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Synthesis, Spectrophotometric and Stoichiometric Studies of Metal Complexes of Four Antipsychotic Drugs in Bulk, Spiked Human Plasma and Pharmaceutical Dosage Forms

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ABSTRACT

A simple, accurate and sensitive spectrophotometric method has been developed and validated for determination of antipsychotic drugs: Levetiracetam, Piracetam, Entacapon, and Carbamazepine. The method was based on the complexation of these drugs with Copper (II) as copper sulphate or chloride. Levetiracetam and Piracetam indirectly determined using Cu (II) Sulfate exchange complexation and subsequent measurement of the excess copper sulphate a colored compound at (λ_{max} at 524 nm). The decrease in the absorption intensity (ΔA) of the colored copper sulphate, due to the presence of these two drugs (I or II) was correlated with their concentration in the sample solution. Entacapon and Carbamazepine react with copper chloride to give stable copper (II) complexes. The absorption intensity (λ_{max} at 448 nm) was correlated with drugs concentration in a linear relationship. Different variables affecting the reaction were carefully studied and optimized. Relationships with good correlation coefficients (0.9985-0.9994) were found between ΔA or A values and the concentrations of the drugs. The method was validated, in terms of accuracy, precision and selectivity; the results were satisfactory. The proposed method was successfully applied to the analysis of the investigated drugs in their pure, spiked human plasma samples and pharmaceutical dosage forms. The Stoichiometry of complexes determined by Job's method and the stability constants were calculated according to the Benesi–Hildebrand equation. The copper complexes with Entacapon and Carbamazepine are separated and characterized mainly by elemental analysis, magnetic moment, electron spin resonance spectroscopy (ESR) and Fourier transform IR (FT-IR).

Key words: Antipsychotic drugs, Copper (II), Spectrophotometry, Stoichiometry, Job's method, Elemental analysis, ESR and FT-IR.

INTRODUCTION

Antipsychotic medications, sometimes referred to as neuroleptics or major tranquilizers, are prescribed to treat schizophrenia and to reduce the symptoms associated with psychotic conditions such as bipolar, psychotic depression, senile psychoses, various organic psychoses, and drug-induced psychoses [1] .

Levetiracetam (I) and Piracetam (II) are antiepileptic drugs approved by the U.S. Food and Drug Administration as an adjunct in partial, myoclonic and tonic-clonic seizures and mono therapy for partial seizures they bind to a synaptic vesicle protein SV2A and are believed to impede nerve conduction across synapses [2]. Carbamazepine (CBZ) (III) is an anticonvulsant agent, extensively used as antiepileptic and mood stabilizing drug. It is also approved to treat bipolar affective disorder like resistant schizophrenia [3]. Entacapon (IV) is therapeutically classified as a selective, reversible and peripheral inhibitor of catechol-Omethyl transferase. Be given as adjunctive therapy to patient with Parkinson's disease [4].

P. Nikolaou [5] developed a sensitive and accurate gas chromatography with mass spectroscopy (GC/MS) using solid-phase extraction (SPE) procedure for determination of Levetiracetam wile Vermeij et al., [6] used nitrogen-phosphorous detectors. Levetiracetam and its metabolite have been separated and quantified chromatographically using hyphenated techniques such as; (LC–MS/MS) [7] method and high performance liquid chromatography-electrospray tandem mass spectrometry (HPLC-ESI-MS/MS) method have been also developed for Levetiracetam determination [8]. Quantification of Levetiracetam in human serum was performed by high performance liquid chromatography HPLC using porous graphitic carbon analytical HPLC-column with Ultraviolet (UV) detector [9], or diode-array detectors [10]. L. Antonilli et al [11] had adopted a Reversed-phase RP-HPLC separation method with UV-detection and HPTLC method for quantitative determination of Levetiracetam in pharmaceutical formulations and in bulk materials. An easy and fast electrochemical method has been developed for Levetiracetam (LEV) determination using new enzymatic electrochemical biosensor and carbon working electrode previously modified by an aryl diazonium salt [12, 13].

Rapid bioanalytical chromatographic methods were evaluated for the simultaneous determination of Piracetam and its metabolites in human microsomal preparations, plasma, pharmaceutical formulations and in bulk materials including; fast ultra-performance liquid chromatography/tandem mass spectrometry (UPLC–MS/MS) [14], (HPLC/UV) [15], TLC-densitometry [16].

H. Yeh et al (2006) [17] had developed a simple micellar electrokinetic chromatography (MEKC) method with UV detection for analysis of Piracetam (II) in cerebrospinal fluid (CSF). Gas chromatographic (GC) method using liquid-liquid extraction and fused-silica capillary column with mass spectrometric detection had been developed for quantitative determination of Piracetam (II) [18]. Capillary electrophoresis (CE) procedure for the determination of Piracetam have been developed and validated using uncoated silica capillary and UV detectors operated at 200 nm [19]. A simple, accurate and sensitive microextraction by packed sorbent-gas chromatography–mass spectrometry (GC-MS) method has been developed [20] for the simultaneous quantification of four antiepileptic drugs such as Carbamazepine (III) in human plasma and urine. A number of LC methods with UV detection for the determination of CBZ had been described [21, 22].

A specific and sensitive liquid chromatography–electrospray ionization mass spectrometry method for the simultaneous determination of Carbamazepine (III) and its metabolites has been developed and validated [23]. S. Thomas et al [24] and A. M. Stolker et al [25] have recently reported Liquid chromatography/tandem mass spectrometry (LC–MS/MS) with quadruple-time of flight mass spectrometry (LC-Q-TOF MS) for the determination of (III).

A number of HPLC methods for simultaneous determination of Carbamazepine (III) and its metabolites using fluorescence polarization immunoassay [26], chemiluminescence [27], Spectrophotometry [28] and spectrofluorometry method [29] had been developed and validated. G. Izzo et al [30] had developed Miceller Kinetic Cappilary chromatography (MEKC) for quantitation of Carbamazepine (III) and its metabolites which is a modification of capillary electrophoresis (CE), where the samples are separated by differential partitioning between micelles (pseudo-stationary phase) and a surrounding aqueous buffer solution (mobile phase). Capillary electrophoresis has been used for the separation of CBZ and its metabolites and monitoring the concentrations of CBZ and its metabolites in plasma [31].

Entacapon as a COMT inhibitor present hydrophobic groups in its chemical structures, so reversed-phase liquid chromatography had been used as the major approach for the determination of such compounds, especially high-performance liquid chromatography coupled to ultraviolet detection HPLC-UV [32], electrochemical detection HPLC-ECD [33], amperometric detection RP-HPLC/Amp [34], mass spectrometry detection HPLC–MSMS [35,36] and liquid chromatography electrospray ionization mass spectroscopy LC–ESI-MS/MS [37]. Regarding the sample preparation, the traditional liquid–liquid extraction (LLE) and solid-phase extraction (SPE) were also the most widely used procedures for extraction of the analytes of interest prior to the analysis of samples. Voltammetric methods have been developed for the determination of Entacapon [38]. Entacapon containing electrolyte enhanced the reduction current signal and the mechanism of reduction has been postulated on the basis of controlled potential electrolysis and coulometer, differential pulse voltammetry (DPV) and square ware voltammetry (SWV).

Our presented work reported simple, sensitive and accurate spectrophotometric method for the analysis of four antipsychotic agents, Levetiracetam, Piracetam, Entacapon, and Carbamazepine. Also we describe the synthesis and characterization of the Cu (II) complexes of Entacapon, and Carbamazepine.

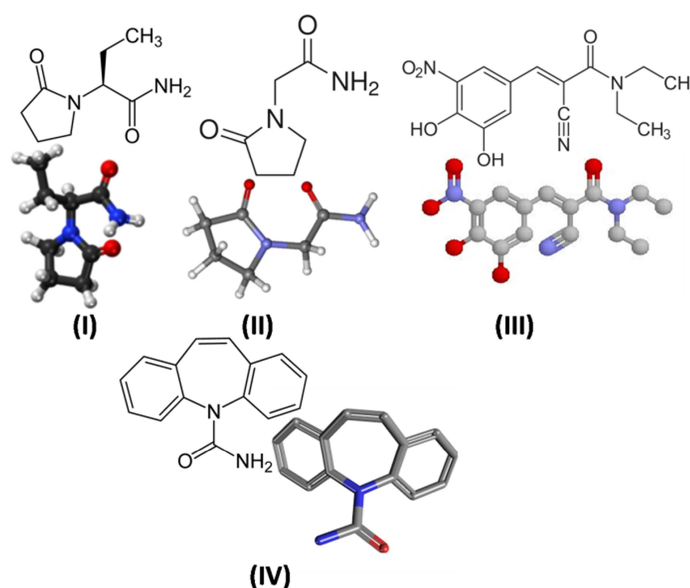

Fig.1 The 1-D and 3D structure of Levetiracetam (I), Piracetam (II), Entacapon (III), and Carbamazepine (IV)

MATERIALS AND METHODS

2.1. Chemicals

1. Levetiracetam, Piracetam, Entacapon and Carbamazepine working standards were provided by Sigma Pharmaceutical Industries Company.
2. Plasma samples were purchased from the central blood bank of Tanta University Hospital. Copper chloride was prepared fresh daily. All reagents used were of analytical grade.
3. Copper (II) chloride and copper sulphate solutions were prepared as a 5 % w/v stock solution in methanol. Aliquots of these solutions were diluted with the same solvent to obtain solutions containing appropriate concentrations to obtain optimal spectrophotometric absorbance for each drug.
4. Tritam Injection (1500 mg/100 ml) and Tritam tablet (500 mg) (Sigma Pharmaceutical Industries.) were purchased from local community pharmacies.
5. Nootropil 400 mg capsule, Nootropil 800 FC tablet, Nootropil 20% syrup and Nootropil 1mg/5ml IV/IM (Sigma Pharmaceutical Industries) were purchased from local community pharmacies.
6. Parkicapon 200 mg f.c. tablet (Sigma Pharmaceutical Industries) were purchased from local community pharmacies.
7. Tegretol 200 mg tablet and Tegretol 2% syrup (Novartis Pharma) were purchased from local community pharmacies.

2.2. Instrumentation

The elemental analysis of the carbon, hydrogen and nitrogen contents were performed using Carlo Erba instruments EA 1110. The UV/VIS absorption spectra of copper chloride and the resulting complexes were recorded over a wavelength range of 200-900 nm using Shimadzu U.V-160A spectrophotometer-double beam. The instrument was equipped with a quartz cell with a 1.0 cm path length. The mid-infrared (IR) spectra (KBr discs) within the range of 5000-400 cm^{-1} for the solid copper complexes were recorded on a Shimadzu FT-IR spectrophotometer. The electron spin resonance was recorded on RT and LNT from VARIAN E-112 ESR spectrometer.

2.3. Preparation of standard stock solutions and spiked human plasma samples

Stock solution for Levetiracetam, Piracetam, Entacapon and Carbamazepine were prepared in methanol to contain 1 mg/ml. Serial standard solutions were prepared in the same solvent having concentrations ranging from 5 to 40 µg/ml, 1 to 17 µg/ml , 1-15 µg/ml and 62-150 of Levetiracetam, Piracetam, Entacapon and Carbamazepine respectively. Serial standard solutions were spiked in human plasma and vortex mixed. Spiked human plasma samples were mixed with methanol and centrifuged for 15 minutes to separate the precipitated protein. The clear supernatant was filtered to obtain solutions in concentrations ranging from 8 to 35 µg/ml, 5 to 15 µg/ml, 4 to 12 µg/ml and from 70 to 120 µg/ml of Levetiracetam, Piracetam, Entacapon and Carbamazepine respectively.

2.4. Procedures

A. Indirect method

1 ml Copper sulphate and specified volume of Na_2HPO_4–citrate acid buffer (pH 6) were added to 1 ml of Levetiracetam and Piracetam standard solutions, assay solution of pharmaceutical preparations and assay solution of spiked human plasma samples in methanol and transferred to 10.0 ml screw capped test tube. The resulting solutions were adjusted to volume with the same solvent and measured at 524 nm [39].

B. Direct method

1 ml copper chloride specified volume of Na_2HPO_4–citrate acid buffer (pH 6) were added to 1 ml of Entacapon and Carbamazepine standard solutions, assay solution of pharmaceutical preparations and assay solution of spiked human plasma samples in methanol and transferred to 10.0 ml screw capped test tube. The mixtures were stirred for specific time. The temperature of the reaction was very important in the color development. The colored products absorbance reached maximum value after keeping in boiling-water bath for specified time, cooled and then transferred to 10.0 ml volumetric flask and the resulting solution was adjusted to volume with the same solvent and measured at 450 to 550 nm [40].

RESULTS AND DISCUSSION

2.5. Spectrophotometric conditions and experimental parameters optimization

Different parameters affecting the reaction between Levetiracetam (I), Piracetam (II), Entacapon (III), and Carbamazepine (IV) with Copper sulphate or copper (II) chloride were studied to optimize the reaction conditions namely; the concentration of Copper sulphate, concentration of Copper chloride, the stirring or reaction time, heating temperature, heating time, buffer pH and buffer volume as shown in table.1. According to solubility data of pharmacopeia all drugs were studied in methanol.

Table .1 Optimal conditions for the direct and indirect spectrophotometric analysis of Levetiracetam (I), Piracetam (II), Entacapon (III), and Carbamazepine (IV) using copper complexation reaction

Drug	Conc.	$CuCl_2$ conc. (W/V %)	Heating temp. 0C	Heating time	Stirring or reaction time (min)	CU (II) sulphate Conc. (W/V %)	Volume of buffer (ml)
(I)	20 µg/ml	NA	25	-	25*	0.25	2.3
(II)	8 µg/ml	NA	25	-	15*	0.15	1.2
(III)	12 µg/ml	0.15	30	14	20		1.5
(IV)	70 µg/ml	0.2	45	10	25		1.8

Effect of copper concentration

The effect of the copper (sulphate or chloride) concentration on the absorbance of complexation system was investigated. Different concentrations (%W/V) of copper were added to the solutions containing a fixed amount of drug and the absorbance of the solutions was recorded at the absorption peak of 524 and 448 nm for copper sulphate and copper chloride respectively, as shown in figures (Fig.2 and Fig.3)

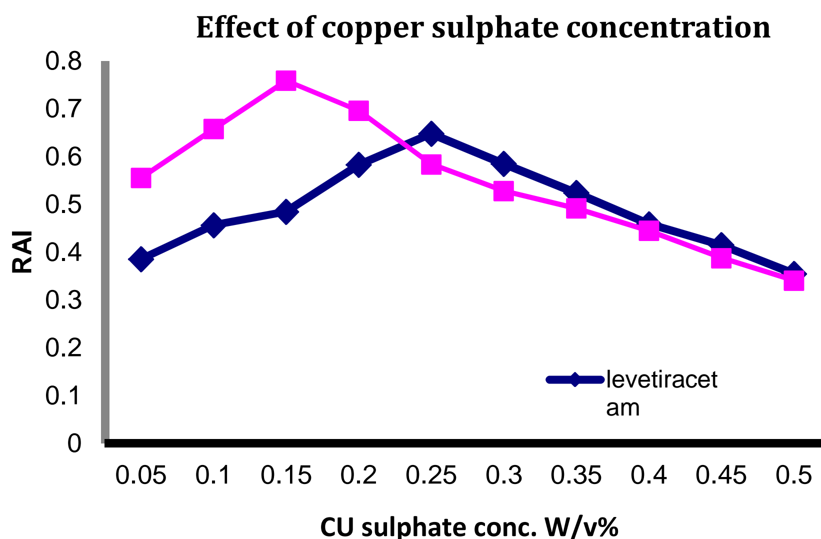

Fig.2: Effect of Copper sulphate concentration (W/V%) on the spectrophotometric intensity of Levetiracetam (I) and Piracetam (II)

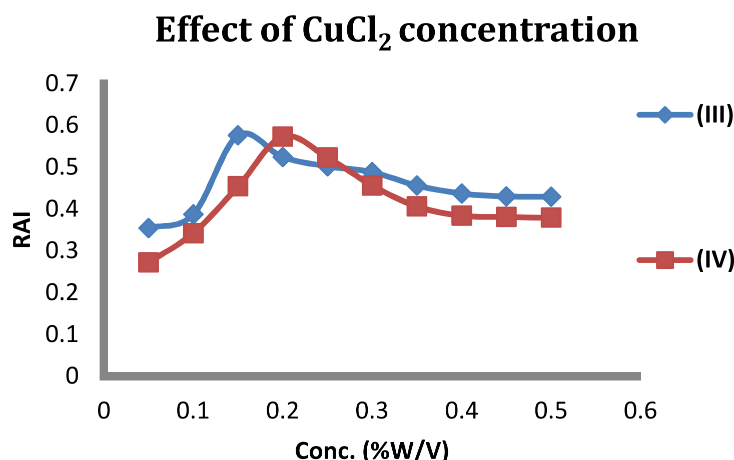

Fig.3: Effect of Copper chloride concentration (W/V%) on the spectrophotometric intensity of Entacapon (III), and Carbamazepine (IV)

Effect of reaction time

The reaction time of the system was then investigated. The absorbance of the Cu^{2+}-drug complexes reached the maximum value at a specified Cu^{2+} concentration as shown in table IV.1 and kept stable in the following 1 h observation. This result indicates that the reaction between drugs and Cu^{2+} is rapid and stable.

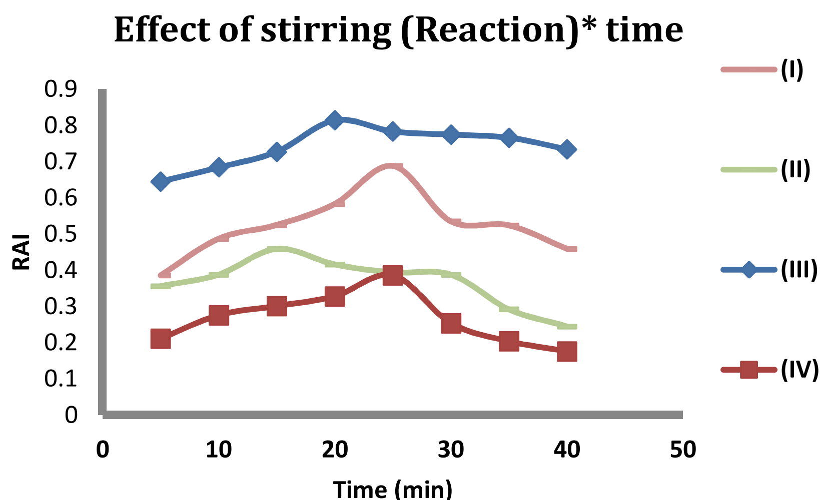

Fig.4: Effect of stirring time or reaction time on the spectrophotometric intensity of Levetiracetam (I), Piracetam (II), Entacapon (III), and Carbamazepine (IV)

Effect of temperature

The effect of temperature in the range of 20–55 °C on the absorbance of the Cu^{2+}-drug complex solutions was studied, and the results are shown in Fig.5 andFig.6. As shown in Fig.5, the absorbance values of Levetiracetam (I) and Piracetam (II), are almost the same at different temperatures, illustrating that temperature has little effect on the complexation of Cu^{2+} ion by (I and II). Therefore, the reaction between Levetiracetam (I), Piracetam (II) and Cu^{2+} ion does not require fine control of temperature.

All the formed complexes of Entacapon (III), and Carbamazepine (IV) with Copper chloride were stable up to 45◦C; On the contrary, at temperature higher than 45◦C, the relative intensity decreases due to dissociation of the complexes at higher temperatures. Therefore, the determination of studied drugs was carried out at 45±2◦C.

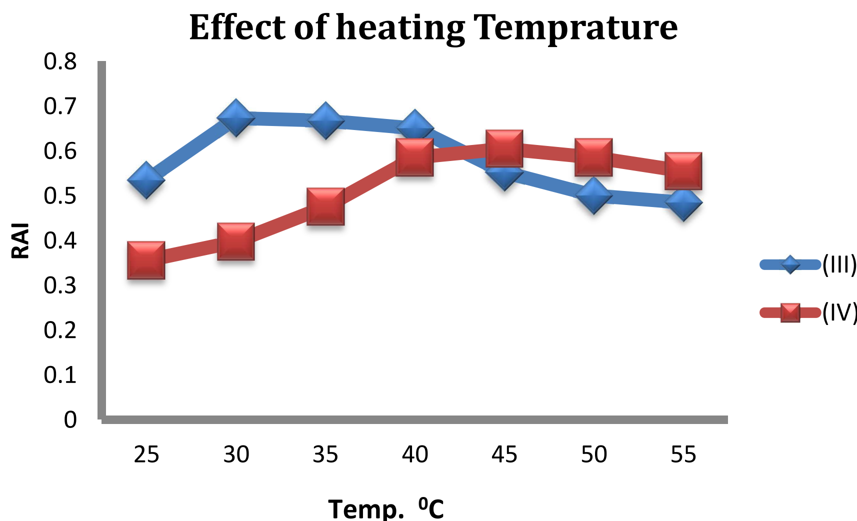

Fig.5: Effect of heating temperature on the spectrophotometric intensity of Entacapon (III), and Carbamazepine (IV)

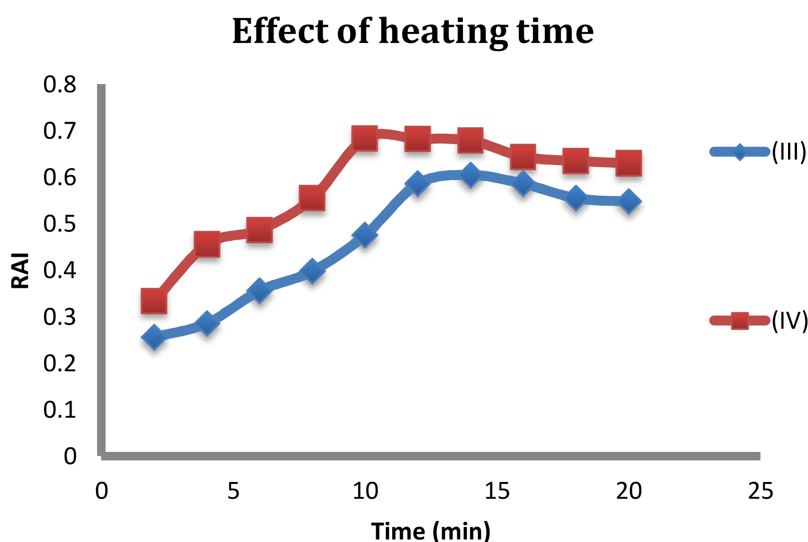

Fig.6: Effect of heating time on the spectrophotometric intensity of Entacapon (III) and Carbamazepine (IV)

The effect of PH

In order to achieve the highly sensitive detection of drugs using the copper (II) ion, the pH value of solutions was studied and optimized as shown in fig.7. We tested the absorbance of Cu^{2+}-drug complex at different pH values. In the presence of Cu^{2+}, the absorption of Cu^{2+}-drug complex is quite different over the wide pH range from 1.8 to 11.6.

In strongly acidic media ($pH < 3.0$), the addition of Cu^{2+} has nearly no effect on the absorption spectrum of the system, which may be attributed to that the amino groups of the drug are well protonated and are thus unable to chelates Cu^{2+} to form the complex.

In alkaline solutions ($pH > 7.0$), the absorbance is not satisfied either, which may result from that partial hydrolysis of Cu^{2+} ion in the alkaline media inhibiting the complex reaction between Cu^{2+} and the drug.

In contrast, in the weakly acidic media (pH 5.5–6.5), the absorbance has high values, suggesting that these weakly acid media can be chosen for the sensitive detection of these drugs with Cu^{2+}. Therefore, we chose the pH 6.0 as the optimum value.

Effects of buffer solutions

When dealing with complex ions, the accuracy of the quantification method depends on the ability of operating without disturbing the complex forming equilibrium. The complex formation can be affected by the aqueous

environment in which the reaction takes place. To find a suitable medium which allows good sensitivity and reproducibility of the response, three different reaction media, such as Na_2HPO_4–citrate acid (PA), acetate buffer (A) and sodium citrate–citrate acid (C), were tested. The results showed (Fig. IV.31) that PA buffer was the best among the buffers, so PA buffer was selected as the proper reaction medium. Subsequently, we investigated the influence of the amount of this buffer. The result (Fig.8) indicated that the volume of PA buffer in the range of 1.2-2.6 ml had nearly no effect on the absorption of the system.

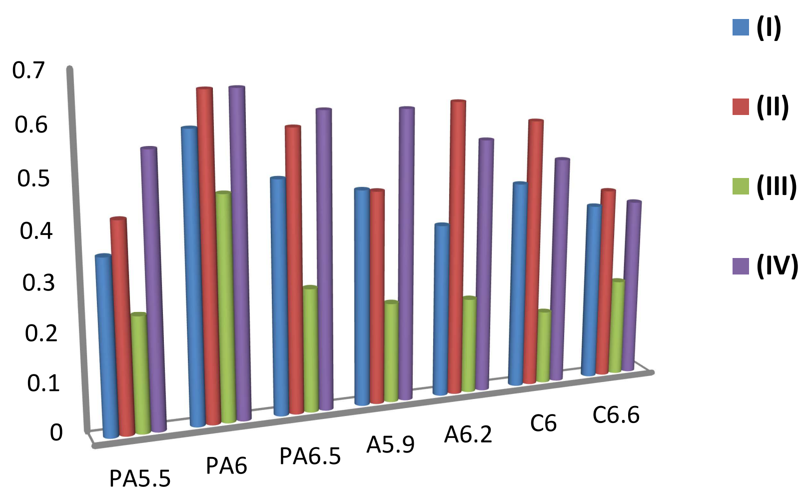

Fig.7: Effect of type and pH of buffer on the spectrophotometric intensity of Levetiracetam (I), Piracetam (II), Entacapon (III), and Carbamazepine (IV)

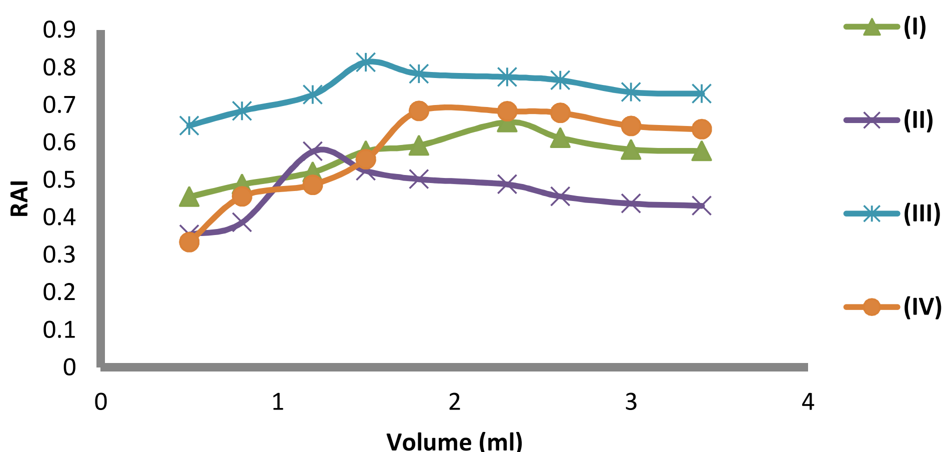

Fig.8: Effect of buffer volume (ml) on the spectrophotometric intensity of Levetiracetam (I), Piracetam (II), Entacapon (III), and Carbamazepine (IV)

2.6. Method validation

The method was validated by using the ICH guideline [41]. The selectivity, limits of detection and quantification, linearity, precision, and accuracy were determined) in both standard solutions (S) and in spiked human plasma samples (P).

a. Linearity

Stock solutions of Levetiracetam (I), Piracetam (II), Entacapon (III), and Carbamazepine (IV) were prepared in solutions and in plasma samples. A series of standard curves were prepared over a concentration range of 5 to 40 µg/ml, 1 to 17 µg/ml , 1-15 µg/ml and 62-150 in methanol solution and 8 - 35 µg/ml, 5 - 15 µg/ml, 4 - 12 µg/ml and 70 - 120 µg/ml in human plasma of Levetiracetam, Piracetam, Entacapon and Carbamazepine respectively.

The data of UV/VIS absorbance versus drug concentration was treated by regression analysis. The standard curves were evaluated for intra-day and inter-day reproducibility. A Linear relationship is obtained for Entacapon and Carbamazepine copper chloride complexes while; a collinear relationship is obtained for both Levetiracetam and Piracetam after reaction with copper (II) sulphate in ammonia buffer. The regression characteristics of the proposed methods are given in Table 2.

Table 2. Regression characteristics of Levetiracetam (I), Piracetam (II), Entacapon (III), and Carbamazepine (IV) using our proposed methods in standard solutions and in spiked human plasma samples

Drug	I*	II*	III**	IV**
		Standard solution		
R^2	0.999	0.997	0.995	0.996
Intercept	0.534	0.603	0.146	0.125
Slope	-0.012	-0.023	0.026	0.005
		Spiked human plasma		
R^2	0.998	0.998	0.996	0.995
Intercept	0.6671	0.7336	0.154	0.101
Slope	-0.0113	-0.0201	0.052	0.004

**with copper sulphate ** with copper chloride*

b. Precision and accuracy

The accuracy of the method was checked for three different concentrations. All estimations were repeated thrice, and the amounts of recovered drug were calculated in both standard solutions (S) and spiked human plasma samples (P). The results were expressed as % recovery, S.D. and C.V. (Table 3).

The excellent mean %recovery values, close to 100%, the values of correlation coefficients and low values of the relative standard deviations indicate high accuracy of the method. The precision of the method was judged by performing intra-day and inter-day (three days intervals) analyses of different concentrations covering the linearity range in both standard solution and spiked human plasma samples. It is determined as relative standard deviation (RSD) and coefficient of variation C.V. The range of standard deviation (SD) and coefficients of variation (CV %) was found to be from 0.254 to 4.854 and from 1.5 to 9.54 % for SD and CV respectively in both standard solution and spiked human plasma samples.

Table 3. Mean values of accuracy parameters Levetiracetam (I), Piracetam (II), Entacapon (III), and Carbamazepine (IV) using our proposed methods in standard solutions and in spiked human plasma samples

Drug		(I)*	(II)*	(III)**	(IV)**
			Standard solution		
Mean	%R	100.53	102.083	100.77	100.19
	S.D.	0.290	0.432	3.221	0.858
	C.V.	1.517	6.921	3.214	1.595
			Spiked human plasma		
Mean	%R	102	100.74	100.041	100.13
	S.D.	0.765	0.193	0.095	1.62
	C.V.	3.60854	2.879	1.162	1.763

**with copper sulphate ** with copper chloride*

The values of the between-day relative standard deviations for different concentrations of the drugs, obtained from experiments carried out over a period of four days. It was found that the within day relative standard deviations are lower than 5 %, which indicates that the proposed method is highly reproducible and copper chloride or copper sulphate reagents can be successfully applied to determine Levetiracetam, Piracetam, Entacapon and Carbamazepine drugs via the copper complexation reaction.

C. Limit of detection and limit of quantification

The LOD is the lowest amount of analyte, which can be detected but not necessarily quantitated as an exact value. The LOQ is the lowest amount of analyte, which can be quantitatively determined with suitable precision and accuracy. The experimentally determined DL and QL both in standard solution and spiked human plasma were determined and was also cross-checked by formulas given below;

$$LOD = \frac{3.3\sigma}{S} \text{ and } LOD = \frac{10\sigma}{S}$$

Where σ is the standard deviation of the response for blank Experiment and S is the slope of the standard curve.

The higher values of detection limits in case of spiked human plasma samples might be rationalized on the basis of possible partial binding of the drug to plasma components which makes the bound part unavailable. Furthermore, the expected higher noise level exerted by various components of plasma contributes to the observed higher DL values in such case as shown in table 4.

Table 4. Calculated and determined detection limits and quantitation limits Levetiracetam (I), Piracetam (II), Entacapon (III), and Carbamazepine (IV) using our proposed methods in standard solutions and in spiked human plasma samples

Drug	I*	II*	III**	IV**
In standard solution				
LOD	3	0.5	0.7	55
LOQ	5	1	1	62
In spiked human plasma				
LOD	6	3	2.5	64
LOQ	8	5	4	70

**with copper sulphate ** with copper chloride*

Based on the above DL and QL limits and peak plasma concentrations of all available dosage forms the developed method would be suitable for monitoring the blood level of Levetiracetam (I), Piracetam (II), Entacapon (III), and Carbamazepine (IV) in patients after administration of a single dose of drug dosage forms.

D. Specificity (selectivity):

The assay results were unaffected by the presence of excipients, as shown by the excellent recoveries obtained when analyzing the studied drugs in the presence of commonly encountered excipients (Table 5).

Table 5. Mean specificity parameters of Levetiracetam(I)*, Piracetam(II)*, Entacapon(III) and Carbamazepine(IV)** in synthetic mixtures**

Drug	First mixture	Second mixture	Third mixture
levetiracetam	Mean %R = 99.157%	Mean %R= 99.272%	NA
	S.D. ± 0.272	S.D. ± 1.748	
	C.V. = 1.906%	C.V. = 1.7612%	
Piracetam	Mean %R = 101.69%	Mean %R= 106.413%	Mean %R= 99.102%
	S.D. ± 0.448	S.D. ± 4.121	S.D. ± 3.665
	C.V. = 5.641%	C.V. = 5.641%	C.V. = 5.641%
Entacapon	Mean %R = 99.85%	NA	NA
	S.D. ± 0.098		
	C.V. = 0.443%		
Carbamazepine	Mean %R = 100.776%	Mean %R = 100.4307%	
	S.D. ± 0.007	S.D. ± 0.289	
	C.V. = 3.349%	C.V. = 1.244%	

**with copper sulphate ** with copper chloride*

2.7. Pharmaceutical preparation analysis

The methods were applied to the determination of Levetiracetam, Piracetam, Entacapon and Carbamazepine in Tritam, Nootropil, Parkicapon and Tegretol different dosage forms respectively. Drug recoveries were satisfactory (Table 6) and the results were in good agreement with label claims and with values obtained using the official British Pharmacopoeia methods.

Table 6. Recovery data Levetiracetam(I)*, Piracetam(II)*, Entacapon(III) and Carbamazepine(IV)** in their pharmaceutical preparation**

DRUG	PHARMACEUTICAL PREPARATION	%RECOVERY ±SD
I	Tritam Injection (1500 mg/100 ml)	101.3%±0.42
	Tritam tablet (500 mg)	99.68%±0.31
II	Nootropil 400 mg capsule	100.73%±0.33
	Nootropil 800 FC tablet	99.87%±0.17
	Nootropil 20% syrup	102.05%±0.15
	Nootropil 1mg/5ml IV/IM	100.14%±0.57
III	Parkicapon 200 mg f.c. tablet	101.51%±0.086
IV	Tegretol 200 mg tablet	100.74%±0.48
	Tegretol 2% syrup	102.12%±0.43

**with copper sulphate ** with copper chloride*

2.8. Stoichiometry

Molar ratio of the reactants (drug: reagent) in copper complexes was determined by the continuous variation method (Job's method) [42, 43] and it was found to be 2:1 for the three drugs: Levetiracetam, Piracetam, Entacapon and Carbamazepine (Fig. 9). These ratios may be due to the presence of amino group moieties which may be included in amide bond as it is less sterically hindered and the most basic one/s.

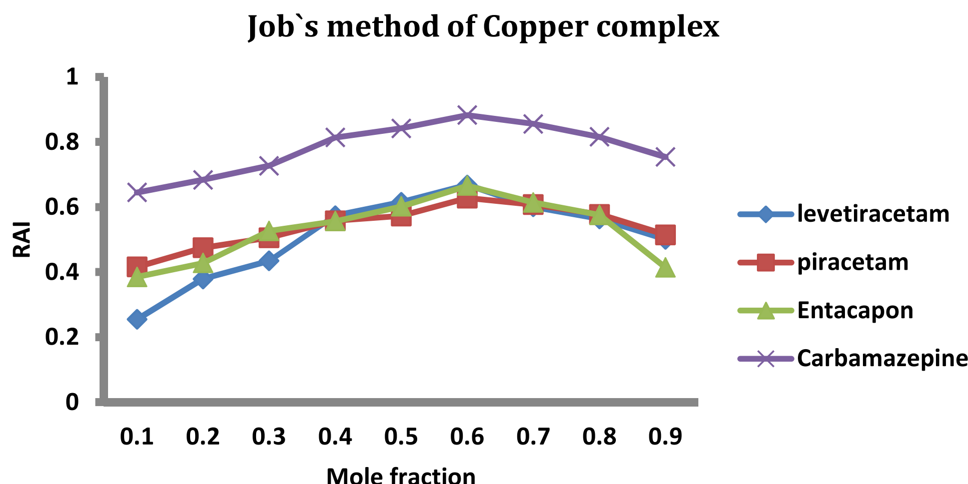

Fig. 9 The Stoichiometry of copper complexes with Levetiracetam (I)*, Piracetam (II)*, Entacapon (III) and Carbamazepine (IV)** determined by Job's method**
with copper sulphate ** with copper chloride

The stability constants (K) for the reported copper complexes were calculated according to the Benesi–Hildebrand equation:
[D]/ΔA =1/([Cu]. ε .K) + 1/(ε)

Where;
1. [D] is the molar concentration of the Drug
2. [Cu] is the sum of the reagent concentration in the complex and in the free State
3. K is the association constant
4. ε is the molar absorptivity of the formed complexes.

The values of ε were found to be equal to 1.36, 3.58, 4.21 and $8.29*10^3$ and the values of K were found to be 3.22, 1.23, 1.04 and 0.53 $*10^3$ for Levetiracetam, Piracetam, Entacapon and Carbamazepine respectively.

The free energy change of the interaction between the n-donor and the π-acceptor is related to the overall association constant, Kc by the relationship.

$$\Delta G^{\circ} = -2.303\, RT \log K_c$$

Where R is the universal gas constant (1.987 cal.mol^{-1} ◦C), T the absolute temperature in Kelvin (273+$^{\circ}$C) and G° is Gibb's free energy (kJ mol^{-1}). The calculated association energies of Levetiracetam, Piracetam, Entacapon and Carbamazepine with copper sulphate or copper chloride were found to be -4.784, -4.214, -4.11, -3.715 and -4.389 kJ mol^{-1} for Levetiracetam, Piracetam, Entacapon and Carbamazepine respectively. The indication is that all complexes can be formed *without* an external supply of energy.

2.9. Investigations on the structure of the Cu complexes

Metal complexes of general formula (L_NM_X) have been widely used in spectrophotometric analysis [44]. For the complexes dealt with in this paper is that their main ligand L is the cited drugs complexes Levetiracetam, Piracetam, Entacapon and Carbamazepine and the M is copper (II) metal. The complexes of Entacapon and Carbamazepine are synthesized, separated and characterized while; complexes of Levetiracetam and Piracetam can't be separated.

2.9.1. Preparation of the complexes

Each drug (III and IV) (0.25m.mol) was dissolved in 2 ml of methanol. Next, a solution of 0.5 m.mol of CuCl2 · $2H_2O$ or $CuSO_4$ in methanol was added and then stirred for 30 min. The final solutions were left to stand at room temperature. A Precipitate suitable for analysis formed after 7 days.

2.9.2. Physical measurements of the separated complexes

A. Elemental analysis

The purity and contribution of elements (CHN) of the synthesized complex were checked by the elemental analysis. The results showed that the copper complex of Entacapon (III) compound (mp.= 244°C) contains C: 55.08% (54.91%), H: 4.95% (4.86%), N: 13.76% (13.45%) and the Carbamazepine (IV) copper complex (mp. = 188 ^{o}C)

contain C: 76.25% (76.71%), H: 5.12% (4.96%), N: 11.86% (12.25%). The data analyzed indicate that the experimentally obtained values were in good agreement with theoretical values (within the bracket). The result confirms the formation of the compound in Stoichiometric proportion and the compound is free of impurities.

B. IR spectral analysis

The uncoordinated ligands show a medium intensity bands in the region of 3765–3130 cm^{-1} and a strong bands at 3346 and 3130 cm^{-1}. The bands at 3446 and 3130 cm–1 are assigned to νOH of phenolic groups of the drug, while a lower frequency band at 2958 cm^{-1} is assigned to arise from N triple bond stretching vibration. Entacapon shows a very strong intensity band in the region of 1681 cm^{-1}, 1223 assigned to *ν*C$=$O, C-N[3] stretching vibration respectively. Entacapon also shows two peaks at 1597.8 and 1317 cm^{-1} assigned to N=O group. The essential features of the IR spectra of the complexes consist of bands at 3445 and 3181 cm^{-1} in the region of 3000–3600 cm^{-1} which are similar to those of the uncoordinated ligands but they are slightly broadened. These bands are assigned to the *ν*OH vibration of uncoordinated phenolic-OH groups

The essential features of the IR spectra of the complexes consist of bands at the region of 3000–3600 cm^{-1} which are similar to those of the uncoordinated ligand but they are slightly broadened. Such features of *ν*OH bands in the ligand and complex rule out the possibility of the involvement of phenolic –OH groups in bonding with the metal centre. Another prominent feature of the IR spectra of the complexes is the appearance of a cluster of medium intensity bands in the region of 2300–2100 cm^{-1} in the complexes. This band is characteristic of the occurrence of stronger intramolecular H-bonding in the complexes involving –OH groups. The ligand bands appearing at 2958 and 1681 due to N triple bond and C=O bond stretching vibrations show a large negative shift by 40 and 60 cm^{-1} in the complex. This shows the coordination of both the N triple bond and C=O groups to the metal centre as shown in (Figure 10).

The second ligand (Carbamazepine) bands appearing at 1788 cm^{-1} due to C$=$O vibration show a large negative shift by 156 cm–1 in the complex. The lower shift of C$=$O band by such a large frequency indicates a strong coordination of the ligand to the metal centre through C$=$O groups. The amide band appearing at 1606 cm^{-1} is shifted to lower frequency by 70 cm^{-1} and appears as a strong shoulder band at 1716 cm^{-1} in the complex.

The negative shift of the amide band in the complexes suggests coordination of the C$=$O group to the metal. The most crucial feature of the IR spectra of the complexes is the appearance of a new strong band in the region of 2287–2760 cm^{-1}, which arises usually due to *ν*NCO^{-} resulting from enolization of the ligand. This indicates the possibility of enolization of ligands in the complexes and suggested the co-ordination of drug to the metal centre in the enol form as shown in (Figure 11). Figure 12 shows the expected copper complex structures for the non synthesized Levetiracetam (I), Piracetam (II) and the synthesized Entacapon (III) and Carbamazepine (IV)

b

a

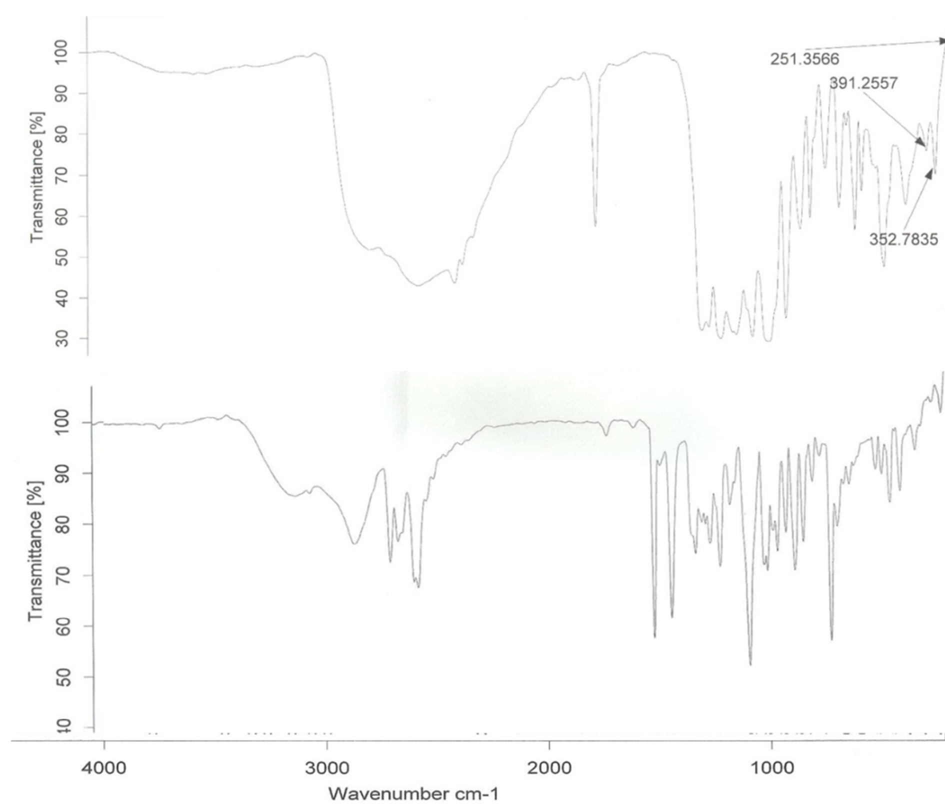

Fig. 10: The IR spectra of (a) free Entacapon (III) and (b) its CuCl2 complex

b

a

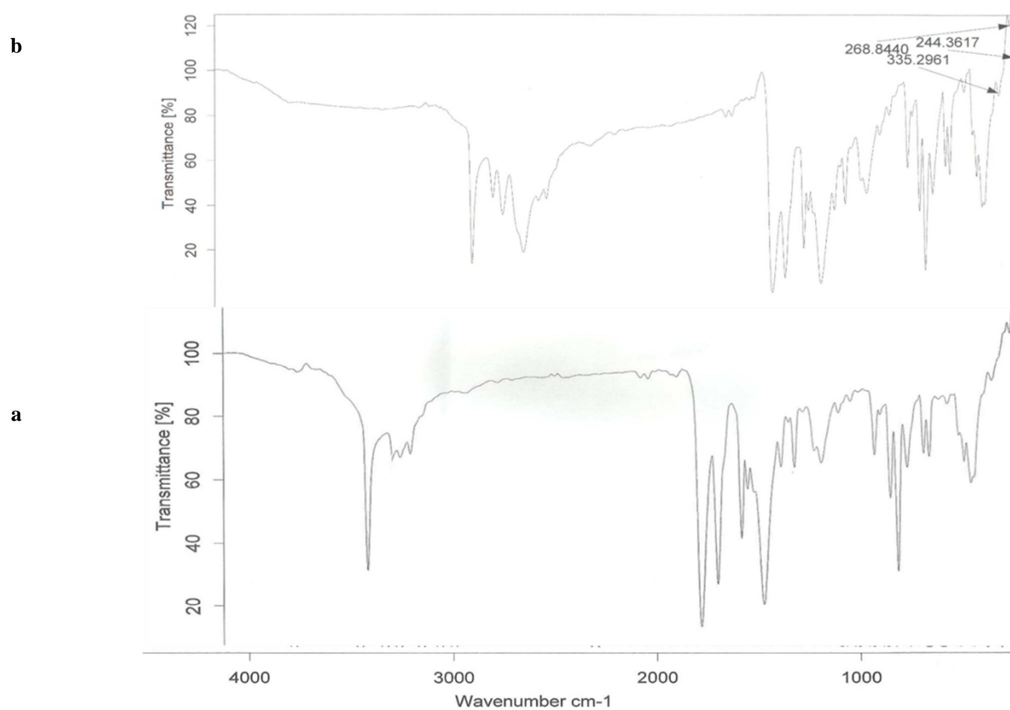

Fig. 11: The IR spectra of (a) free Carbamazepine (IV) and (b) its $CuCl_2$ complex

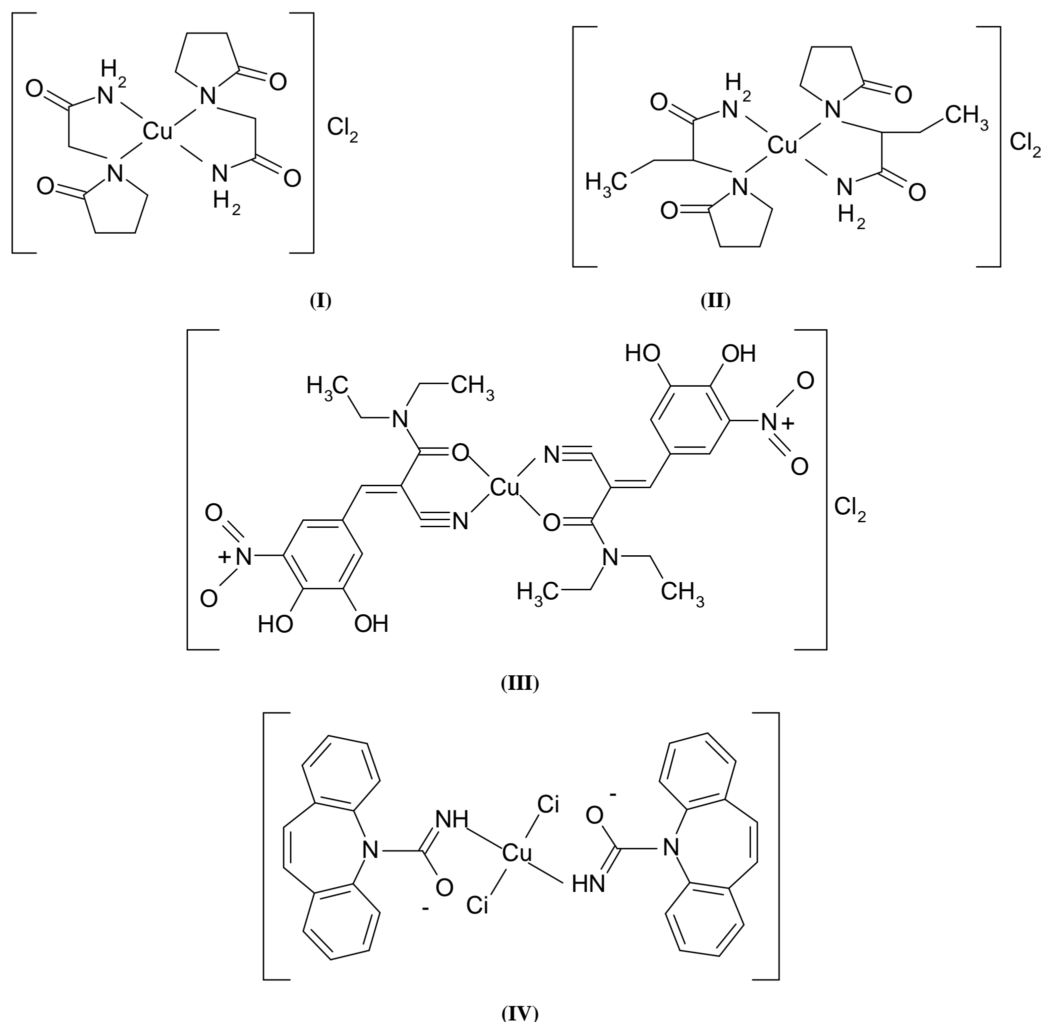

Fig. 12 The structure of copper complexes Levetiracetam (I)*, Piracetam (II)*, Entacapon (III) ** and Carbamazepine (IV) **

C. Molar conductance

It is clear from the conductivity data, compared with the values of free ligands that the complexes present seem to be electrolytes. Also the molar conductance values indicate that the anions may be exhibits outside or absent or inside the coordination sphere [45].

This is in case confirmed by Carbamazepine (IV) complex, the molar conductance ($\Lambda M = 20$) value are too low to account for an ionic complex; therefore, these complexes are considered to be neutral.

The molar conductance (ΛM) value for Entacapon (III) complex corresponds to a 2:1 electrolyte ($\Lambda M = 80$) indicating that both the coordinated anions are replaced by solvent molecules. These complexes are hex coordinated and its probable geometry may be octahedral.

D. Magnetic moment

Copper (II) complexes show magnetic moment values in the range of 1.85–1.87 BM. This value is close to the spin-only value of 1.73 BM [46]. This indicates the absence of any appreciable spin–spin coupling between unpaired electrons belonging to different copper atoms. According to (B.N. Figgis) [47], a magnetic moment value >1.90 BM indicates a tetrahedral stereochemistry while <1.90 BM is indicative of square planar as well as octahedral stereochemistry.

E. Electron spin resonance spectroscopy (ESR)

The monometallic complexes with Entacapon and Carbamazepine have been studied by ESR spectroscopy at LNT in a glassy state.

The complexes show anisotropic spectra and have almost similar ESR spectral features. The $g\|$ value for the complexes lies in the region of 2.273–2.291 while $g\perp$ value lies in the region of 2.093–2.097. The $g\|$ and $g\perp$ values depart considerably from the free ion value. The shifting of g values from 2.0023 in transition metal complexes is due to mixing, via spin–orbit coupling of the metal orbitals containing the unpaired electron(s), with the empty or filled ligand orbitals. When the mixing is with empty ligand orbitals, the result is a negative g shift, whereas the mixing with the filled ligand orbitals leads to a positive g value shift. The shift depends on the amount of unpaired electron density at the donor sites of the ligands i.e., on the degree of covalency of the complex and the ESR spectra of the complexes (III) and (IV) show splitting in the $g\|$ region. The nuclear quantum number of copper is 3/2, hence it should show four signals. However, all the signals are not observed in the complexes.

(D. Kivelson et al., 1961) [48] have reported that the $g_{\|}$ value in copper complexes can be used as a measure of the nature of the metal–ligand bond. If this value is more than 2.3, the environment is essentially ionic and values less than this limit are indicative of a covalent character. The fact is that $g_{\|}$ values for the complexes (III) and (IV) are less than 2.3. This indicates that the metal–ligand bonds in these complexes have covalent character. Also the shape of the ESR lines indicates that the geometry around the copper (II) ion is not trigonal bipyramidal in these complexes since the low field side of the ESR spectrum is less intense than the high field side and the order of $g_{\perp}$ values is not in accordance with the range suggested for trigonal bipyramidal complexes [49] ($2.00 > g_{\|} > g_{\perp}$). The magnetic parameters indicate $g_{\|} > g_{\perp} >$ free spin (2.0023) which shows that the unpaired electron is in the d_{x2-y2} orbital of the Cu (II) centre. The in-plane α covalency parameter, X^2_{Cu} was calculated for Entacapon (III) and Carbamazepine (IV) Cu (II) complexes using the equations of these literatures (S. Sujatha et al.) [50] and (A. Syamal, R.L. Dutta) [51].

The X^2_{Cu} value accounts for the fraction of unpaired electron density on the copper ion. Smaller the value of X^2_{Cu}, more covalent is the bonding nature. For example X^2_{Cu}=0.5 indicates complete covalent bonding, but X^2_{Cu}=1 suggests complete ionic bonding. The X^2_{Cu} values for the Cu (II) complexes are in the range of 0.794–0.852 < 1 indicating that the Cu (II) complexes have considerable amount of covalent character.

V. Suresh Babu and S. Djebbar-Sid [52, 53] had reported that $g_{\|} > 2.4$ for copper-oxygen bonds and 2.3 for copper–nitrogen bonds. The Cu (II) complexes (III and IV) have $g_{\|}$ values between 2.273–2.291 and are in agreement with the presence of mixed copper-oxygen and copper–nitrogen bonds.

The nature of the ligand forming the complex is evaluated from G values obtained by using the following equation:

$$G= (g_{\|}-2)/ (g_{\perp}-2)$$

If $G < 4.0$, the ligand forming the complex is regarded as a strong field ligand. For the square planar complexes G is usually in the range of 2.03–2.45. G value for the complexes (III and IV) lies in the range of 2.814–3.129 at LNT which suggests that Entacapon and Carbamazepine ligands have a sufficiently strong field in the complexes.

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