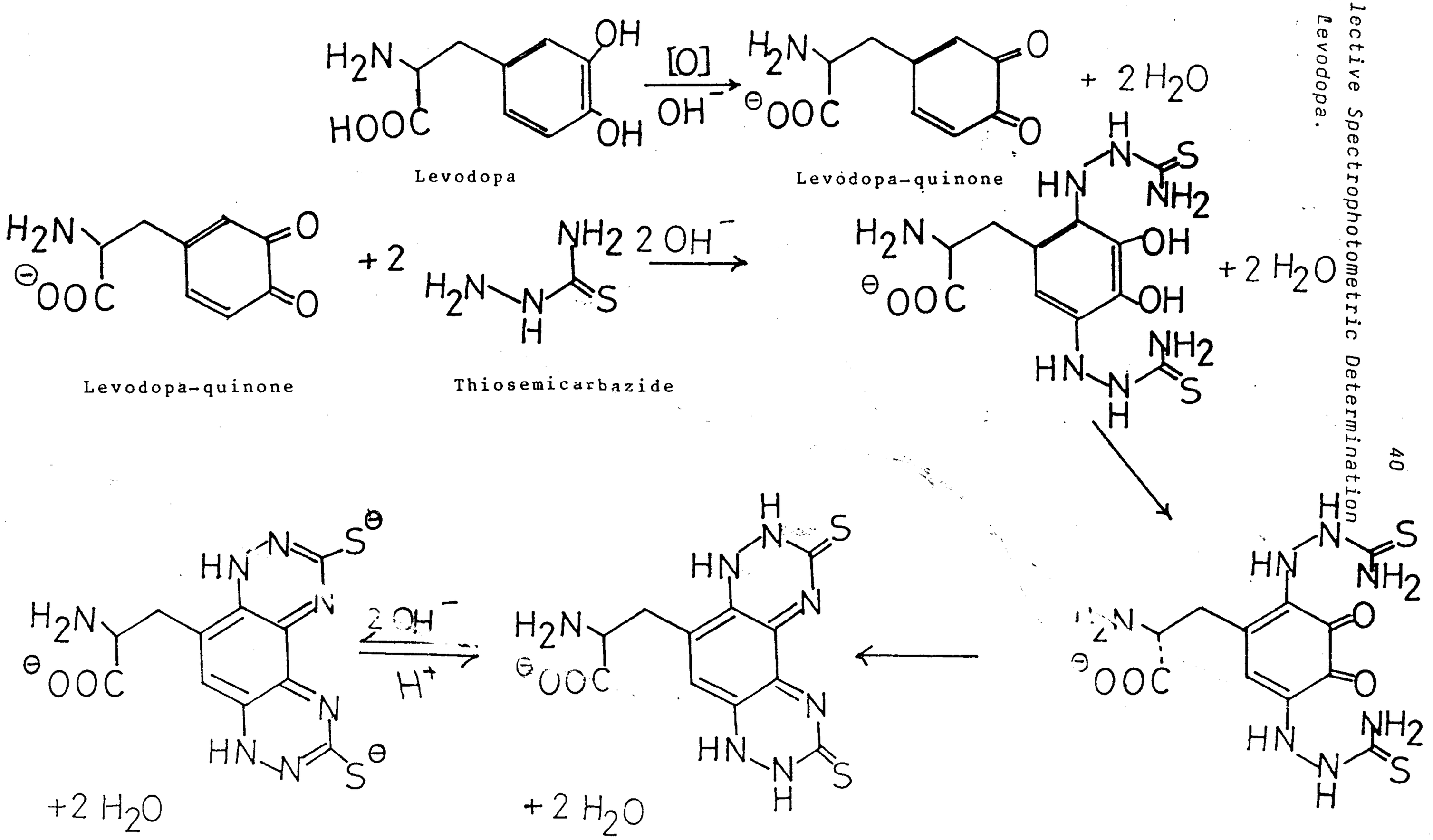
Statistical evaluation of the regression equation gives the following data :

Standard deviation (S)=0.0101
Variance ratio (F) = 9411.64
Probability of correlation > 0.995

Beer's plot can be used also for calculation of concentration. The reproducibility of the procedure was determined by running 10 replicate samples, each containing 5 mc[illegible] of levodopa per ml in the final assay solution. At this concentration level, the coefficient of variation was 0.84 %.

Application to Bulk Drug and Dosage Forms :

The suggested method was applied to the quantitative determination of levodopa in bulk and in Larodopa tablets in comparison with the U.S.P. XXI method (Table 1). The values of student's t and F ratio show no significant difference between the thiosemicarbazide and the pharmacopoeial methods.


Levodopa
Levodopa-quinone
Levodopa-quinone
Thiosemicarbazide

Scheme 1.

Table 1: Assay of levodopa in bulk drug and dosage form by the thiosemicarbazide method.

Sample or tablets	Amount taken, mg	Recovery %±SD.%*			
		Thiosemicarbazide method	Official method	t[a]	F[b]
Bulk drug	25	100.1+0.79	99.9±1.22	0.308	2.38
Bulk drug	50	99.7±0.86	99.8±1.17	0.154	1.85
Bulk drug	75	99.9±0.75	100.1±1.20	0.316	2.56
Larodopa tablets	100	98.5±0.82	98.8±0.92	0.544	1.26
Larodopa tablets	200	98.9±0.76	99.1±1.04	0.347	1.87

* Mean of 5 determinations

a Tabulated t for 4 degrees of freedom at P 0.05=2.776

b Tabulated F for(4,4)degrees of freedom at P 0.05=6.39

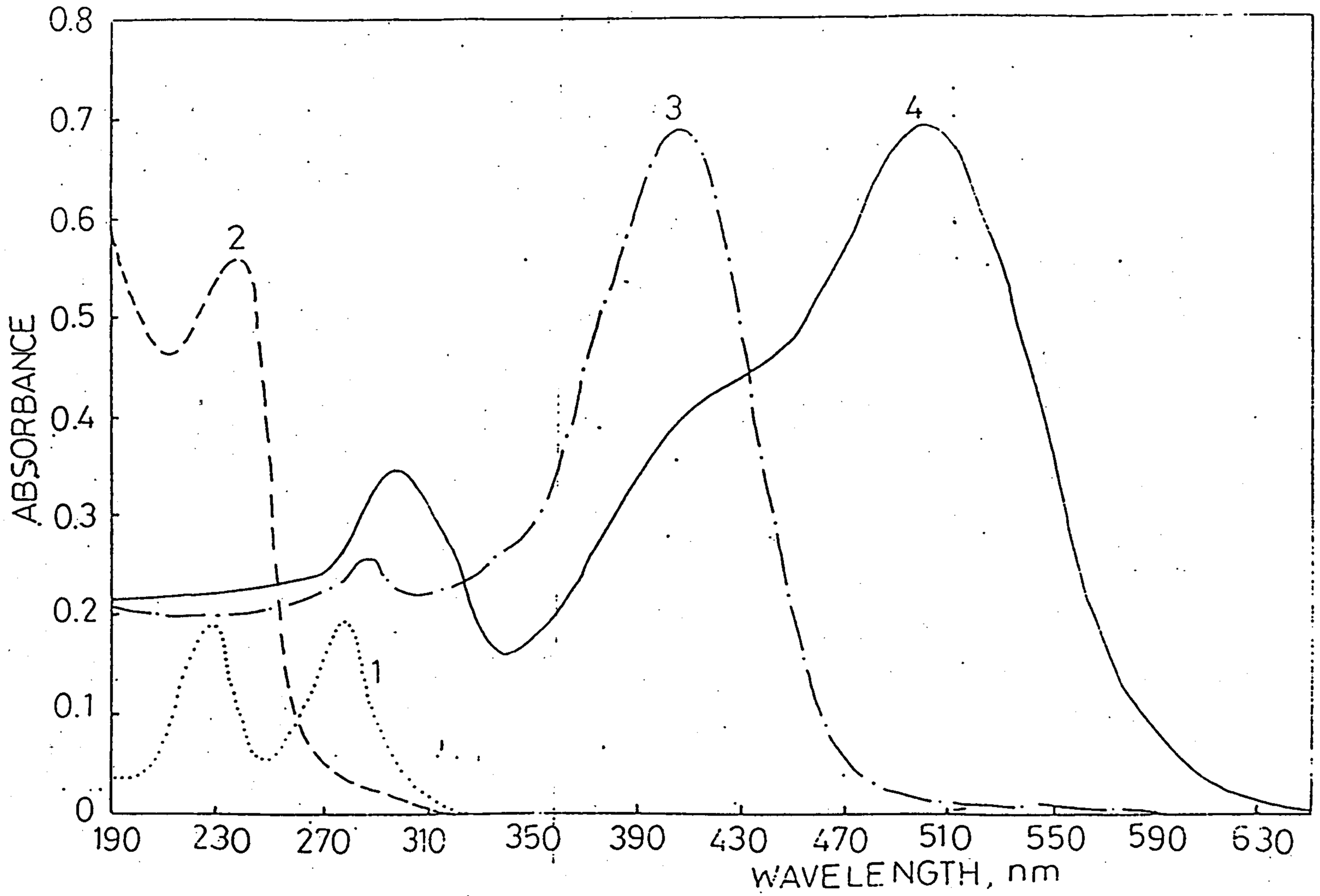


Fig. 1. Absorption spectra of : (1) levodopa, (2) thiosemicarbazide, (3) coloured product in 0.03 N H_2SO_4 and (4) coloured product in 0.03 N NaOH. Final concentration 5.3 μg/ml.

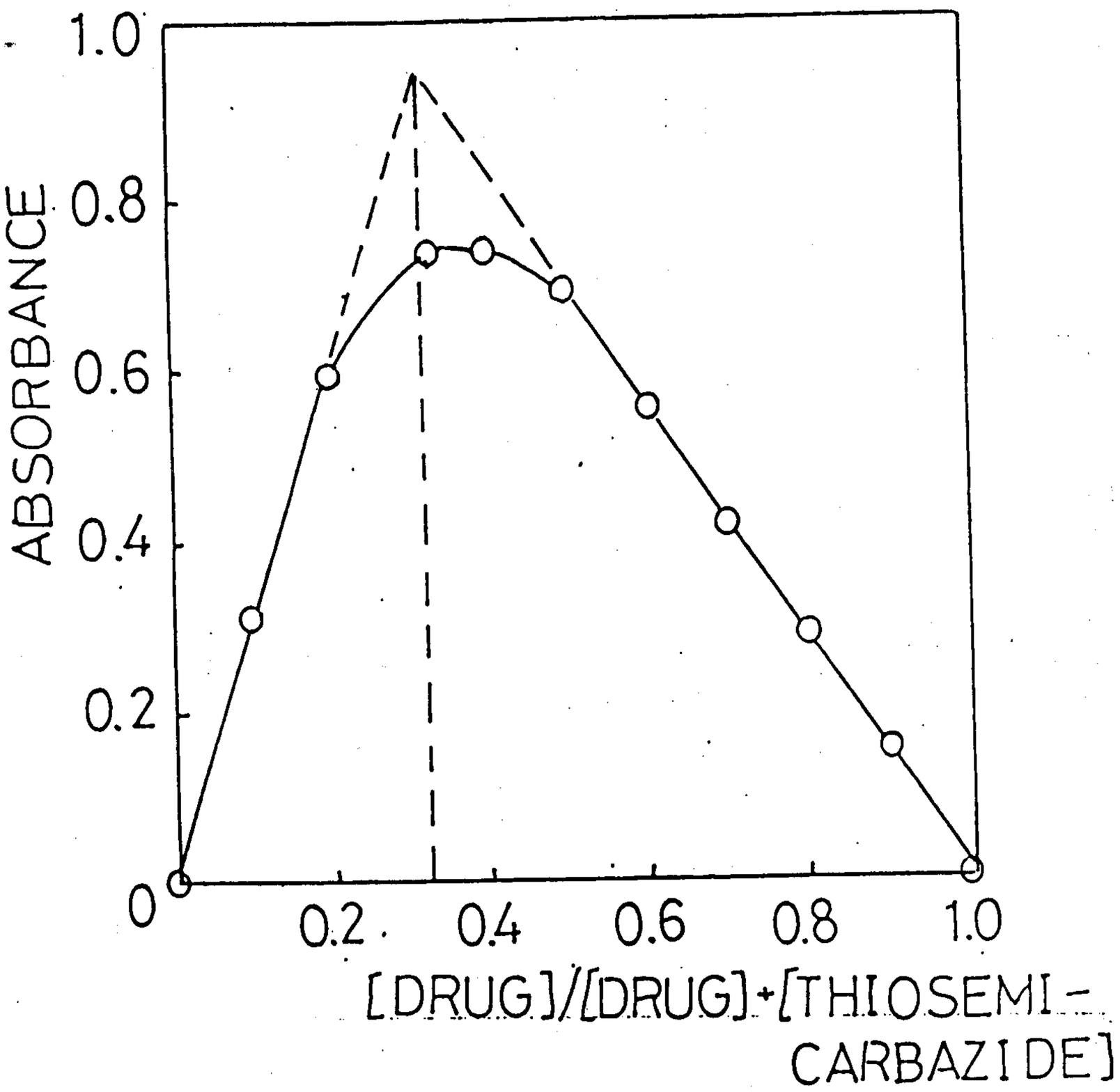


Fig. 2. Continuous molar variation plot.

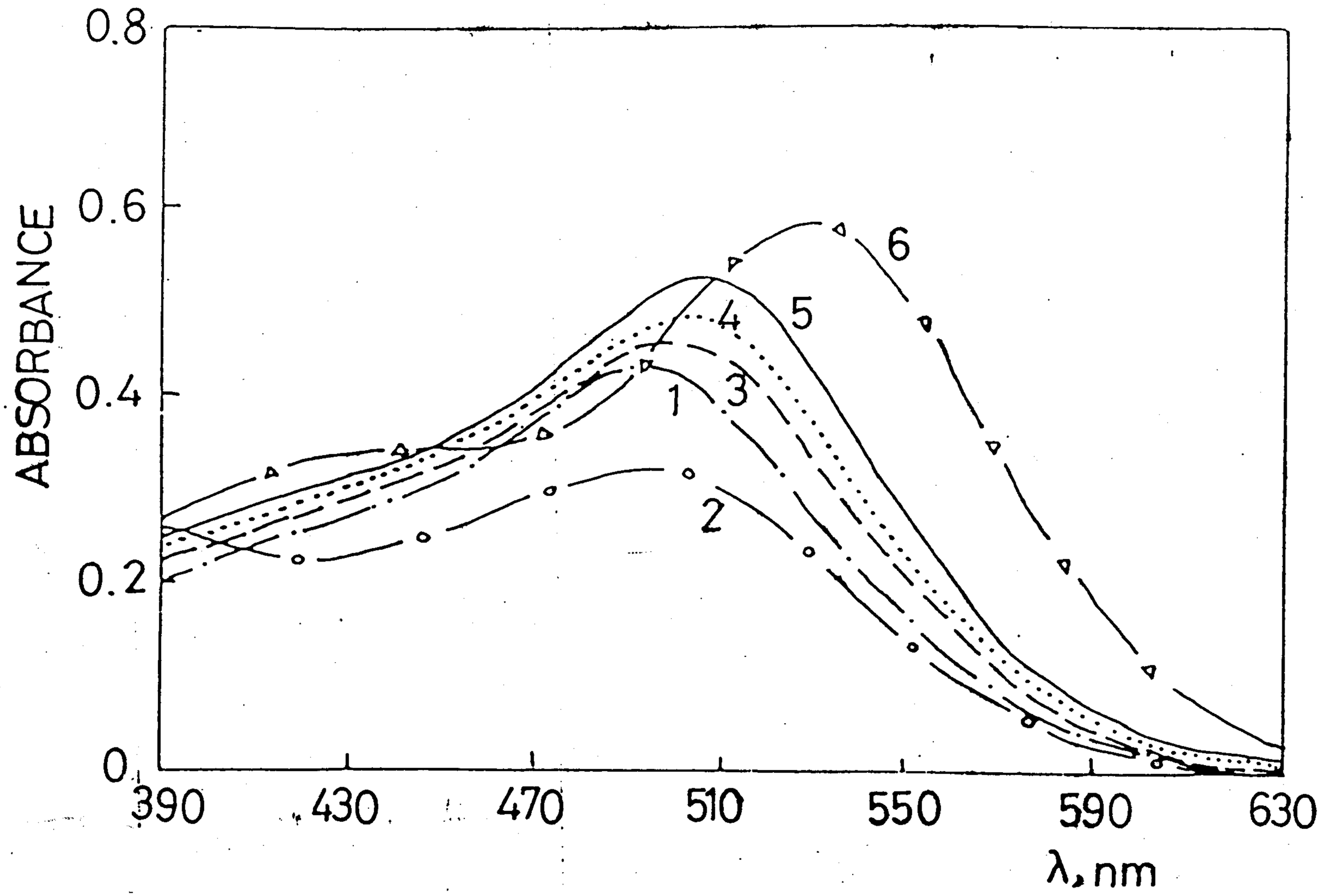


Fig. 3. Absorption spectra of levodopa - thiosemicarbazide reaction product in : (1)water, (2)methanol, (3)ethanol, (4)propan-1-ol, (5)propan-2-ol and (6)DMSO. Final concentration, 3.9 μg/ml.

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طريقة طيفية انتقائية لتعيين ليفودوبا

ميشيل ايليا القمص

قسم الكيمياء الصيدلية ـ كلية الصيدلة ـ جامعة أسيوط

فى هذا البحث تم التوصل الى طريقة طيفية انتقائية للتعيين الكمى لمادة الليفودوبا وهو دواء يستخدم فى علاج مرض الباركنسون • وتعتمد الطريقة على تفاعل الليفوديا مع ثيوسيميكارباذيدفى وسط قلوى لانتاج مركب أحمر اللون له ذروه أمتصاص عند ٥٠٠ ن م وتصل درجة الامتصاص الجزيئ للجوهر الملون الى ٢٥ر٨٠٠ •

ولقد تمت دراسة طيف الجوهر الملون الناتج فى الوسط الحامضى والوسط القلوى وأيضا تم تقدير النسبة الجزيئية لليفودوبا وثيوسيميا كاربازيد فى التفاعل حيث وجد أنها ١ : ٢ وبناء عليه تم اقتراح ميكانيكية التفاعل •

كما تمت دراسة كل العوامل التى تؤثر على التفاعل لمعرفة أحسن الظروف للتعيين الكمى من حيث الحساسية والسرعة •

وقد استخدمت الطريقة فى تحليل ليفودوبا فى صورته النقية وأيضا فى صورة أقراص لارودوبا (شركة روش) ووجدت النتائج متطابقة مع نتائج طريقة دستور الادوية الامريكى ١٩٨٥ •

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