تطوير طريقتين طيفيتين لتقدير الامتربتلين في المستحضرات الصيدلانية بالاعتماد على دراسة المحددات أحاديا وبطريقة السمبلكس للوصول إلى الظروف الفضلى

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الخلاصة

اقترحت طريقتان طيفيتان سهلتان وبسيطتان لتقدير الامتربتلين بشكله النقي وفي الأقراص.تعتمد الطريقة الأولى على تكوين معقد ازدواج شحنة بين الامتربتلين واهبا للالكترونات مع تترابرومثيلين مثل مستقبل باي.اظهر ناتج التفاعل أقصى امتصاص عند طول موجي مقداره 470 نانوميتر في مذيب الاسيتونتريل عند دالة حمضية مقدارها 9. وفي الطريقة الثانية قيست امتصاصية معقد الازدواج الأيوني المتكون من تفاعل لدواء قيد الدراسة مع البروموكريزول الأخضر عند طول موجي مقداره 415 نانوميتر عند دالة حمضية مقدارها 3.5

طبقت طريقتا تغير المحددات أحاديا التقليدية وطريقة السمبلكس المحورة في دراسة المتغيرات للوصول إلى الظروف الفضلى للتفاعلين باستخدام برنامج السمبلكس الهندسي ثلاثي الإبعاد .أظهرت النتائج التي تم الحصول عليها مطاوعة لقانون بيير في مدى تركيز يتراوح من 6–70 و 8 – 100 مايكرو غرام.مل$^{-1}$ وبقيم معامل امتصاص مولاي مقدارها 2275 و 1475 لتر.مول$^{-1}$ سم$^{-1}$ لطريقتي تترابرومثيلين والبروموكريزول الأخضر على التوالي.كانت قيم حدود الكشف مساوية إلى 0.043 و 0.034 مايكرو غرام.مل$^{-1}$ وقيم حساسية ساندل هي 0.122 و 0.188 مايكرو غرام.سم$^{-1}$ للطريقتين على التوالي. طبقت الطريقتان بنجاح لتقدير الامتربتلين في أقراص دوائية من مناشى مختلفة وكانت دقة وتوافق النتائج مقبولة.

Development of Two New Spectrophotometeric Methods for the Determination of Amitriptyline in Pharmaceutical Preparation Using Univariate and Simplex Optimization

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Abstract

Two simple and sensitive spectrophotometric methods are proposed for the determination of amitriptyline in its pure form and in tablets. The first method is based on the formation of charge-transfer complex between amitriptyline as n-donor and tetracyano-ethylene (TCNE) as π-acceptor. The product exhibit absorbance maximum at 470 nm in acetonitrile solvent (pH =9.0) . In the second method the absorbance of the ion- pair complex, which is formed between the soughted drug and bromocresol green (BCG), was measured at 415 nm at (pH=3.5) .
In addition to classical univariate optimization, modified simplex method (MSM) was applied in the optimization of the variable affecting the color producing reaction by a geometric simplex in three dimensions of space.
Beers' law was obeyed in the concentration ranges 6.0-70 and 8.0-100 $\mu g.ml^{-1}$ with molar absorbitivites of 2275 and 1475 $l. mol^{-1} cm^{-1}$ for TCNE and BCG methods respectively. The limits of detection of the two methods are 0.043 and 0.034 $\mu g.ml^{-1}$ and their Sandells sensitivity values are 0.122 and 0.188 $\mu g.ml^{-1}$ respectively.

Introduction

Amitriptyline is chemically 3-(10,11-dihydro-5H-dibenzo[a,d]cycloheptene-5-ylidene)-N,N-dimethyl-1-propanamine, a tricyclic antidepressant drug, widely used for treating clinical depression, neuropathic pain , nocturnal enuresis, and attention-deficit hyperactivity disorder (ADHD) [1] , but it has also been used successfully for headache, anxiety, smoking cessation, bulimia nervosa, persistent hiccups and as an adjunct in schizophrenia [2].

The vital importance of this drug prompted the development of various analytical methods for its determination , these methods include capillary electrophoresis [3,4] high performance liquid chromatography [5,6], gas chromatography [7,8], potentiometric [9,10] , chemometric [11], and spectrophotometry [12-18].

π-acceptors such as 2,3-dichloro-5,6-dicyano-p-benzo-quinone(DDQ), 7,7,8,8-tetracyanoquino-dimethane(TCNQ), tetracyano-ethylene(TCNE), 2,4,7-trinitroflurene-9-one(TNF), 2,5-dihydroxy-3,6-dichloro-p-benzoquinone(p- chloranilic acid) are known to yield charge transfer complexes and radical ions with a variety of electron donors such as cephalosporins [19] , gabapentin [20] , loratadine [21] and ranitidine [22] . On the other hand, ion- pair extraction spectrophotometry has been received aconsiderable attention for quantitative estimation of pharmaceutical compound . Bromophenol blue (BPB), methylene blue (MB) , bromocresol purple (BCP) and thymol blue (TB) were widely used as ion- pairing reagents for the quantitative analysis of many pharmaceutical compounds [23-25] .

In experimental chemistry, the optimization of technical systems is the process of adjusting the control variables to find the levels that achieve the best optimization. Usually, many conflicting response must be optimized simultaneously. In lack of systematic approaches the optimization is done by " trial-and- error", or by changing one control variable at a time while holding the rest constant, such methods requires a lot of experiments to be carried out.

Simplex optimization of experimental parameters was first introduced by Spendley [26] and then modified by Nelder [27] and Aberg [28] . The method found a lot of applications in the felid of analytical chemistry [29-32] , because it offers the capability of optimizing several factors simultaneously depending on a statistical design search to find the maxima or minima of response, by rejecting the point producing the worst response and a replacement of it by the new point which is obtained statistically.

The present work established an improved spectrophotometric method for the determination of amitriptyline by exploiting its basic nature and electron- donating property. The determination is based on charge- transfer reaction with TCNE and ion – association complexation with Bromocresol green (BCG) and the optimization of chemical spectrophotometric variables of the proposed methods namely pH, reagent amount and reaction time were studied by using both classical univariate and modified simplex. The multivariate simplex optimization was carried out via computer program [33] adapted to fit personal micro-computer.

Experimental

Apparatus:

A Shimadzu (model 1601 UV- visible spectrophotometer from Shimadzu, Koyoto, Japan) with 1cm glass cells was used for absorbance measurements.pH – meter model PW-9421 from Philips was used for all pH measurements .

Materials and Reagents

All chemical used were of analytical reagent grade unless otherwise- amitriptyline-hydrochloride standard powder materials (purity 99.8%) were provided from the State Company for Drug Industries and Medical Appliances Samara-Iraq (SDI).

1- TCNE 1.6×10^{-3} M solution, prepared by dissolving 20.5 mg of the reagent in 100 ml of acetonitrile by using volumetric flask.

2- BCG 1×10^{-3} M solution prepared by dissolving 36.0 mg of the reagent in 50 ml distilled water by using volumetric flask.

3- Phthalate buffer (pH=3.60).To 250 ml of 0.2M potassium hydrogen phthalate 11.90 ml of 0.2M HCl was added and then the solution was diluted to a final volume 1000ml with distilled water [34] .

Standard amitriptyline solutions:

1- Solution for TCNE procedure (250 $\mu g.ml^{1-}$): 50 mg of amitriptyline base was dissolved in 50 ml of methanol, then the solution was made alkaline (pH=9.0) with a dropwise of 0.2N sodium hydroxide.The solution was quantitatively transferred into a separating funnel and shaken with four 10 ml portions of chloroform.The extracts were pooled by filtration through a filter paper containing anhydrous sodium sulphate into a 200 ml volumetric flask and diluted to volume with chloroform. This was diluted to get a working concentration of 100 µg/ml .

2- Solution for BCG procedure (250 $\mu g.ml^{-1}$): 50 mg of amitriptyline base was dissolved in 200 ml of methanol by using volumetric flask.

Procedures:

Calibration graphs

1- **TCNE method:**
Serial volumes of standard solution ranging from 0.60 to 7.0 ml were transferred to 10 ml volumetric flasks , then 1.50 ml of TCNE reagent was added, and allowed to stand for 30 min at 25^0 C and then diluted to volume with acetonitrile. The absorbance was measured at 470nm against reagent blank.

2- **BCG method**:
Serial volumes of standard solution ranging 0.32 to 4.0 ml were transferred individually into 25 ml separating funnel, then 1 ml of BCG solution and three ml phthalate buffer (pH= 3.6) were added.The formed complex was extracted for 2 min with two 5 ml portions of chloroform. The extracts were pooled by filtration through a filter paper containing anhydrous sodium sulphate into a 10 ml volumetric flask and diluted to volume with chloroform, and then the absorbance was measured at 415 nm against reagent blank.

Procedure for the determination of amitriptyline in pharmaceutical preparation:

TCNE method:

Ten tablets were finely powdered and mixed .An accurately weighed quantity equivalent to the drug base concentration mentioned in the standard solution preparation was dissolved by shaking with 50 ml distilled water . The solution was made alkaline (pH=9.0) with a drop wise addition of 0.2N sodium hydroxide. The resulted solution was quantitatively transferred into a separating funnel and shaken with four 10 ml portions of chloroform. The extracts were pooled by filtration through a filter paper containing anhydrous sodium sulphate into a 200 ml volumetric flask and diluted to volume with chloroform. The procedure was continued as described under the preparation of calibration graphs [24].

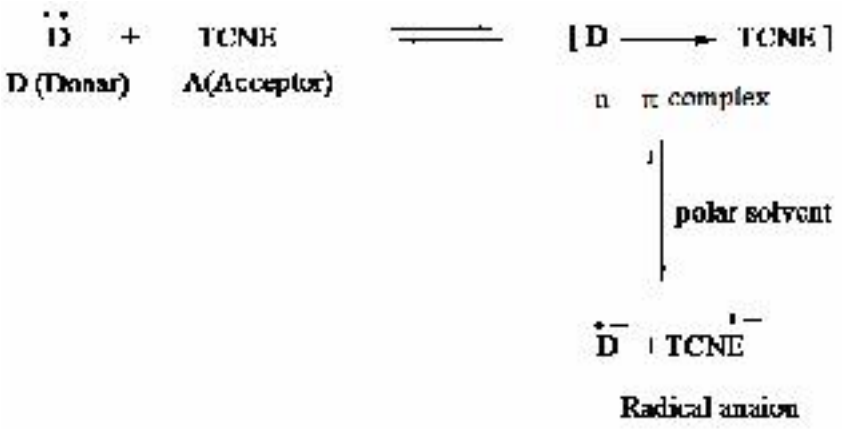
BCG method :

Ten tablets were finely powdered and mixed .An accurately weighed quantity equivalent to the drug base concentration, mentioned in the standard solution preparation, was dissolved in 200 ml of methanol by using volumetric flask .The procedure was continued as described under the preparation of calibration graghs [24].

Results and Discussion

Charge transfer complexation:

The reaction of amitriptyline with TCNE in acetonitrile solvent results in the formation an intense red brown color complex, which exhibits an absorption maximum at 470 nm (Fig 1). This absorption band formed is the results of the formation of charge-transfer complex through the interaction of TCNE as a π - acceptor and the studied drug as n-donor followed by the formation of colored radical anion according to the following scheme [22,24,35,36]:



Formation of a radical anion in such molecular interactions was confirmed by electron-spin resonance measurements [37].

Optimization of experimental variables:

i. Univariate method

Effect of pH:

The effect of pH on the development of the colored complex between amitriptyline and TCNE is shown in (Fig 2). The pH being adjusted with few drops of 0.1 M HCl and 0.1 M NaOH. Maximum and constant absorbance were obtained in the pH range 9.0-9.5.The absorbance decreased at pH value above 9.5 and below 9.0. Hence a pH of 9.0 was used in all the subsequent experimental work.

Effect of reagent:

Various volumes of TCNE solution were added to 40µg.ml^{-1} of amitriptyline solution. 1.5 ml of 1.6x 10^{-4} M of TCNE was found to be enough to develop the color to its full intensity and was considered to be the optimum for the concentration range of amitriptyline 6.0-75 µg.ml^{-1} (Fig3).

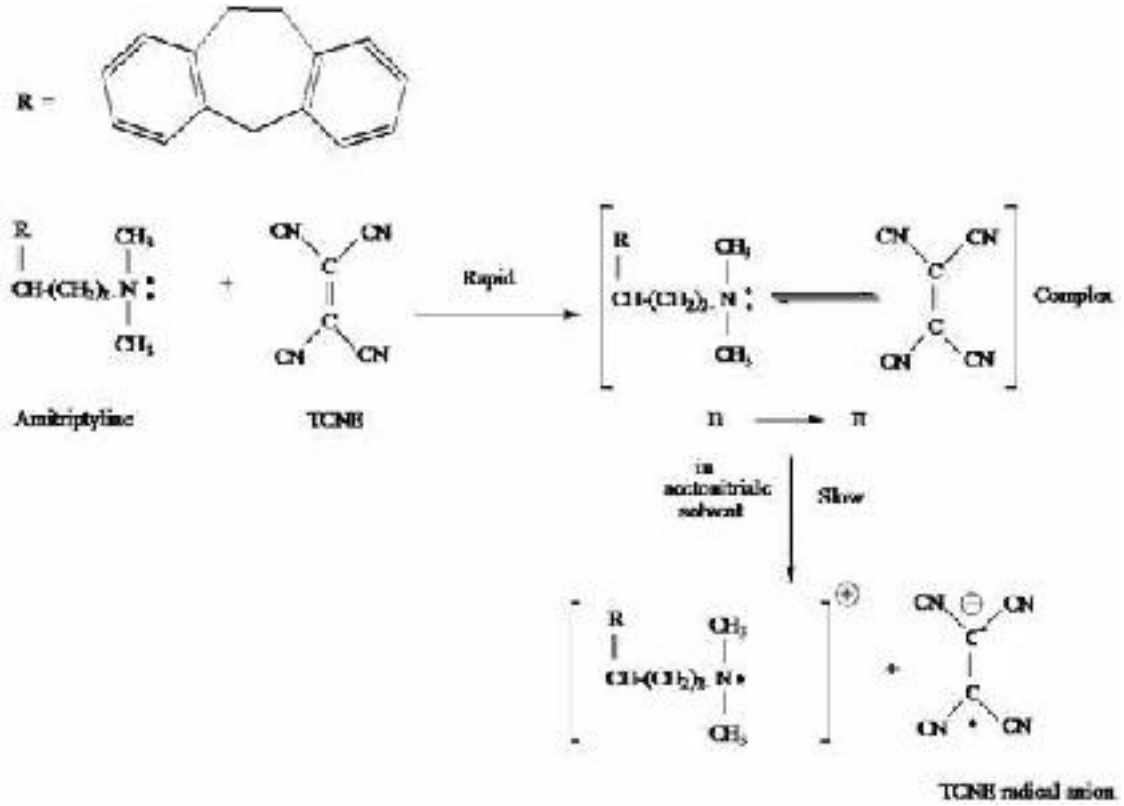
Effect of reaction time:

The color intensity reached a maximum after the amitriptyline was reacted with TCNE for 30 minutes (Fig 4), therefore 30 minutes development time was selected as optimum in the general procedure . The color obtained was stable for at least 2 hours.

Stoichiometry of the complex :

The stoichiometry of the reaction between amitriptyline and TCNE was studied by mole ratio method (Fig 5).

The results obtained shows that 1:1 amitriptyline to TCNE was formed at 470nm, therefore the formation of the complex can be represented as in following scheme [22,35] :



ii. Simplex optimization:

To set the simplex optimization of the three studied variables, four experimental conditions should be chosen involve values for pH, reagent volume and standing time. The values of the four experimental conditions were selected with specific boundaries for each at which it affects the absorption signal of the colored product (tablel).

The absorbencies of these four initial experiments were measured and the results were feed to the computer program. The program then starts simplex by searching the worse absorption signal and reflects it in ahyper-plane of the remaining points to produce a new set of experimental conditions, which were applied to carry out the experiment and the measured absorption signal was feeded again to the program. The process is repeated successively until optimum conditions were obtained (i.e. conditions yielding highest absorption signal). The procedure is continued for further few experiments to ensure that the optimum conditions are reached (Table 2 and Figure 6). Values of the results obtained by applying simplex program are shown in Figure 7.

Calibration graph:

Employing the experimental conditions described under procedure, a linear calibration graph for amitriptyline is obtained (Fig 8), which shows that Beers law was obeyed in the concentration range 6.0-70 $\mu g.ml^{-1}$.

Ion-pair complexation:

The amitriptyline solution reacted with BCG solution in aqueous solution in acidic medium to form a yellow color ion pair complex, which exhibits an absorption maximum band at 415nm against reagent blank (Fig 9).

Optimization of experimental variables:

i. Univariate method:

Effect of pH:

In order to established the optimum pH range , amitriptyline solution was mixed with aspecified volume of BCG , and then the pH was adjusted to a value between 2.0-6.0 with a few drops of 0.1N NaOH or 0.1N HCl .Maximum and constant absorbances were obtained in the pH range 3.5-4.0 (Fig 10) . The absorbance was decreased at pH value above 4.0 and below 3.50. Hence a pH of 4.0 was used in all the subsequent experimental work.

Effect of reagent:

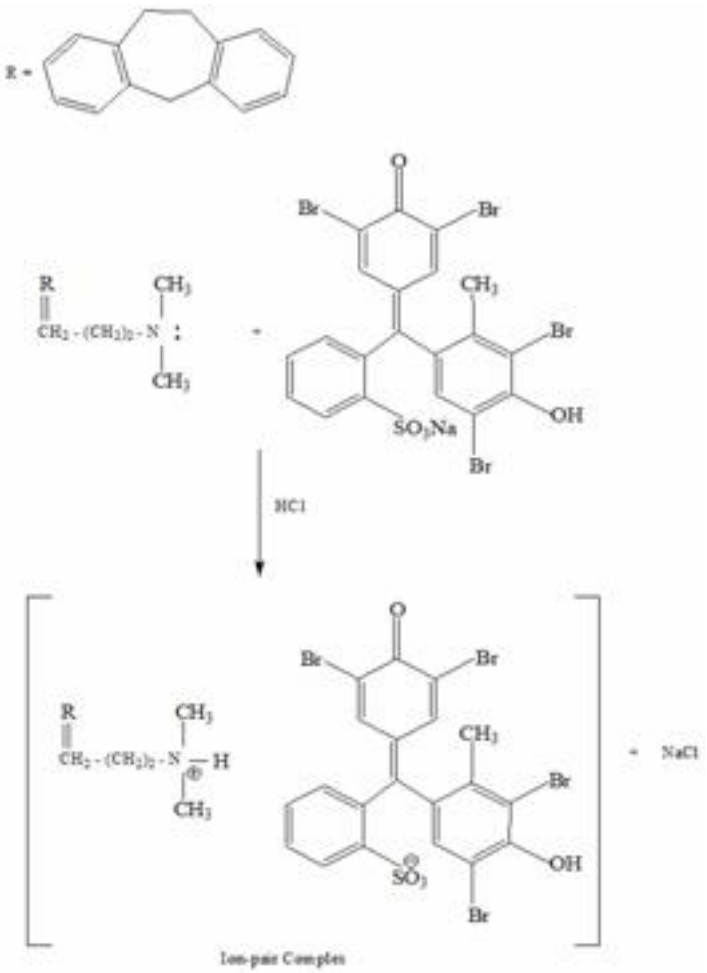
The influence of excess reagent concentration on the absorbance of the complex is illustrated in (Fig 11). One ml of $1.6x10^{-4}$ M solution of BCG was found enough to develop the color and reached its maximum intensity.

Effect of shaking time:

The optimum shaking time for the complete extraction of the ion pair complex with chloroform was studied from 30 second to 3 minutes (Fig12). It was found that the minimum shaking time for complete extraction was 2 minutes at room temperature ($25\pm1\ C^{o}$) .

Stoichiometry of the complex:

The stoichiometry of the reaction between amitriptyline and BCG was studied by mole ratio method (Fig13). The results obtained shows that 1:1 amitriptyline to BCG was formed at 415 nm, therefore the formation of the complex can be represented as in following scheme [24,25] :



ii. Simplex optimization:

The same steps of simplex program, within the given boundary conditions (Table 3), were followed to optimize the experimental conditions. Results are shown in Figure 14, 15 and Table 4.

Calibration graph

Employing the experimental conditions, a linear calibration graph for amitriptyline is obtained (Fig 16), which shows that Beers law was obeyed in the concentration range of 8.0-100 $\mu g.ml^{-1}$.

Spectral characteristic of the two proposed methods:

Under the optimum experimental conditions of the two proposed methods, the regression plots showed that there were linear dependence of absorbance signals on the concentration of the drug in the ranges given in Table 5. The regression equations and correlation coefficients, which were obtained by the linear least –squares treatment of the results in addition to the molar absorptivites, detection limits , and Sandell sensitivities are given in Table 5.

The accuracies of the two proposed methods were established by performing seven replicate analyses on standard solutions containing three different amounts of drug and calculating the percentage error. The precisions were determined by calculating the relative standard deviations (RSD) for seven determinations at each level Table 6. It is clear from the results that at all of the three studied levels , the values of the mean ($\bar{X} - \mu$) were less than the values of indeterminate error ($\pm ts/\sqrt{N}$), indicating that no significant differences existed between the mean and the true values.

Table 7 shows that the two proposed methods have acceptable linearity ranges, acceptable precisions and accuracies when they compared with other methods.

Analytical application:

The proposed methods were applied to determine amitriptyline in pharmaceutical preparation tablets. The results, presented in Table 8, reveal that the recoveries were in the range of 94.56 to 100.20 %, reflecting high accuracies and precisions of the proposed methods as indicated by low RSD values.

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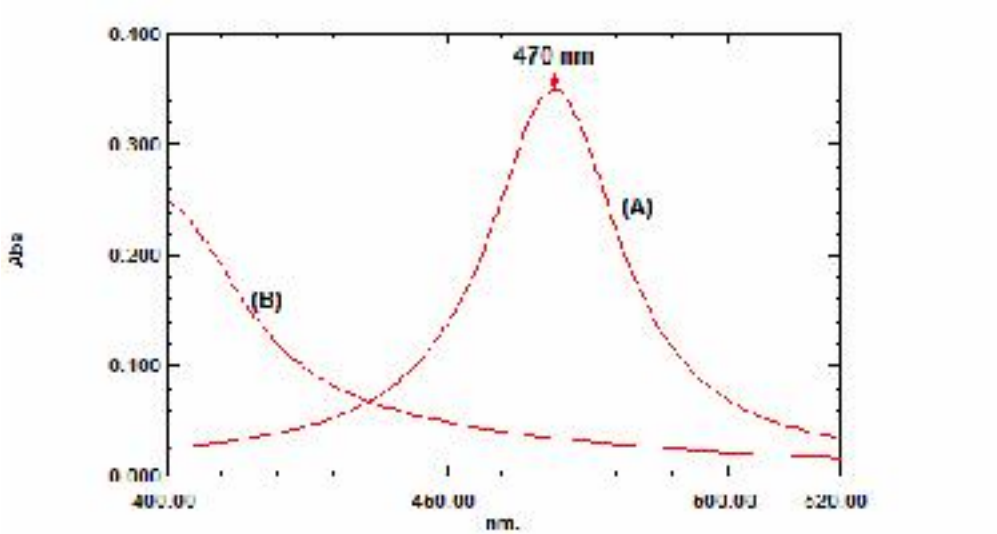

Fig.(1): Absorption spectra of: (A) 40 µg.ml^{-1} amitriptyline, 2.4 x 10^{-4} M TCNE at pH=9.0 against reagent blank; (B) 2.4 x 10^{-4}M TCNE in acetonitrile against distilled water.

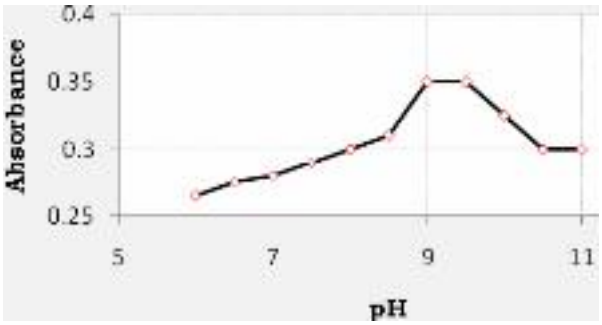

Fig.(2): Effect of pH on the absorbance of : 40 µg.ml^{-1} amitriptyline, 2.4 x 10^{-4} M TCNE at 470 nm

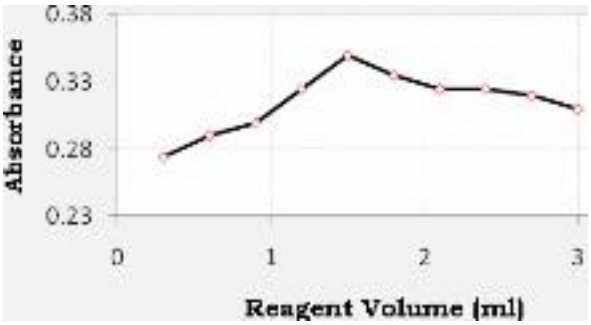

Fig.(3): Effect of reagent volume on the absorbance of 40 µg.ml^{-1} amitriptyline solution.

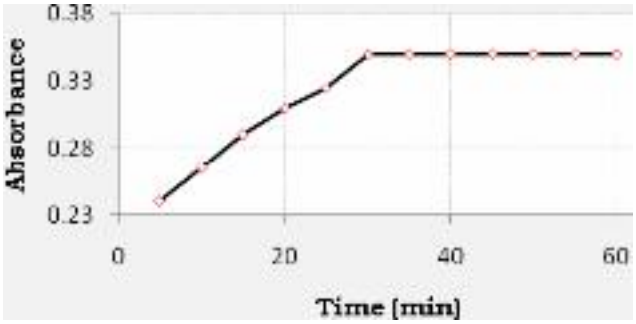

Fig.(4): Effect of standing time on the absorbance of 40 µg.ml^{-1} amitriptyline, 2.4 x 10^{-4} M TCNE at pH=9.0.

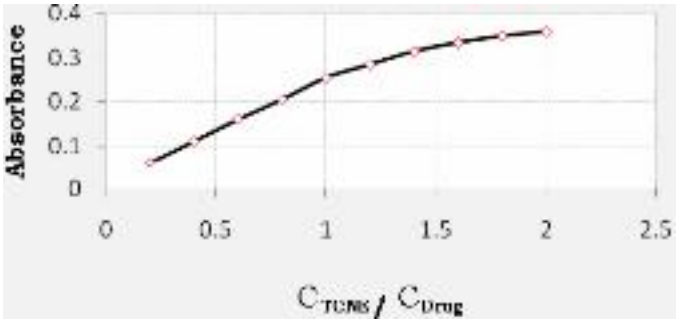

Fig.(5): Mole ratio plot of amitriptyline determined via charge-transfer method.

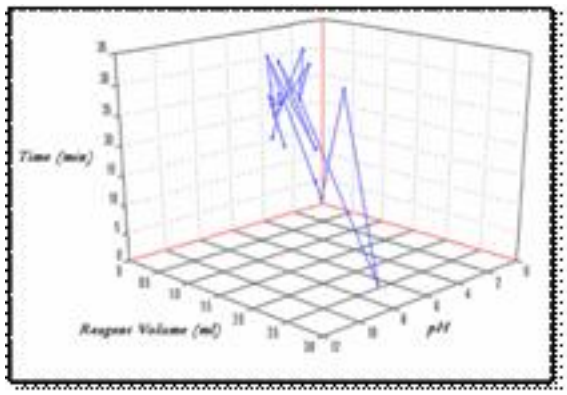

Fig.(6): Experimental simplex study of variables resulting in pH=9, reagent volume=1.5ml, and standing time=30min.

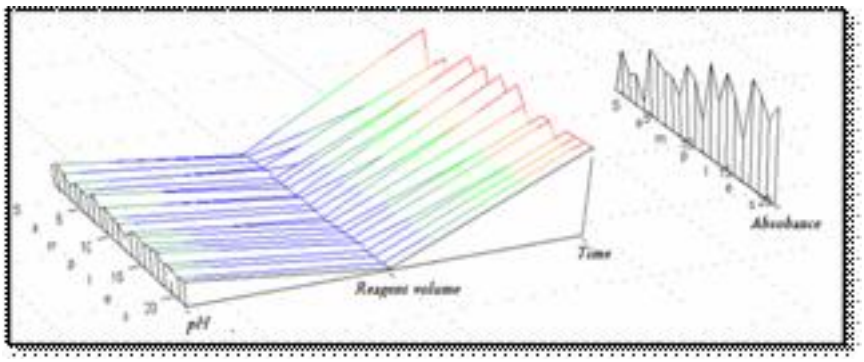

Fig. (7): Optimization of absorption signal via simplex changing of pH, reagent volume, and standing time.

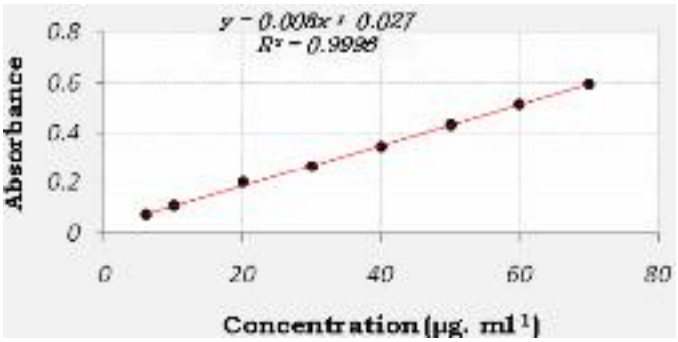

Fig.(8): Calibration graph of amitriptyline with 2.4 x 10^{-4} M TCNE at 460 nm.

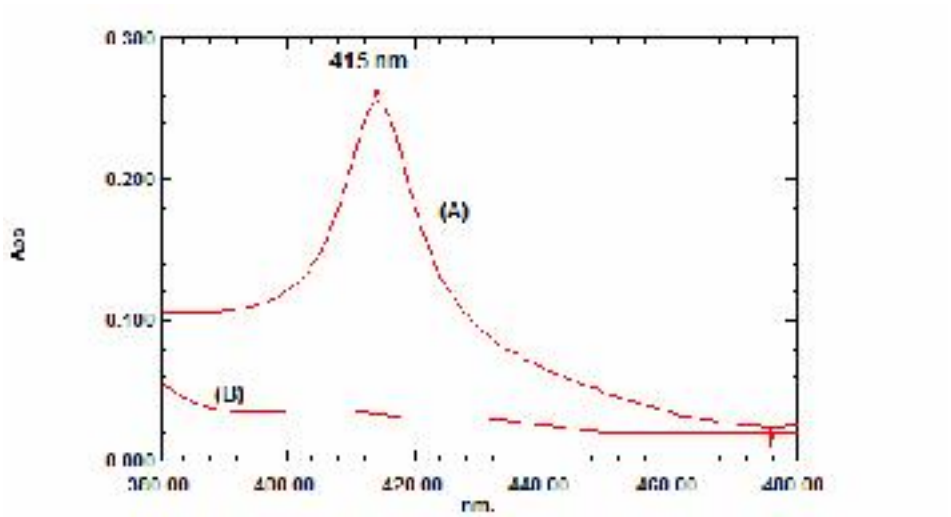

Fig.(9): Absorption spectra of: (A) 45 µg.ml^{-1} amitriptyline, 1.0 x 10^{-4} M BCG at pH=3.5 against reagent blank; (B) 1.0 x 10^{-4} M BCG in chloform against distilled water.

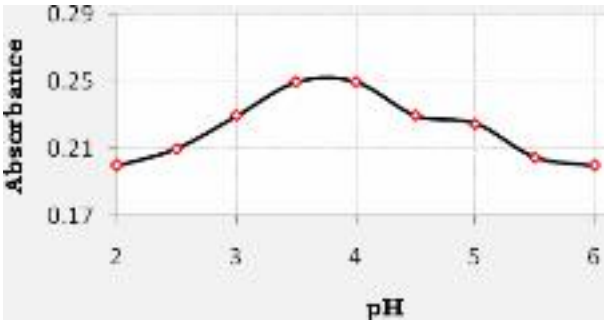

Fig.(10): Effect of pH on the absorbance of : 45 µg.ml^{-1} amitriptyline, 1.0 x 10^{-4} M BCG at 415 nm

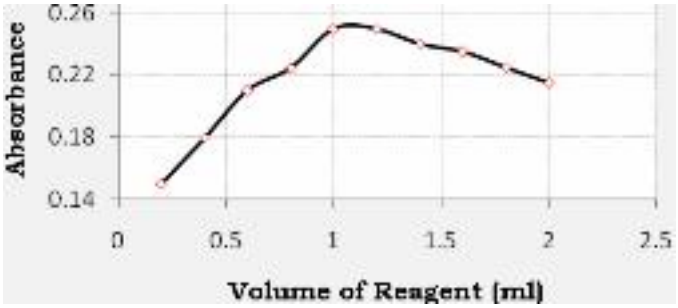

Fig.(11): Effect of reagent volume on the absorbance of 45 µg.ml^{-1} amitriptyline solution.

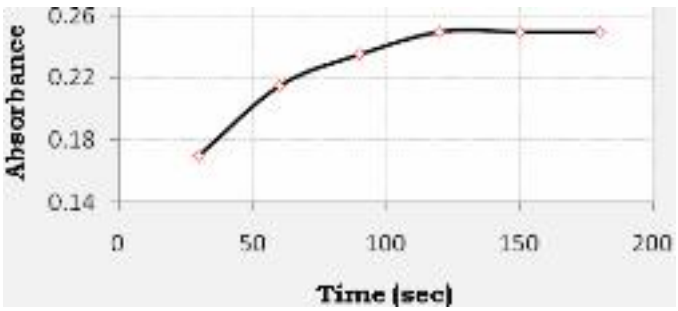

Fig.(12): Effect of shaking time on the absorbance of 45 µg.ml^{-1} amitriptyline, 1.0 x 10^{-4} M BCG.

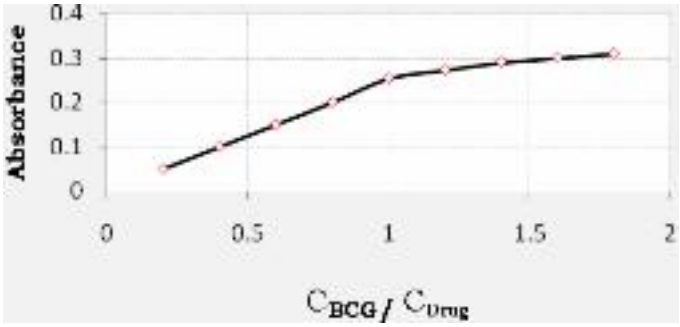

Fig.(13): Mole ratio plot of amitriptyline determined via ion-pair method

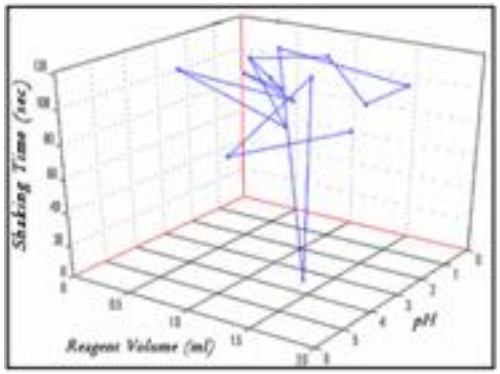

Fig.(14): Experimental simplex study of variables resulting in pH=1.2, reagent volume=1.2ml, and shaking time=90 sec.

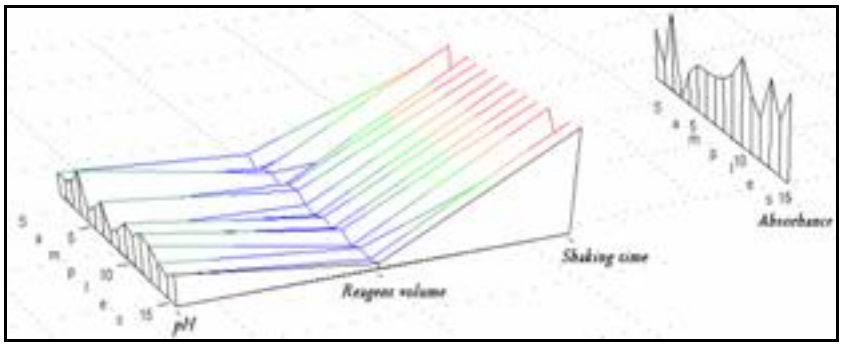

Fig.(15): Optimization of absorption signal via simplex changing of pH, reagent volume, and shaking time.

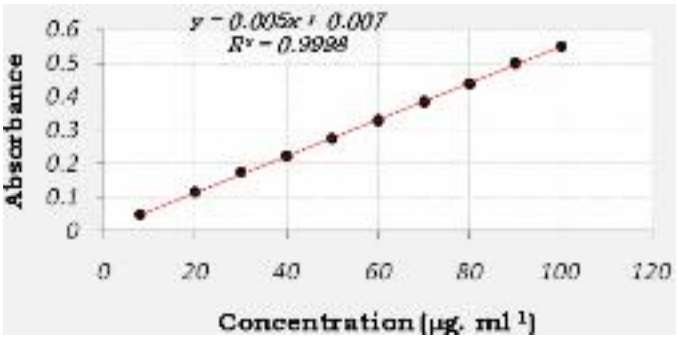

Fig.(16): Calibration graph of amitriptyline with 1.0 x 10^{-4} M BCG at 415 nm.

Table (1): Boundary conditions for the studied variables

Variable	range
pH	6-11
Reagent volume (ml)	0.3-3.0
Standing time (min)	5-60

Table(2): Absorbance for each of the simplexes in the optimization of color producing reaction variables

Operation	pH	Reagent Volume(ml)	Time (min)	Absorbance
Simplex 1	8.0	1.2	35	0.200
Simplex 2	10.0	2.4	20	0.267
Simplex 3	8.5	2.1	15	0.239
Simplex 4	6.0	1.8	30	0.246
Simplex 5	9.0	3.0	5	0.212
Simplex 6	8.5	1.8	30	0.297
Simplex 7	8.0	1.8	35	0.287
Simplex 8	11.0	2.1	30	0.279
Simplex 9	8.0	1.2	35	0.279
Simplex 10	10.0	2.4	25	0.259
Simplex 11	8.5	1.5	35	0.310
Simplex 12	8.5	1.8	30	0.297
Simplex 13	6.0	1.2	35	0.246
Simplex 14	9.5	1.8	30	0.335
Simplex 15	10.0	1.8	25	0.289
Simplex 16	9.5	1.8	30	0.335
Simplex 17	10.0	1.8	30	0.311
Simplex 18	10.5	2.1	25	0.259
Simplex 19	9.0	1.5	30	0.350
Simplex 20	9.5	1.8	30	0.335
Simplex 21	8.5	1.5	30	0.310
Simplex 22	9.5	1.8	30	0.335

Table (3): Boundary conditions for the studied variables

Variable	range
pH	2-6
Reagent volume (ml)	0.2-2.0
Standing time (sec)	30-180

Table (4): Absorbance for each of the simplexes in the optimization of color producing reaction variables

Operation	pH	Reagent Volume(ml)	Time (sec)	Absorbance
Simplex 1	3.0	1.6	90	0.203
Simplex 2	3.0	0.6	60	0.166
Simplex 3	3.5	1.4	120	0.240
Simplex 4	5.5	1.8	30	0.138
Simplex 5	2.0	0.8	120	0.180
Simplex 6	3.0	2.0	120	0.198
Simplex 7	4.5	2.0	120	0.198
Simplex 8	2.0	1.2	120	0.200
Simplex 9	3.0	0.8	120	0.207
Simplex 10	4.5	1.4	120	0.221
Simplex 11	4.0	1.0	120	0.250
Simplex 12	5.0	1.6	120	0.212
Simplex 13	4.5	0.6	120	0.193
Simplex 14	3.5	1.2	90	0.250
Simplex 15	3.0	0.8	120	0.207
Simplex 16	4.0	1.2	120	0.250

Table(5): Analytical characteristics for the two methods.

Parameter	TCNE method	BCG method
linear dynamic range ($\mu g.ml^{-1}$)	6.0-70	8.0-100
Regression equation	Abs=0.008Conc.+0.027	Abs=0.005Conc.+0.007
Slope (b) ($l.mg^{-1}.cm^{-1}$)	0.008	0.005
Intercept (a)	0.027	0.007
Correlation coefficient	0.9998	0.9999
Molar absorptivity $l.mol^{-1}.cm^{-1}$	2275	1475.50
Detection limit ($\mu g.ml^{-1}$)*	0.043	0.034
Sandell sensitivity ($\mu g.ml^{-1}$)	0.122	0.188

*Calculated for single analysis at 99.9 confidence limit.

Table(6): Evaluation of accuracy and precision of the two methods.

Item	TCNE method			BCG method		
Concentration of amitriptyline taken ($\mu g.ml^{-1}$)	15.00	25.00	35.00	30.00	20.00	50.00
Concentration of amitriptyline found ($\mu g.ml^{-1}$) *	15.03	24.85	34.75	30.13	20.24	50.21
Error %	0.20	0.60	0.71	0.43	0.24	0.42
Standard deviation	0.11	0.59	0.59	0.39	0.41	0.30
R.S.D % (n= 7)	0.76	2.40	1.71	1.29	2.06	0.61
$\bar{X} - \mu$	0.03	0.15	0.25	0.13	0.24	0.21
$\pm ts/\sqrt{N}$	0.10	0.52	0.52	0.34	0.37	0.27

* Mean value of seven determinations (N) at each level.
$\bar{X}$=mean value , μ= true value.
t= 2.36 for n=7 at 95% confidence level.
s =standard deviation.

Table (7): Comparison of linearties of the two proposed methods with those from other spectrophotometric methods for determination of amitriptyline in pharmaceutical formulation.

Reagent	Beers law limit $\mu g.ml^{-1}$	References
Niobium (V) thiocyanate	1.0-12	16
-	1.0-30	12
Potassium thiocyanate *	3.0-60	14
Ammonium molybdate	1.0-140	15
Bromocresol purple *	30-200	13
Chloranilic acid	8.7-90	18
TCNE	6.0-75	This work
BCG	8.0-100	This work

*** Extractive procedure**

Table (8): Results of analyses of pharmaceutical preparations containing amitriptyline by TCNE method and BCG method.

Method	Depresol * 25mg				Tryptizol ♦ 25mg				Amitriptyline ◊ 25 mg			
	Taken ($\mu.ml^{-1}$)	Found ($\mu.ml^{-1}$)	§Mean Recovery%	§R.S.D.%	Taken ($\mu.ml^{-1}$)	Found ($\mu.ml^{-1}$)	§Mean Recovery%	§R.S.D.%	Taken ($\mu.ml^{-1}$)	Found ($\mu.ml^{-1}$)	§Mean Recovery%	§R.S.D.%
Charge-transfer method	15.00	14.95	99.68	0.98	15.00	14.84	98.94	1.98	25.00	24.84	99.38	1.18
	25.00	24.84	99.38	1.18	30.00	29.42	98.09	0.90	35.00	34.84	94.56	0.81
	45.00	44.52	98.94	0.71	45.00	44.52	98.94	0.71	50.00	49.77	99.55	1.84
Ion-pair method	15.00	14.92	99.46	0.82	15.00	14.91	99.40	0.71	20.00	19.74	98.71	0.66
	30.00	29.87	99.56	0.66	35.00	35.07	100.20	0.50	40.00	39.89	99.75	0.91
	60.00	59.71	99.51	0.87	60.00	59.60	99.33	1.01	70.00	69.92	99.88	0.41

§ n = 4

* Marked by S.D.I, Iraq.

♦Marked by MSD-USA.

◊Marked by HM-Holden BV-Holland

37-M.E.Abdel Hamid, M. Abdel Salam, M.S. Mahrous and M.M.Abdel-Khalek, Utility of 7,7,8,8-tetracyanoquinodimethane and p-chloranilic acid in the qualitative and quantitative analysis