Developing spectrophotometric method for Carbamazepine (antiepileptic drug) determination through preparation and Characterization of Fe(III) Complex With Mixed Ligand Containing Carbamazepine and Salicylaldehyde

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Abstract

The research includes developing an easy and sensitive analytical spectrophotometric method to determine Carbamazepine (CBZ) ,which consider among antiepileptic and antisezuires drugs.

The method was depended on preparation of mixed ligand [dibenzo[b,f]azepine-5[N-(O-hydroxybenzyliden)] amide from CBZ reaction with salicylaldehyde. It was found that the ligand has an ability to form colored complex with ferric ion at ratio (2:1) L: M , that have λ_{max} at 524 nm in aqueous solution at pH 6.5. The optimum conditions for Complexing CBZ with ferric ion including the volume of ferric solution (1.2 ml), time period (10 minutes) and temperature ranges (30-35 ^{o} C) were identified. The structural formula of the formed complex was proposed due to the spectroscopic study including UV and IR spectrums. Stability constant of CBZ-Fe complex in aqueous solution was (1.3×10^{9} L^{2}. mol^{-2}), and the complex was stable for 24 hours in the aqueous solution. Calibration curve obeyed the Beer's law at (1.0 -25) $mg.l^{-1}$ for CBZ with a correlation coefficient (r = 0.9996). The molar absorptivity coefficient was 2.17×10^{3} l. $mol^{-1}cm^{-1}$. The statistical processing of the results showed that relative standard deviation 1.1%, the average of relative error 1.42%, while method recovery is 100.2 %. The detection limit was 0.25 $mg.l^{-1}$ and limit of quotation was 0.83 $mg.l^{-1}$. The method reveals high accuracy and precision. This method is characterized by no need for solvent extraction. The proposed method was applied on samples of drugs and plasma which provided encouraging resultants.

الخلاصة

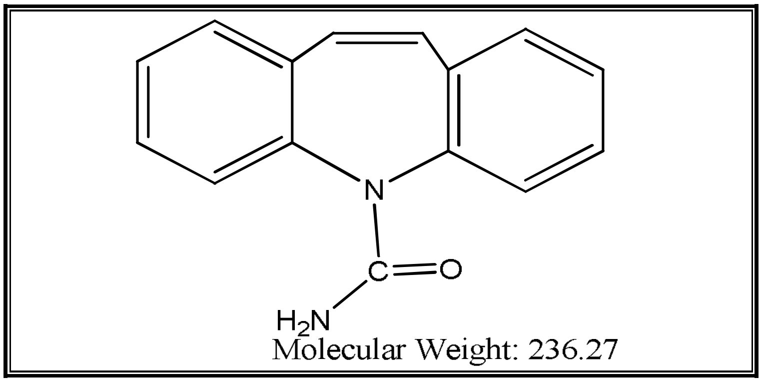
يتضمن البحث تطوير طريقة تحليلية طيفية سهلة وحساسة لتقدير الكاربامازبين CBZ المعروف بالتكريتول الذي يعد من الادوية المضادة للصرع والتشنجات . أعتمدت الطريقة على تحضير ليكاند مختلط

(dibenzo[b,f]azepine−5[N−(O−hydroxybenzyliden)] amide) من تفاعل CBZ مع السالسلديهايد والذي تبين ان له قابلية تكوين معقدات ملونة مع ايون الحديد الثلاثي بنسبة 2 : 1 (ليكاند : فلز) باستخدام طريقة النسبة المولية والتغيرات المستمرة . كان الطول الموجي للامتصاص الأعظم للمعقد (524) نانومتر في المحلول المائي وعند دالة حامضية 6.5، تم تحديد الظروف الفضلى لتقدير CBZ مثل حجم محلول أيون الحديديك (1,2 مل) والفترة الزمنية المثلى التي كانت 10 دقائق ودرجة الحرارة التي تراوحت بين 30 و 35 مْ. درس طيف الأشعة تحت الحمراء (IR) للمعقدالناتج وتمت مقارنتها مع طيف الليكاند و CBZ وشخصت المجاميع الوظيفية المشاركة في عملية التناسق مع اقتراح الصيغة التركيبية للمعقد المتكون. تضمن البحث دراسة ثابت استقرار نواتج المعقد في الوسط المائي، حيث وجد ان ثابت الاستقرار كان $1,3\times 10^{9}$ لتر^{2}/مول^{2} ، كما أنّ المعقد مستقر لـ 24 ساعة في الوسط المائي عند الدالة الحامضية المثلى. يطاوع منحني المعايرة قانون بير من 1 الى 25 ملغم/لتر ، وكان معامل الارتباط ($r = 0.9996$)، والامتصاصية المولارية للمعقد ($2,17\times 10^{3}$) لتر.مول$^{-1}$.سم$^{-1}$. بينت المعالجة الإحصائية للنتائج أنّ معدل قيم الانحراف القياسي النسبي (RSD) 1,1 % ومعدل الخطأ النسبي 1,42 % ، في حين ان معدل نسبة الاستردادية 100,2 % وحد الكشف 0,25 ملغم/لتر ، مما يدل على أن الطريقة تتمتع بالدقة والضبط الجيد . تمتاز الطريقة أنها لاتحتاج إلى عملية الاستخلاص بالمذيب ، وقد تم تطبيق الطريقة المقترحة بنجاح على نماذج من الأدوية وبلازما الدم .

Key words: Carbamazepine , Spectrophotpmetric , Antiepileptical , Salicylaldehyde , Ferric ion

Introduction

Carbamazepine (CBZ) is a white powder, soluble in polar solvents (propane , glycol, ethanol and acetone), but very poorly soluble in water. (1) Systematic (IUPAK) name: 5H-dibenz[b, f] azepine-5-carboxamide or 5h-dibenzazepine-5-carboxamide. Formula:$C_{15}H_{12}N_{2}O$, Molecular mass: 236.27g/mol , melting point (189-193) ^{o}C. Fig(1). CBZ was considered the primary drug for the treatment of partial and tonic-clonic seizures (2), epilepsy (3), and alcohol withdrawal (4). CBZ has been sold under the names Tegretol , Biston, Calepsin, Carbatrol, Epitol, Trimonil, and Epimaz. (5). The peak plasma concentration after one 400 mg tablet is 4.5 mg/l. (6). CBZ in human serum was determined by gas-liquid chromatography (7-12), liquid-Chromatography (13) and thin layer chromatography(14) . Also method based on the combination of liquid chromatography and mass spectrometry (LC-MS) has been developed for the quantitative analysis of CBZ (15). CBZ was determined by HPLC in serum, (16) in both plasma and saliva with detection limit= 0.02 µg.ml^{-1} (17) serum, saliva and urine samples (18) in human hair (19) and in human breast milk (20). Spectrofluorimetric method for the determination of CBZ in serum was developed with linear range of (0.04–140) µg ml^{-1} and detection limit (0.01) µg ml^{-1} (21)A flow injection spectrofluorimetric approach was used for determination of CBZ in serum with detection limit (80) µg ml^{-1} (22) and in pharmaceutical formulations. (23) In the present work, a new ferric complex with mixed ligand {Carbamazepine (CBZ) and salicylaldehyde) have been prepared. The complex was characterized physico-chemically and used analytically for carbamazepine drug determination.



Molecular Weight: 236.27

Fig. 1: The chemical structure of CBZ M.mass=236.27 g/mol

Experimental: Starting materials:

Carbamazepine standard was supplied by Sigma-Aldrich; all chemicals used were of analytical grade from Aldrich 'BDH and Merck. CBZ solution was prepared by dissolving 0.1 gm in 50 ml of ethanol, the solution stable for at least 3 months at 4°C. (24) , and Salicylaldehyde solution (2%) in ethanol was also prepared.

Synthetic methods:

1- The (CBZ- salicylaldehyde) ligand: (dibenzo [b, f] azepine-5-carboxylic acid-2-hydroxy-benzylidenamide):

CBZ (0.5 gm)(0.002 mol) dissolved in 20ml ethanol, then added to (0.244 gm)(0.002 mol) salicylaldehyde , The solution was refluxed for two hours. The mixture was allowed to stand overnight and concentrated. The crude material was recrystallized twice with methanol.

2- The Complex: Ferric (III) - (dibenzo [b,f]azepine- 5-carboxylic acid-2-hydroxy-benzylidenamide)

Equimolar solution (0.036M) of ligand and ferric hydroxide were prepared: A (0.2448 gm)(0.00072 mol) of Ligand (Molecular mass: 340 g/mol) dissolved in 10 ml absolute ethanol was mixed with (0.252 gm) (0.00072 mol) of $Fe(NO_3)_3.6H_2O$ (Molecular mass:350g/mol) (dissolved in 10ml of absolute ethanol , the mixture then refluxed for two hours , allowed to cool in ice bath and stand overnight and concentrated. The crude material was recrystallized with ethanol.

3-Analytical and Physical Measurements:

The color change or precipitate formation was noticed upon reaction of the prepared Ligand ($1X10^{-3}$ M) with 19 metal ions (0.1 M) in acidic and alkaline media with reflux for 30 minutes to 60 °C.

4- The optimum conditions for complex preparation:

The effect of ferric (Ш) concentration was studied by using different aliquots (0.3-4) ml of (100 mg/l) ferric solution was transferred into a series of 5ml standard flasks. To each flask, 0.5 ml of ligand solution (100 mg/l) was added and kept aside for 10 min. The volume was then made up with distilled water and absorbance of each solution was measured at λ_{max} (524nm). Using optimum ferric(Ш) concentration with standard solution of 0.5 ml Ligand (100ppm) , the absorbance was measured at λ $_{max}$ (524nm) with different pH value ranging from 1 to 11 using 0.1N HCl or 0.1N NaOH .

The stability of the CBZ complex with optimum value of ferric ion volume and pH were studied with different time intervals (0-24 hours) by measuring absorbance at λ $_{max}$ (524nm). The Effect of temperature on CBZ complex formation was studied at a range from (10 -80°C) using optimum volume of ferric ion volume and pH by measuring absorbance at λ $_{max}$ (524nm).

The stoichiometry of the formed complex was studied by mixing a solution of ferric ion and the ligand having identical concentrations (4.2×10^{-4}) M . The absorbance at λ_{max} (524nm) is then plotted by Job's method mole ratio method.

The infrared spectrum has been recorded on FT-IR Shimadzu, FTIR-8000-Japan in the range 400-4000 cm^{-1} using KBr pellets. Electronic spectra has been recorded on JENWAY double-beam UV-6405 UV-Visible Spectrophotometer for solutions of the ligands and their complexes

Stability constant of the resultant complex was calculated depending on mole ratio method with optimal conditions (25). If mole ratio of (ligand: metal) is (2:1): Solutions in equalmolr concentration (4.2×10^{-4}) M for ligand and ferric ion were mixed, then absorbance (As) measured at λ $_{max}$(524 nm) for the dissociated complex. Other solutions were prepared containing the same amount of iron ion and an increase concentration of ligand (four double) and then absorbance (Am) was measured at λ $_{max}$ (524 nm) for the composed complex ML2.

Determination CBZ in pharmaceutical drug CARBASAM by direct Method:-

A 10 tablets of the CARBASAM (each weighing 0.294 gm, a single disc to 200 mg) was provided from the State Company for Drug Industries and Medical Appliances (IRAQ-NDI), after they crushed well , (0.2000 gm) dissolved in 10 ml of ethanol , the volume is made up to 100 ml volumetric flask (2000 ppm) , a 10 ml of this solution was added to 2 ml of 0.2% salicylaldehyde (2000) ppm , then the mixture is heated in water bath at 60 °C for 30 minutes and left to cool , the volume is made up to 20 ml.

X ml of ligand was transferred to volumetric flask capacity of 5 ml, to each 0.5 ml of standard ferric solution (4.2×10^{-3}) M was added under controlled optimum conditions, the absorbance measured at λ_{max} (524 nm).

Results & Discussion

Preparation of the CBZ-salicylaldehyde ligand: (dibenzo[b,f]azepine-5[N-(O-hydroxybenzyliden)] amide) :

The reaction is depending on Schiff's bases reactions between CBZ and salicyladehyde . The prepared ligand has a melting point (184-186) °C , comparing with pure CBZ (189-191) °C , Fig (2) . Preliminary tests results for the reaction of pure CBZ with 19 studied cations shows no clear color change or precipitate formation. While the results (table (1)) from the reaction of the prepared ligand with 19 metal ions shows that Fe^{+3} gives a clear change in color (Violet) upon the reaction with ligand.

Table 1: Preliminary tests results for reaction of Ligand with some cations

Ions	Color					Conclusion
	Solution	after Ligand addition	at 60 °C	in acidic medium	in basic medium	
Al^{+3}	colorless	No change	No change	No change	yellow	Reaction
Ag^{+}	colorless	No change	No change	White ppt.	Black ppt.	No reaction
Mn^{+2}	colorless	No change	No change	No change	Brown ppt.	No reaction
Na^{+}	colorless	No change	No change	No change	Yellow	Reaction
K^{+}	colorless	No change	No change	No change	Yellow	Reaction
Cr^{+3}	Light green	No change	Green ppt.	Light green	Yellow	Reaction
Ca^{+2}	colorless	No change	No change	No change	Yellow	Reaction
Fe^{+2}	Colorless	No change	No change	No change	Red ppt.	Reaction
Ce^{+3}	Colorless	No change	No change	No change	Yellow ppt.	No reaction
Zn^{+2}	Colorless	No change	No change	No change	Gelatin ppt.	No reaction
Co^{+3}	pink	No change	No change	Light pink	Orange	Reaction
Fe^{+3}	colorless	Violet	No change	Light violet	Yellow	Reaction
Cd^{+2}	Colorless	No change	No change	No change	White ppt.	No reaction
Ni^{+2}	Light green	No change	No change	No change	Yellow ppt.	No reaction
Cu^{+2}	Blue	No change	No change	No change	Yellow ppt.	No reaction
Pb^{+2}	Colorless	No change	No change	White ppt.	Yellow ppt.	No reaction
Mg^{+2}	Colorless	No change	No change	Colorless	Yellow	Reaction
Hg^{+2}	Colorless	No change	No change	No change	Yellow	Reaction

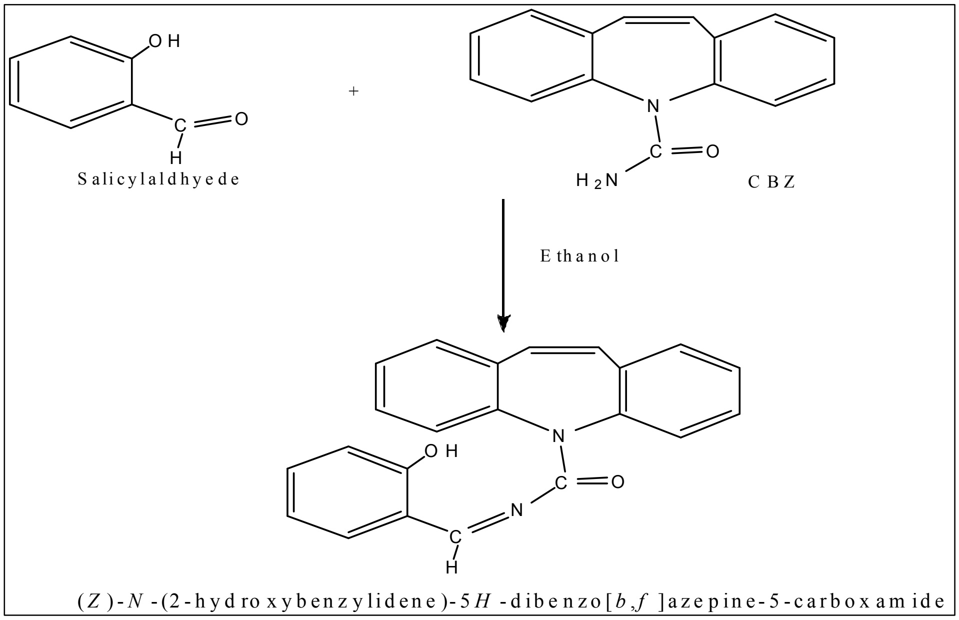


Fig. 2: The structure of the product ligand from reaction between CBZ and salicyladehyde

Preparation of complex: The prepared complex from the ligand and ferric ion is charred between (98-100)^{o}C, while the melting point of the CBZ and ligand was (189-192) ^{o}C and(184-186) ^{o}C respectively. The prepared complex have a peak at UV spectrum differ from that of ligand and the ferric ion. Ferric ion was used in this study as the ion of interest for preparing the complex ferric (III)- (dibenzo [b,f]azepine-5-carboxylic acid-2-hydroxy-benzylidenamide) .

UV spectrum study:

The UV Spectrum was recorded for (20 mg/ L) CBZ and the prepared ligand using ethanol as blank solution. The prepared ligand spectrum shows three peaks, at 219 nm , 255nm and 325nm due to $\pi \rightarrow \pi^{*}$ transition (fig. 3, 4). The absorption spectrum of the colored complex has been studied and shows new absorption peak at (524)nm recurring to electron transfer $\pi \rightarrow \pi^{*}$, indicate a red shift compatible with ligand, due to the access link between the ligand and metal ion. (Fig.5).This maximum absorption at (524) nm was used as λ_{max} in all subsequent experiments. The resultant complex has a violet color which differs from that of the ligand and ferric (Ш) solution.

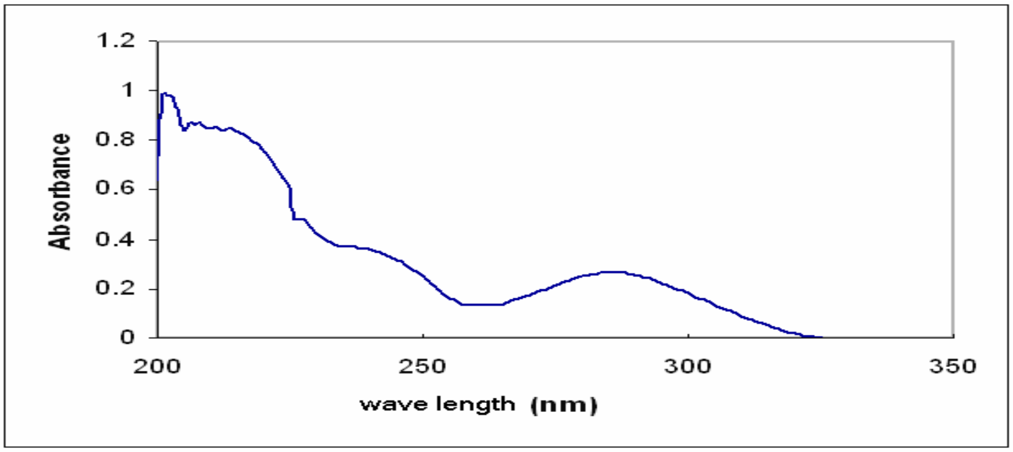


Fig.3: U.V. Absorption spectra of CBZ

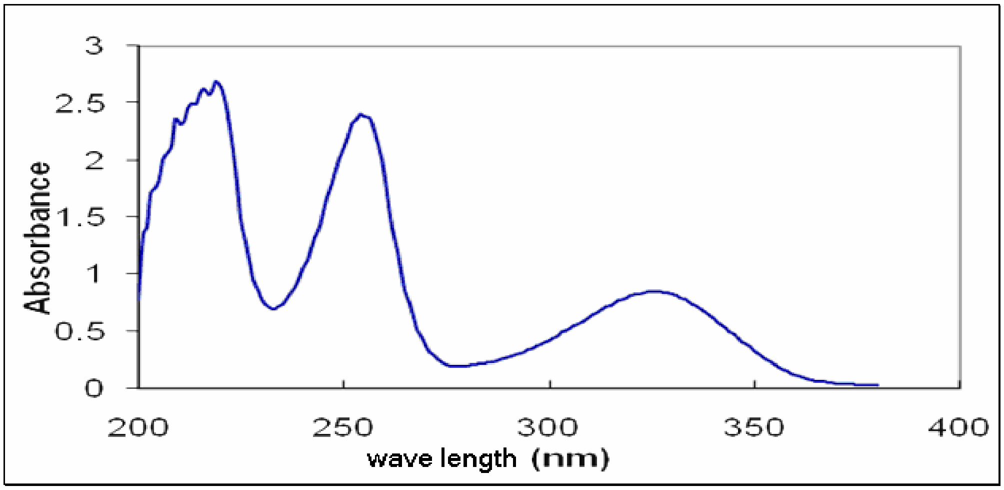


Fig.4: U.V. Absorption spectra of Ligand

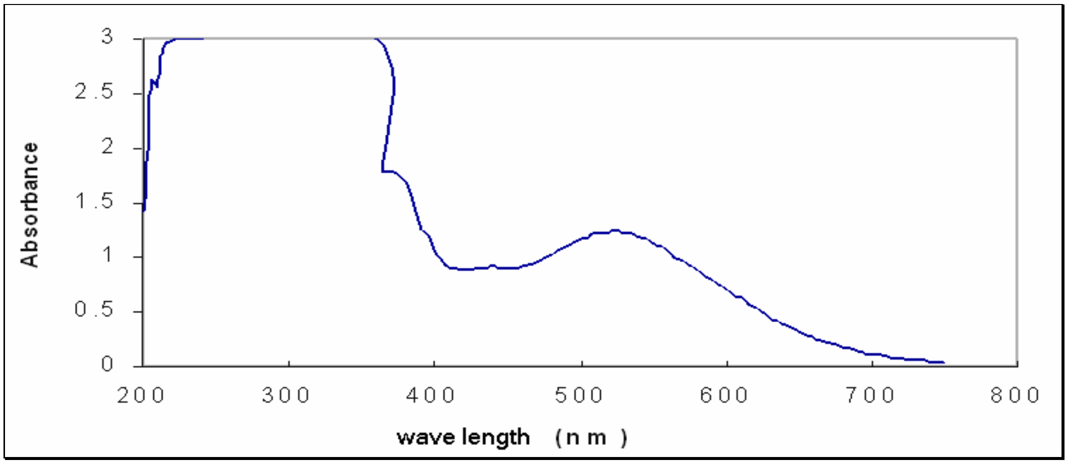


Fig.5: Absorption spectra of ligand –Fe complex(λmax=524nm)

Infrared spectrum study:

1- IR spectrum region (1700 – 4000 cm$^{-1)}$:

Pure CBZ have two peaks (3500-3625) cm^{-1} attributed to stretching vibration of N-H in amid group. The prepared ligand spectrum shows disappearance of N-H peak and appearance of new stretching vibration at 3500 cm^{-1} due to (O-H) indicating the reaction of CBZ and salicylaldehyde with disappearance of C-H peak of salicylaldehyd at 2600 cm^{-1} . While the complex spectrum shows disappearance of two absorption band due to the ($-NH_2$) stretching of CBZ in the position (3500-3625) cm^{-1} and appearance of new stretching vibration at 3000-3500 cm^{-1} due to (O-H) indicating the combination of oxygen in OH with metal ion

2- IR spectrum region (400-1700 cm$^{-1)}$:

Indicates very important peaks for the prepared ligand showing the changes resulted due to Schiff base formation and the coordination with metal ion. Pure CBZ shows absorption band at 1715 cm^{-1} due to (C=O) of amide group, and two bands at 1380 and 1600 cm^{-1} attributed to(C-N) and (H-N) groups. While the ligand shows the disappearance of (C=O) band that return to amide of CBZ (1715) cm^{-1}, and disappearance of salicylaldehyde (C=O) band (1690) cm^{-1} and amide (C=O) band(1715) cm^{-1} which shifted toward low frequency at (1681) cm^{-1} indicating the ligand formation.

The disappearance of N-H peak of CBZ at (1600) cm^{-1} and appearance of new stretching vibration at 1596 cm^{-1} due to (C=N) group of ligand, give strong evidence to the reaction between amide group of CBZ and salicylaldehyde which indicate the success of this reaction, this band shifted to low frequency at 1581 cm^{-1} indicate participation of schiff's base in the reaction. So, the evidence is clear that ferric ion combined with (C=N) group not with (C=O) because this lead to unstable eight membered ring. (Fig.6) ,table (2).

Table (2) IR spectrum for some functional groups of the studied compounds

Functional group	CBZ	Salicylaldehyde	Ligand	complex
$-NH_2$	3500-3625	--------------------	-------------	-----------------
O-H	-------------	3050-3350 broad	3500	3000-3500 broad
C=O	1715	1690	1681	1680
C= N	--------	---------	1596	1581
C-N	1380	----------------	--------------	---------------
C-H	----------	2760	----------------	---------------
H-N	1600 bending	--------------	---------------	---------------

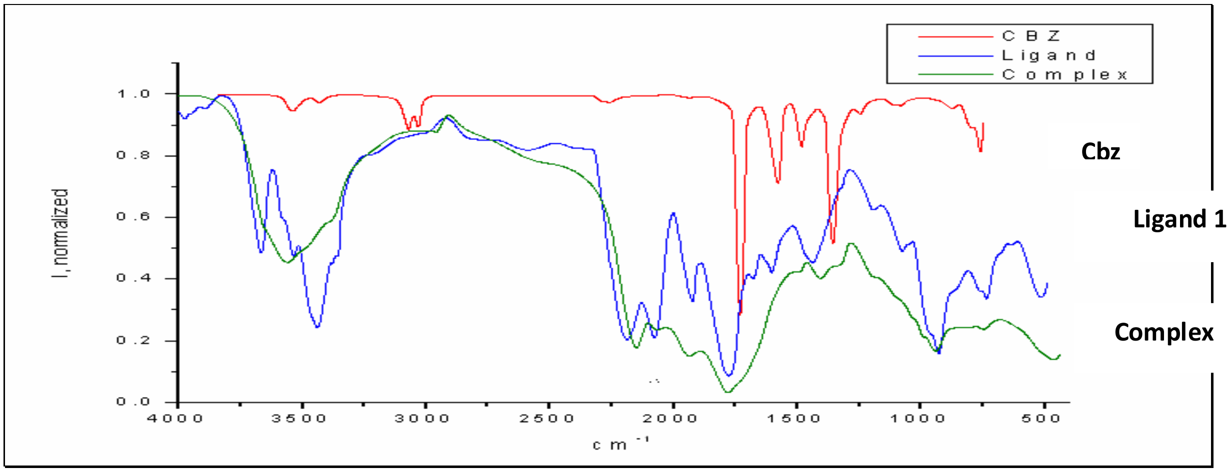


Fig. 6 IR Absorption spectra of CBZ , ligand and ligand–Fe complex

Optimization of the conditions:

1- Ferric ion concentration: The absorbance of the complex changes during the addition of different volume of ferric ion (100 mg/l) . (Fig.7) shows that optimum volume of ferric ion (100 ppm) was 1.2 ml with 0.5 ml ligand solution (100 mg/l) at λ_{max} (524) nm.

2- pH value: It was found that there is a decrease in absorption value in acidic medium , because of strong acid proton linking with amide nitrogen of CBZ leading to lowering the rate of reaction with ferric ion solution and then a decrease in rate of formation of complex or dissolution of complex. The absorbance value decrease in basic medium due to the hydrolysis of ferric ion forming ferric hydroxide decreasing the rate of reaction with the ligand. (Fig. 8). The color of the complex varied with pH change and the most stable violet color was stable at slightly acidic medium, It was found that the optimum pH is 6.5 at λ_{max} (524) nm .

3- Effect of time: The absorbance of the complex changes with time increasing (pH 6.5, λ_{max} 524 nm) was estimated. The complex was stable for 24 hours, indicating the high stability. It was found that the maximum absorbance of complex was at ten minutes after reaction and it was the optimum time for reaction completion. (Fig. 9)

4- Effect of temperature: The absorbance of the complex measured was decreased with increasing in temperature due to complex dissociation. It has found that the optimum absorbance of the complex (pH 6.5, λ_{max} (524) nm) was at 30 ^{o}C. (Fig. 10)

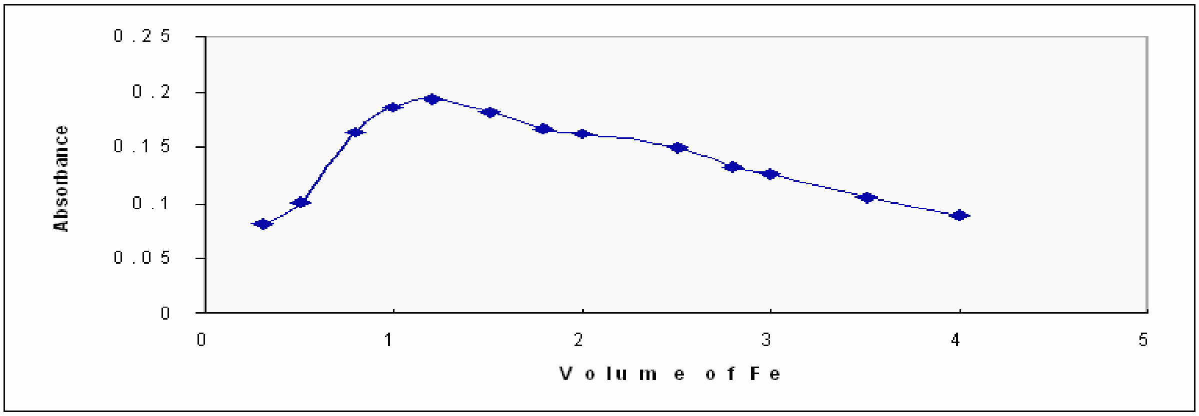


Fig. 7: The optimum volume of ferric ion solution (100 mg/l)

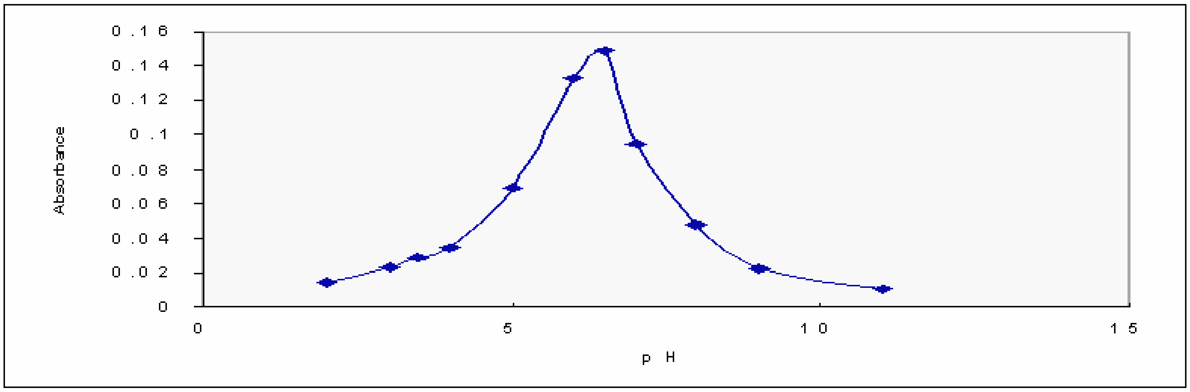


Fig. 8: The effect of pH on ligand –Fe complex formation

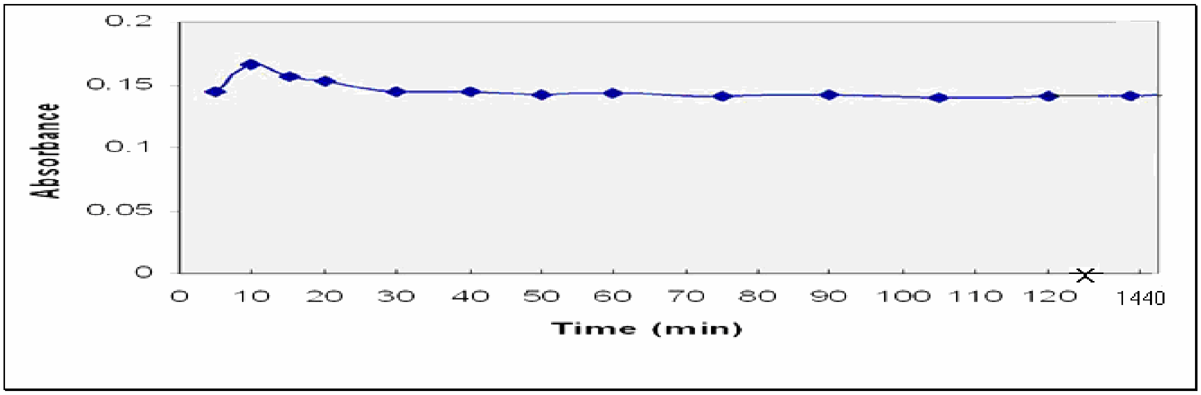


Fig. 9: The effect of time on ligsnd –Fe complex formation

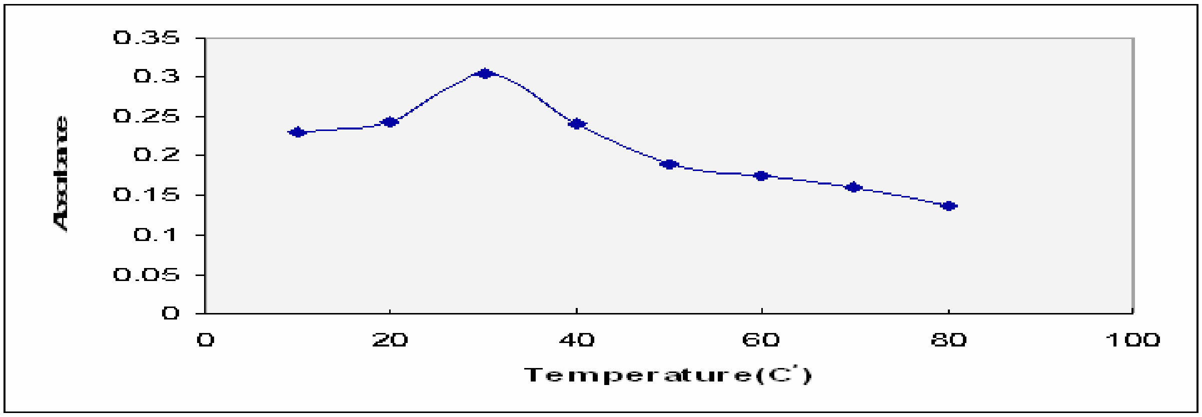


Fig. 10: The effect of temperature on ligend –Fe complex formation

Stoichiometry of the complex:

Continuous variations and Mole ratio methods indicated that the ratio of ligand to ferric ion was (2:1) .(Figures 11 and 12). Literature survey doesn't show any signal about any study or preparation methods including this complex. According to the results including UV, IR spectrum and ligand to metal ratio we can propose the structural formula for the resultant complex (Fig 13).

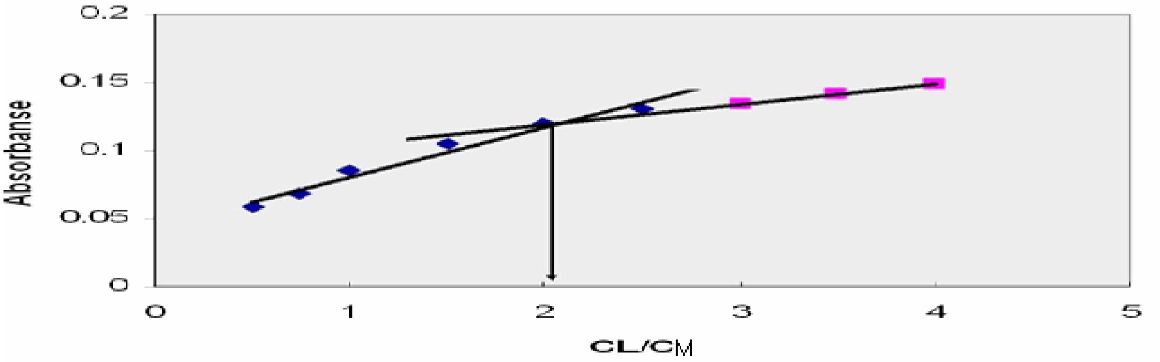


Fig. 11: Mole ratio plot for ligand –Fe complex

C_L: ligand , C_M:Metal

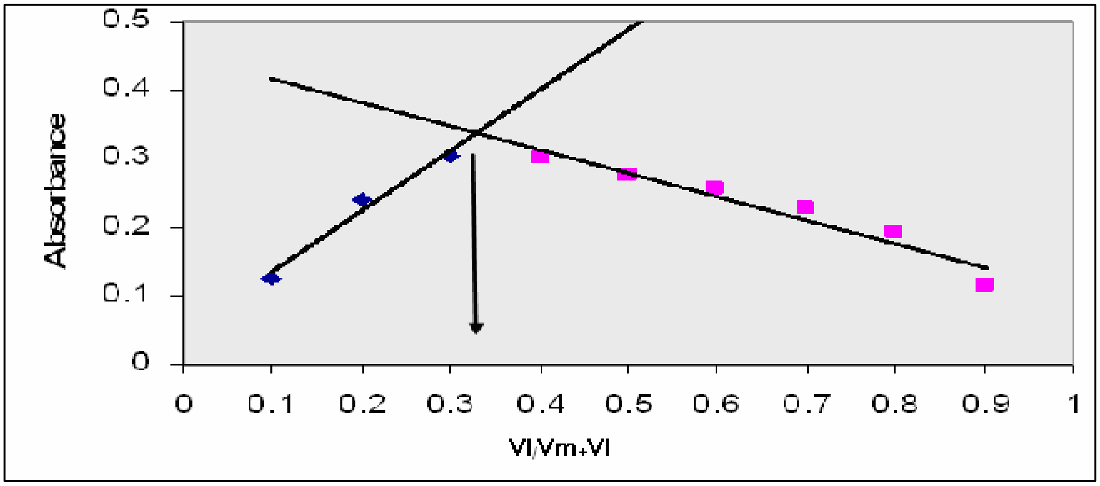


Fig. 12: Continuous variation plot for ligand –Fe complex

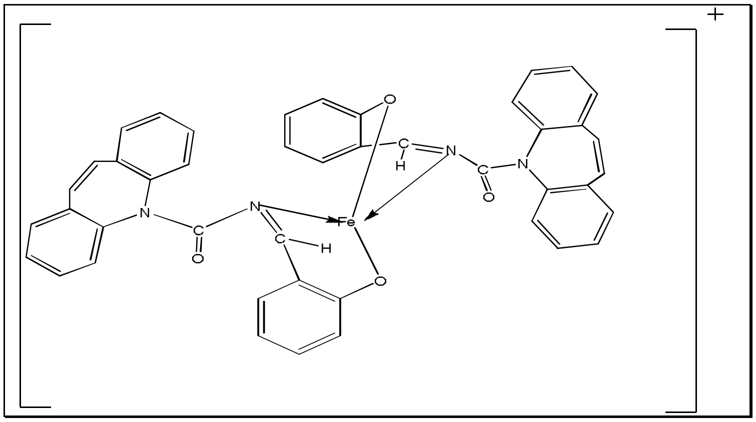


Fig. 13: ligand –Fe (III) complex (2L:1M)

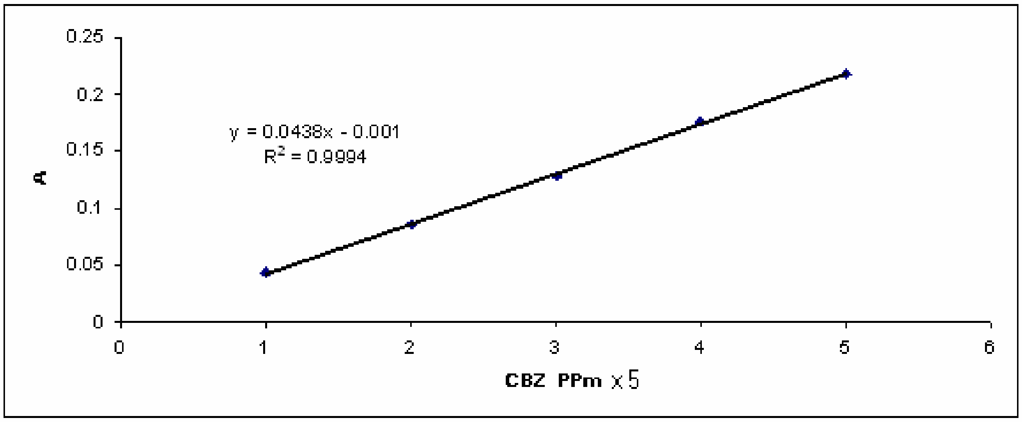


Fig. (14): Linear calibration for determination of CBZ

Disintegration degree and stability constant for the prepared complex were calculated according to mole ratio method under the optimum conditions. For the reaction between a metal and ligand giving ML_2 complex, Degree of dissociation and stability constant was calculated **[25].**

$$\underset{\alpha c}{M} + \underset{2\alpha c}{2L} \rightleftharpoons \underset{(1-\alpha)c}{ML_2} \qquad \text{----------} \quad \mathbf{(1)}$$

$$K = \frac{(1-\alpha)c}{\alpha c(2\alpha c)^2} = \frac{(1-\alpha)c}{4\alpha^3 c^3} = \frac{1-\alpha}{4\alpha^3 c^2} \qquad \text{-----------} \quad \mathbf{(2)}$$

$$\alpha = \frac{A_m - A_s}{A_m} \qquad \text{-----------} \quad \mathbf{(3)}$$

K = Stability constant. C = Final complex concentration. α = Degree of dissociation

The average of the calculated value of stability constant of complex was $13X10^9$ lit^2 mol^{-2} under optimal experimental conditions and this shows that the complex is of high stability and can be easily studied. (Table3). From all the data obtained it is clear that there is high possibility to use ferric ion in determination of CBZ in spectroscopic methods due to the stable violet color of the complex formed and strong absorption peak at 524 nm. The value of molar absorptivity coefficient (ε) was (2.17×10^3) $l.\ mol^{-1}\ cm^{-1}$.

The calibration curve of CBZ complex obeys Beer's law 1.0-25 mg/lit at λ_{max} (524) nm, and the correlation factor close to one (r = 0.9996) , which demonstrates that there is a high correlation between variables, concentration (x) and absorbance (y). For expression of the precision for the suggested analytical method, statistical study shows the high sensitivity of the method for the detection of low concentration of CBZ . The average of relative error (E %= 1.42) and recovery (Rec.%= 100.2) and relative standard deviation (RSD %=1.1) was calculated using different concentrations of CBZ[26,27]. Table (4) . Then the limit of detection (LOD) and Limits of quantitation (LOQ) was calculated and they are 0.25 mg/l and 0.83 mg/l respectively [28] :

Determination of CBZ in pharmaceutical drug CARBASAM : The direct calibration method was used in estimating CBZ in the pharmaceutical .The results in table (5) indicate the possibility of applying the proposed method, where it was consistent with the original content of pharmaceutical products, at relative standard deviation 2.25%, the average of relative error 2.53%, while the method recovery is 99.6%.

Determination of CBZ in serum: The direct calibration method was used in estimating CBZ in serum at the optimum condition after protein separation .The results in table (6) indicate the possibility of applying the proposed method, where it was consistent with the original content of pharmaceutical products, at the relative standard deviation 0.62%, the average of relative error 4.63%, while the method recovery is 95.6%.

The proposed method was applicable for samples of drugs and plasma which provided encouraging resultants. This method is characterized by no need of solvent extraction , solvents, fast and sensitive.

Table (3) : Disintegration degree and stability constant values for complex

Con. Of CBZ (M)	A_s	A_m	α	K st ($lit^2.mol^{-2}$)
1ml (4.2×10^{-4}M)	0.094	0.125	0.25	17×10^8
2ml (4.2×10^{-4}M)	0.119	0.149	0.20	9×10^8

Table (4): Accuracy and precision of suggested analytical method

CBZ con. (M)		Recovery%	Relative Error %	Relative Standard Deviation %
Taken	Found			
8.5×10^{-5}	8.35×10^{-5}	98.24	-1.76	2.12
13×10^{-5}	13.3×10^{-5}	102.30	2.30	0.60
17×10^{-5}	17.03×10^{-5}	100.17	0.17	0.59

*Each value is average of four reading.

Table (5): Determination of CBZ in pharmaceutical by standard curve

CBZ con. (M)		Recovery%	Relative Error %	Relative Standard Deviation %
Taken	Found			
2.54×10^{-5}	2.48×10^{-5}	97.64	-2.36	3.20
3.4×10^{-5}	3.51×10^{-5}	103.20	3.20	1.54
4.2×10^{-5}	4.11×10^{-5}	97.90	-2.10	2.00

*Each value is average of four reading.

Table (6): Determination of CBZ in serum by direct calibration curve method

µg/ml of CBZ		Recovery %	R. Error %	R. Standard Deviation %
True Value	Analytical Value			
40	**39.4**	**98.20**	**1.80**	**0.91**
50	**46.5**	**93.80**	**6.60**	**0.36**
60	**54.1**	**94.80**	**5.50**	**0.59**
70	**69**	**98.70**	**1.4**	**0.68**
80	**71.8**	**89.40**	**12.1**	**1.96**
90	**86**	**95.50**	**4.60**	**2.10**
100	**94.5**	**94.60**	**5.80**	**2.80**

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