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Lanthanide Ions Complexes of 2-(4-amino antipyrine)-L-Tryptophane (AAT): Preparation, Identification and Antimicrobial Assay

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Abstract

In this work, L-Triptophan was coupled with diazotized 4-aminoantipyrine to give novel Mono azo ligand 2-(4-aminoantipyren)-L-Tryptophane (AAT). This prepared ligand was coordinated with chloride salts of [Ln=La(III), Ce(III), Nd(III), Eu(III) and Gd(III)] ions. The structural feature of the ligand and it's metal complexes were identified by elemental analysis FTIR, HMMR, UV-Vis spectroscopy, magnetic susceptibility and molar conductivity. Chloride ion content was also appointed by Mohr method. The spectral data and physicochemical studies declares that the ligand act as neutral N,O-bidentat Pentagonal bipyramidal structure has been suggested for all prepared complexes which were exhibited (1:2) metal:ligand ratio at optimum condition as well as were formulated as $[Ln(AAT)_2Cl_3]$. The nature of all prepared Ln- complexes were performed after install the optimum pH and molar concentration that obeyed Lambert-Beer's law over a concentration range of ($2\text{-}4\times10^{-4}$ M) at the maximum wave length (λ_{max}). Furthermore the stability constant (K) and Gibbs free energy (ΔG) have also been studied. The in vitro antibacterial and antifungal assay of all prepared compounds have been evaluated.

Keywords: Mono azo ligand, Lanthanide complexes, spectral studies, antibacterial and antifungal assay

معقدات ايونات اللانثانات مع 2-(4-امينوانتيبايرين)-تربتوفان: تحضير، تشخيص وفحصها كمضاد للميكروبات

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الخلاصة

حُضر ليكاند أُحادي الأزو جديد 2-(4-امينو انتيبايرين)-تربتوفان من خلال تفاعل الأزدواج لملح الدايزونيوم لـ 4- امينو انتيبايرين مع التربتوفان ومن ثم مفاعلة الليكاند المحضر مع كلوريدات الايونات [La(III), Ce(III), Nd(III), Eu(III) and Gd(III)] شخصت المعقدات المحضرة والليكاند من خلال تحليل العناصر ودراسة أطياف الأشعة التحت الحمراء والفوق البنفسجية – المرئية والرنين النووي المغناطيسي، اضافة الى قياسات الحساسية المغناطيسية و التوصيلية المولارية والطاقة الحرة لكبس وايجاد ثابت الأستقرارية للمعقدات.عينت نسبة ايون الكلوريد باستخدام طريقة مور. أظهرت النتائج ان الليكاند المحضر ذو سلوك ثنائي السن O،N ونسبة مولية (ليكاند : فلز) (2 : 1) عند الظروف المثلى ليعطي الصيغة المقترحة ثنائي الهرم خماسي القاعدة ، وخضعت محاليل جميع المعقدات المحضرة لقانون لامبرت-بير ضمن التراكيز (2-

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4×10^{-4} مولاري)عند الطول الموجي الاعظم.(λ_{max}). وكما اظهرت جميع المركبات المحضرة فعالية متباينة ضد انواع من البكتريا و الفطريات المنتجة.

Introduction

In recent years, the lanthanide complexes have been attracted widely attention and extensively investigated in material science because of the magnetic and optical properties of these ions [1].

It could show a very wide range of coordination numbers (6-12) but the numbers at 2, 3, or 4 are known, with (8 or 9) being favourite [2]. Due to the 4f-electrons are inside the 5S and 5P electrons are core like in their behavior, being shielded from the ligands, thus taking no part in bonding and having spectroscopic and magnetic properties largely independent of environment. In addition the ability to form π bonds is also absent, and thus there are none of the (M=O) or (M≡N) bonds are found for transition metals. The organometallic chemistry is appreciably different from that of transition metals [3]. Azo dyes of 4-amino antipyrine are well known as polydentate ligands coordinating in neutral or ionic forms [4] . A wide spread interest has also been recorded on the synthesis , identification and their abilities as chelating ligands with metal or metal ion and forming stable colored complexes of different geometries owing to the excellent donor properties of conjugated chromospheres azo moiety (-N=N-) , that correlate with pyrazolone ring, as a result hetro azo compounds have high intensity color [5] , therefore they have been used as dyes and pigments for long time [6] , furthermore they are tremendously used in many fields such as food [7] , paints [8] , cosmetics [9] analytical chemistry [10] and medicinal chemistry [11]. Among the pharmaceuticals they are used as analgesic , antipyretic , anti-rheumatic, anti-histaminic and anti-inflammatory drugs [12]. This study has been devoted to prepare and characterize the AAT ligands and its complexes with [Ln=La(III), Ce(III), Nd(III), Eu(III) and Gd(III)]. The in vitro antibacterial and antifungal assay of all prepared compounds have also been performed.

Experimental

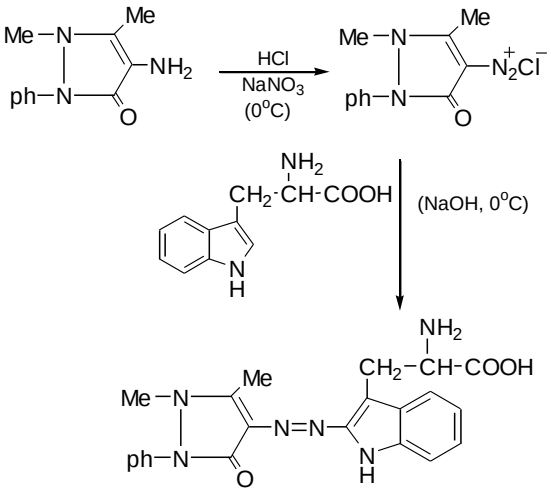
Materials: All the following reagents and solvents are of high purity and used as received from commercial sources.

Instrumental analysis:

The microelemental analysis (C,H,N) were carried out by using (Eurovector EA 3000A elemental analyser) . The lanthanide content of the complexes was measured using atomic emission spectroscopy measurement by an applied research laboratories model 3410 monitors sequential inductively coupled plasma spectrometer (I.C.P.) .The molar conductance measurements were measured by using (10^{-3}M) of Ln-complexes solutions in ethanol and DMF at 25℃ using (HANA instrument / conductivity tester) . magnetic susceptibilities were obtained by using Broker magnet B.M.G instrument at 25℃ . pH measurements were performed using (HANA instruments pH tester / pocket pH tester) stuart melting point apparatus was used to measure the melting points of the ligands and its complexes . FTIR – spectra were carried out on shimadzu , FTIR – 8400 s fourier form infrared spectrophotometer (200 – 4000) cm^{-1} with samples prepared as CsI discs. UV-Vis spectra were obtained for (10^{-4}M) at an ethanolic solution of all prepared compounds by using Shimadzu UV-160A ultraviolet visible spectrophotometer and 1 cm quartz cell in the range (200 – 1100) nm. The HNMR spectra were recorded on a jeol Ex 270 MHz, bucker 500 MHz) using DMSO as a solvent and TMS as a reference.

Synthesis of ligand

AAT ligand was synthesized as in scheme-1 following the method reported in the literature [7] . 4-aminoantipyrine (10^{-3} mole, 0.203 gm) was dissolved in 10 ml of 4M hydrochloric acid and cooled to 0℃ in an ice bath , then diazotized with 10 ml of aqueous 2.5% sodium nitrite and kept stirred for 1 hr. The resulting diazonium chloride solution was then slowly added to (10^{-3} mole, 0.204 gm) of Tryptophan which was dissolved in (15 ml, 1 M) sodium hydroxide at 0℃.The resulted solution was neutralized with (1M, HCL) and continuously stirred for 2hr . The yellow precipitate was filtered and re-crystallized from (1:1) ethanol : water then dried under vacuum . The yield was 80.12%. The molecular structure of the ligand was conforimed by microelemental (Table-1).



Scheme 1- Preparation of 2-(4-aminoantipyrine) tryptophan (AAT)

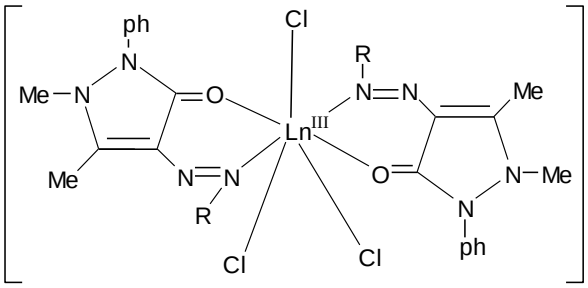
Table 1- Physiochemical properties, (M:L) molar ratio, optimum condition, conductivity and magnetic moment for the ligand (AAT) and it's Ln-complexes

compound (M.wt. gm/mole)	Color	m.p. [°C] (yield%)	elemental analysis found (cal.)					Λ ohm^{-1}.mol^{-1}.cm^2		μ B.M	optimum pH	optimum molar conc. ×10^{-4}	λ_{max} (nm)	M:L
			C	H	N	M	Cl	EtOH	DMF					
AAT (480)	Yellowish Orange	310 (80.12)	62.98 (63.15)	5.22 (5.26)	20.10 (20.09)	-	-	-	-	-	-	-	400	-
$[La(AAT)_2Cl_3]$ (1081.4)	Reddish Orange	213 (66)	48.78 (48.82)	4.11 (4.06)	15.42 (15.53)	12.81 (12.84)	9.72 (9.84)	17.47	15.24	Dia	7	3.5	515	1:2
$[Ce(AAT)_2Cl_3]$ (1084.67)	Orange	189 (72)	48.39 (48.67)	3.95 (4.05)	15.41 (15.48)	12.87 (13.10)	9.83 (9.81)	17.98	16.14	2.5	7	3.5	455	1:2
$[Nd(AAT)_2Cl_3]$ (1086.74)	Red	196 (78)	48.46 (48.58)	3.96 (4.04)	15.44 (15.53)	13.16 (13.27)	9.65 (9.79)	18.81	14.06	3.2	7	3.5	468	1:2
$[Eu(AAT)_2Cl_3]$ (1093.75)	Purple	220 (70)	48.18 (48.27)	3.88 (4.02)	15.33 (15.36)	13.78 (13.82)	9.70 (9.73)	18.52	12.67	3.3	7	3.5	571	1:2
$[Gd(AAT)_2Cl_3]$ (1099.75)	Redish Purple	203 (69)	47.97 (48.01)	4.08 (4.00)	15.18 (15.27)	14.22 (14.29)	9.66 (9.68)	17.38	11.94	7.1	7	3.5	564	1:2

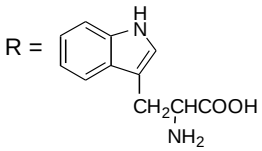
Preparation of Ln-complexes:

The Ln-Complexes were prepared by adding with stirring an ethanolic solution of (2mmole,0.836gm) of the ligand (AAT) to stoichiometric amount (1mmole) of lanthanide chloride [La(III), Ce(III), Nd(III), Eu(III) and Gd (III)] dissolved in the optimum acetate buffer solution. The product solution was stirred at room temperature for (1hr).The color solid products were filtered and washed with hot EtOH:H_2O and dried under vacuum. All analytical and physical data are tabulated in Table-1. The expected stereochemical structure of the complexes is pentagonal bipyramidal as illustrated in Scheme-2.

$LnCl_3 + 2(AAT) \rightarrow [Ln(AAT)_2Cl_3]$
Where: Ln (III)=La, Ce, Nd, Eu and Gd



where:
Ln(III) = (La, Ce, Nd Eu and Gd) complexes



Scheme 2- The proposed Structure of the prepared complexes

Preparation of Buffer Solutions

Buffer solution, in the range of pH values (4-10), of (0.01M, 0.7708 gm) was prepared by dissolving ammonium acetate in (1L) of doubly distilled deionized water. The required pH was got by the adding of either glacial acetic acid or ammonium hydroxide.

Preparation of Standard Solution

The standard solution of lanthanide ions were prepared by dissolving appropriate weight of $LnCl_3$ salts [Ln=La(III), Ce(III), Nd(III), Eu(III) and Gd(III)] in the buffer solutions in the pH range (4-10), with in the concentration ranging between (10^{-2} -10^{-5} M). Ethanolic solutions of the ligand (AAT) at the same concentration (10^{-2}-10^{-5} M) were also prepared.

Antimicrobial Assay

The invitro recognition of antibacterial and antifungal activity was performed depending on the diffusion technique [13]. The bacteria *Escherichia coli (E-Coli)* and *staph aureus* were grown in nutrient broth at (37℃) for (24 hr) . *Aspergillus niger* and *Candida albicans* were grown in malt broth at (28℃) for (48 hr). All the prepared compounds were tested by using the diffusion technique on solid media. The deactivation dimeters were recorded by measuring the zones of growth inhibition circling the disc.

Results and Discussion

Identification of ligand and its Ln-complexes

The mono azo ligand (AAT) was yellow crystalline solid, and has been derived from 4-aminoantipyrine as the diazo component, which is a versatile key intermediate in the preparation of azo ligand and L-Tryptophan was used as coupling component as illustrated in schem(1). The prepared ligand (AAT) exhibited high chelating ability when reacted with [Ln(III) =La, Ce, Nd, Eu and Gd] to form colored complexes,(Scheme(2)) and enhanced as N,O bidentate ligand through nitrogen atom of azo moiety near L-Tryptophan and oxygen atom of carbonyl moiety to form stable five member - chelating ring. All prepared compounds were soluble in most organic solvent but not soluble in water, and stable in air at room temperature. The physiochemical properties are listed in Table-1. From the microanalysis formula of the ligand and its complexes, were in agreement with the calculated data and indicate that the Ln-complexes have stoichiometry (1:2) [Ln:AAT] which gives the formula $[Ln(AAT)_2Cl_2]$. The purity of all prepared compounds was tested by TLC technique. Calibration curve was constructed following a general procedure [14] by plotting absorbance against a range of molar concentration (10^{-5}-10^{-3}M) of mixed aqueous-ethanolic solutions of the (AAT) and lanthanide ions (2-4×10^{-4}M) obeyed Beer's law and appeared in perspicuous intense color. The straight lines were obtained with correlation factor R > 0.9799 as is shown in Figure-1.

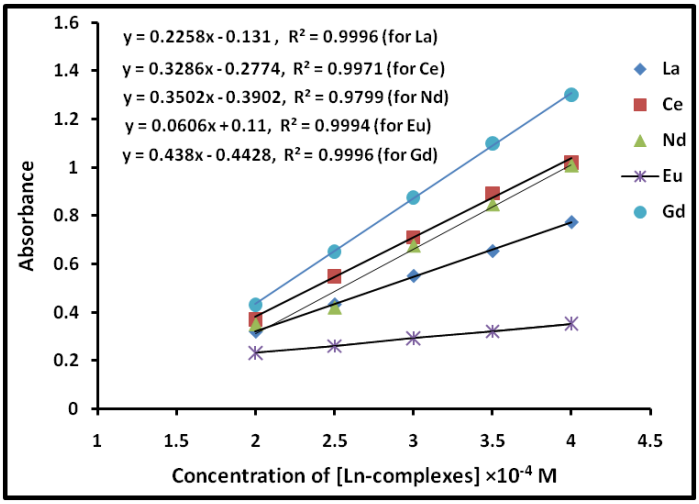


Figure 1- Linear relationship for [Ln-complexes]

Chromogenic of the ligand(AAT) and Ln-complex solutions

The interaction of the studied lanthanide ions and the ligand(AAT) have been investigated spectrophotometrically in solution, using ethanol as reference. When the selected Ln(III) chloride solutions were added , the yellow color of (AAT) solution was changed to red or purple with changes in the electronic spectra after mixing (Figure-2). A high bathochromic shift in the visible region was noticed in the (λ_{max}) from (400)nm for the free (AAT) to (455-571)nm of the prepared Ln-complexes solutions. This change in the spectra was considered important to explore the optimum conditions for the prepared complexes.

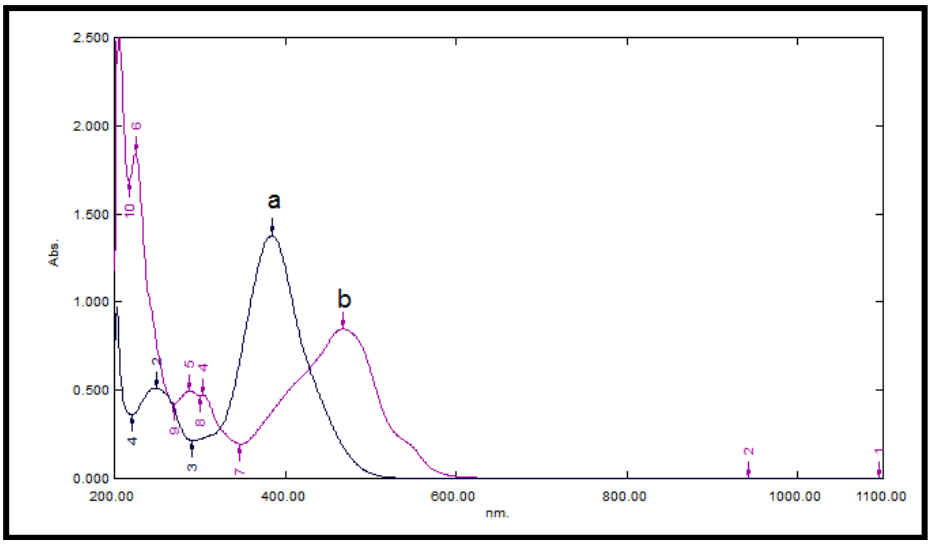


Figure 2- The electronic spectra of : a- free lingand, b- [Nd-AAT] mixed solution

Determination of the optimum conditions

The optimum conditions for the preparation of the complexes were studied first through the investigation of the UV-Vis spectra of mixing solutions for the (AAT) and Ln-complexes to obtained optimum pH and concentration, as well as the maximum wave length (λ_{max}). The optimum concentration was selected for Ln-complex solution based on which solution gave the maximum absorbance at fixed (λ_{max}) with different pH, the results are described in Table-1. At the same time the effect of pH on the absorbance for Ln-complexes were studied in the pH range (4-9). It was found that all prepared complexes had optimum performance at (pH=7) at certain(λ_{max}) as is plotted in Figure-3. The pH value was decreased in the absorbance at (7>pH>7) may be attributed to the hydrolysis of Ln-complex [15]

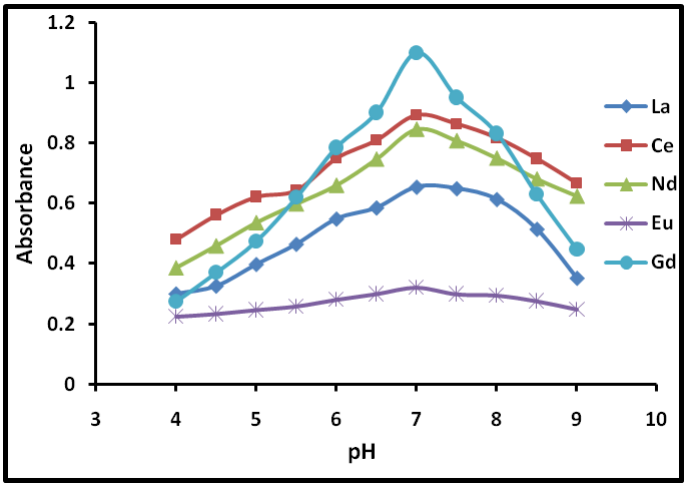


Figure 3- Influence of pH at optimum concentration and (λ_{max}) for (Ln- complexes) solutions

Composition of complexes:

The (Ln:AAT) of the prepared complexes was determined by studying the complex formation in solution by mole ratio method under the optimum condition employed of pH and concentration to obtain maximal and constant absorbance signal (λ_{max}). The solutions of the prepared complexes showed increased absorbance of the complexes solutions to get to the intersection point and the absorbance still constant at passing this point which indicate that the complex was formed [16]. The mole ratio was approximately (1:2) (Ln:AAT) at pH=7 and (3.5×10^{-4}M), (Figure-4). All the results are in agreement with the values reported and are listed in Table-1.

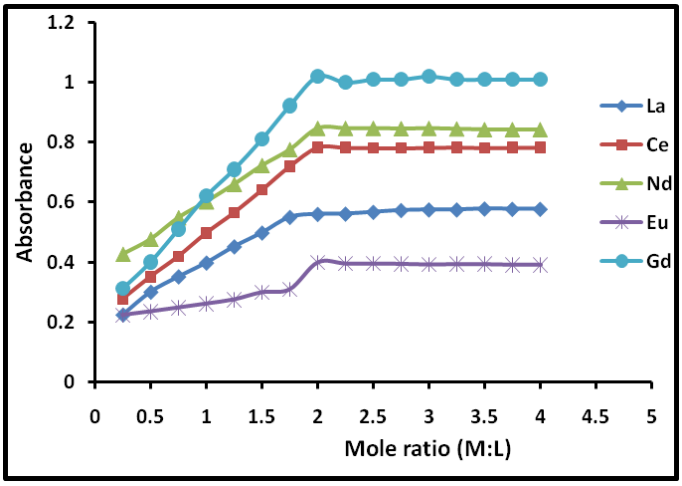


Figure 4- Mole ratio for [Ln-complexes] solutions at optimum pH, concentration and λ_{max}

Influence of Time on Absorbance:

The influence of the reaction time and the intensity of the colored Ln-complex solutions were studied under optimum conditions described before. Figure-5 showed that the reaction was completed after about (10min) at room temperature with constant absorbance of the prepared complexes for at least (24hr). This indicates that the ligand (AAT) showed strong chelation with all selected lanthanide ions.

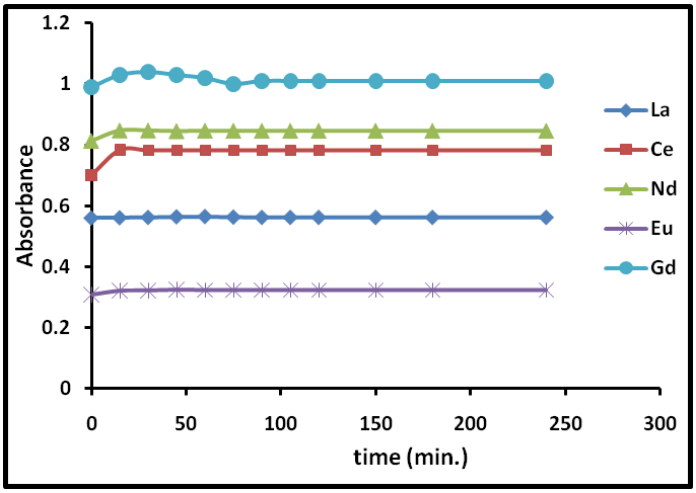


Figure 5- Effect of time on stability of [Ln-complex] solutions

Molar Conductance Measurement

The molar conductance data were measured at room temperature in (10^{-3}M) ethanol and DMF. All prepared Ln-complexes were exhibited low value of molar conductivity which indicates that non-electrolyte and non-conductive species exist in these solvent, and are in a good agreement with that reported in the literature for(1:1) electrolytes in both DMF and methanol solvent[17]. Table-1 shows all data.

Calculation of Ln-Complexes Stability Constant

In order to calculate the stability constant (K) for each (1:2) (Ln:AAT) complex, the spectroscopy method was used by the following equations[18,19]:

$$K = \frac{1-\alpha}{4\alpha^3 C^2} \; ; \qquad \alpha = \frac{A_m - A_s}{A_m}$$

Where:

C = the molar concentration of the complex solution

α = degree of dissociation

A_m and A_s = the absorbance of the fully and partially formed chelating complex respectively at optimum condition and (λ_{max}) of solution. All Data of (K) and log K are recorded in Table-2.

The thermodynamic parameters of Gibbis free energy (ΔG) were also studied. So the (ΔG) values were calculated from relationship [19]:

$\Delta G = - R T \ln K$

Where:

R = gas constant =8.3 $J.mol^{-1}.K$

T = absolute temperature (Kelvin)

The negative value of (ΔG) (Table-2) indicates that the interaction between (AAT) and selected lanthanide ions are spontaneous.

Table 2- The stability constant and Gibbis free energy for the ligand (AAT) and it's Ln- Complexes .

Compound	Log K	ΔG $J.mol^{-1}$
Ligand (AAT)	-	-
$[La(AAT)_2Cl_3]$	7.76	-40524.48
$[Ce(AAT)_2Cl_3]$	7.40	-38608.96
$[Nd(AAT)_2Cl_3]$	7.78	-40600.44
$[Eu(AAT)_2Cl_3]$	7.50	-39179.36
$[Gd(AAT)_2Cl_3]$	7.55	-39408.8

Magnetic Susceptibility:

The organometallic chemistry of lanthanide metals is appreciably different from that of transition metals. The 4f orbital penetrate the xenon core, due to this, they can't overlap with ligand orbital and therefore don't participate significantly in bonding. As a result of their isolation from the influence of the ligand, crystal-field effects are very small and can be regarded as a perturbation of the free-ion states, thus electronic spectra and magnetic properties are essentially unaffected by environment. Hence, these may be accounted for using the Russell-Saunders coupling scheme in which the electron spins are coupled together separately from the coupling of the orbital angular moment at electrons, and the orbital moment is unquenched [20]. The effective magnetic moment (μ_{eff}) of the prepared complexes showed that all prepared complexes are paramagnetic except lanthanum complex is diamagnetic. The results obtained were compared with the actual values and showing close agreement with calculated values (Table-1)

FTIR Spectra:

The most group vibrations of FTIR for the ligand (AAT) and it's prepared Ln-Complexes were listed in Table-3 (Figure (6 and 7)).The spectrum of (AAT) shows well-defined peaks at (3459 and 3144 cm^{-1}) are assigned to the $\nu(O-H)$ of carboxyl moiety and $\nu(N-H)$ indole moiety, respectively [21].In the spectra of the all prepared complexes these two peaks almostly appeared at the same frequency as that of the free ligand, indicating that they don't participate in coordination [22]. Doublet bands were also noticed at the range characteristic (1690-1683 cm^{-1}) in the spectrum of the free AAT. These bands were attributed to $\nu(C=O)$ and $\nu(C=C)$ which were shifted to lower frequency with change in shape and intensity on coordination with lanthanide ion, indicating that the carbonyl oxygen of the antipyrine moiety has been involved in the chelating ring.The spectra of all prepared complexes revealed that the bands characteristic of the azo moiety assigned to ν(C=N–N=C), ν(N=N) and ν(C–N=N–C) where shifted to lower frequency, indicating the engagement of the azo nitrogen atoms in the coordination with lanthanide ions [23].

The FTIR spectra of complexes exhibited new bands at (552-535 and 463-414 cm^{-1}), were assigned to ν(M–O) and ν(M–N) respectively. An additional band appeared at (277-229 cm^{-1}), was assigned to ν(M–Cl) [24].

Table 3- The characteristic FTIR spectral data (cm^{-1}) for the ligand (AAT) and its Ln-complexes

Compound	ν(O-H)	ν(N-H) indol	ν(C=O) ν(C=C)	ν(C=N=N=C)	ν(N=N)	ν(C-N=N-C)	ν(M-O)	ν(M-N) azo	ν(M-Cl)
Ligand (AAT)	3459 st, br	3144 w	1690, 1683 d	1616, 1560 d	1440 m	1217, 1168 st, d	-	-	-
$[La(AAT)_2Cl_3]$	3450 m, br	3141 vw	1650 m, sh	1514 w	1394 m	1203, 1180 st, d	550 vw	422 w	260 m
$[Ce(AAT)_2Cl_3]$	3456 st, br	3141 vw	1627 st, sh	1512 m	1396 w	1230, 1188 st, d	552 m	455 w	277 st
$[Nd(AAT)_2Cl_3]$	3453 st, br	3139 w	1633 m, d	1498 w	1409 m	1213, 1159 st, d	545 m	438 w	267 m
$[Eu(AAT)_2Cl_3]$	3447 m, br	3140 w	1676, 1649 w, d	1492 w	1404 m	1189, 1143 m, d	540 w	463 w	264 st
$[Gd(AAT)_2Cl_3]$	3454 m, br	3142 w	1665, 1643 m, d	1486	1407 w	1166, 1137 m, d	535 w	414 w	229 m

St= strong; sh = sharp; m = medium; w = weak; vw = very weak; d = doublet

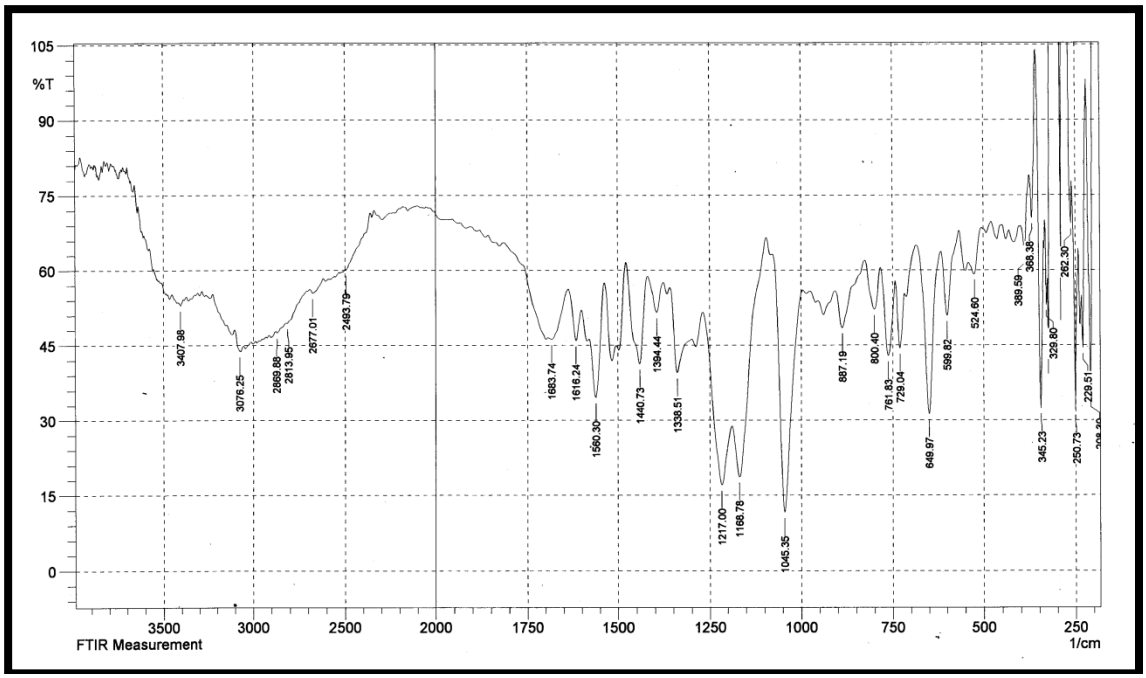


Figure 6- FTIR spectrum of the ligand AAT

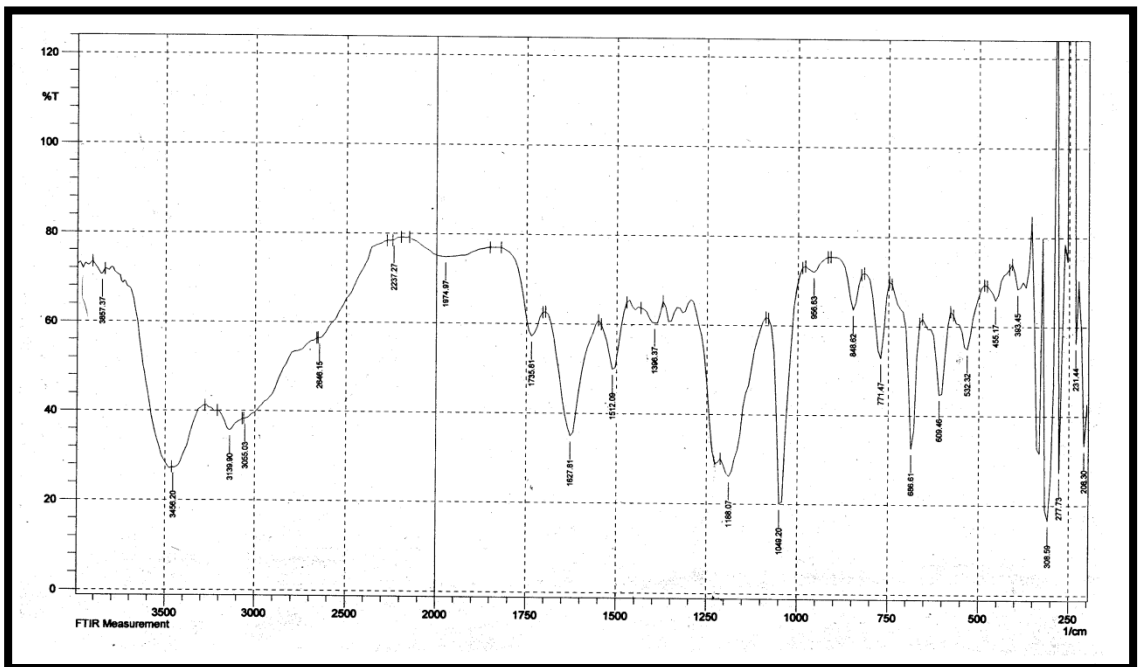


Figure 7- FTIR spectrum of [Ce $(AAT)_2Cl_3$]

Electronic spectra:

In order to attain a wider insight into the properties of the coordination field of prepared complexes, the electronic spectra of the ligand (AAT) and it's Ln-complexes (Table-4) were performed in absolute ethanol(10^{-4}M) measured against ethanol as reference (Figure (8 and 9)).The spectrum of the free ligand (AAT) exhibited two absorption bands. The first band at (400 nm, 25000 cm^{-1}) was assigned to the (π–π*) transition owing to the intermolecular charge transfer taken place through azoic moiety. This band appeared strong red shifted due to coordination with lanthanide ion and proposing the involvement of the ligand in the bond formation. The second band was observed at (257 nm, 38910 cm^{-1}) due to (π–π*) transition for the heterocyclic moiety [25]. Most lanthanide ions absorb electromagnetic radiation, particularly in the visible region of the spectrum, exciting the ions from its ground state to a higher electronic state, as a consequence of partly filled 4f- orbital. The f-f transitions are excited both by magnetic dipole and electric radiation. Normally the magnetic dipole transitions

are parity- allowed and would not be seen, whereas the electric dipole transitions are parity forbidden (Laporte-forbidden) and much weaker. As a result this transitions didn't exist in the spectra of Ln-complexes as they were very weak and were disappeared by the intense absorption of the bands for the (AAT) [26]

Table 4-Electronic spectra for the ligand (AAT) and it's Ln - Complexes

Compound	λ_{max} (nm)	Absorption band (cm^{-1})	$\varepsilon \times 10^4$ $L.mol.^{-1}.cm^{-1}$	Transition
Ligand (AAT)	400 257	25000 36363	0.919 0.321	$\pi \rightarrow \pi^*$ $\pi \rightarrow \pi^*$
$[La(AAT)_2Cl_3]$	515	19417	0.654	MLCT
$[Ce(AAT)_2Cl_3]$	455	21978	0.893	MLCT
$[Nd(AAT)_2Cl_3]$	468	21367	0.846	MLCT
$[Eu(AAT)_2Cl_3]$	571	17513	0.321	MLCT
$[Gd(AAT)_2Cl_3]$	564	17730	1.099	MLCT

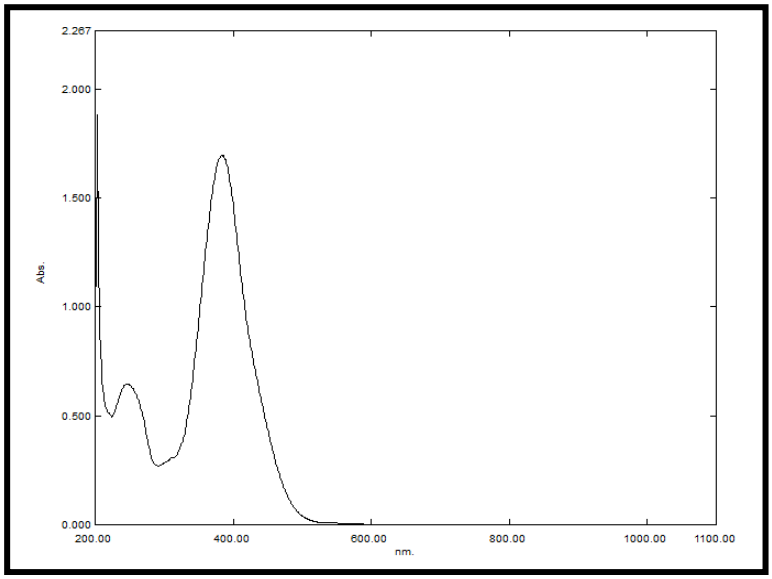


Figure 8- The electronic spectrum of the ligand (AAT)

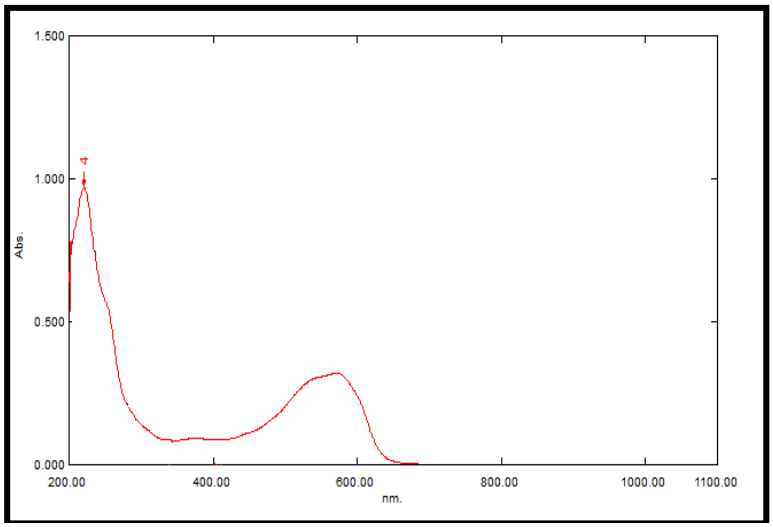


Figure 9- The electronic spectrum of $[Eu(AAT)_2Cl_3]$

The ^{1}HNMR Spectra

Figure (10 and 11) show the ^{1}HMNR spectra off the ligand (AAT) and [La(III) – AAT) complex in DMSO solution with TMS as an internal standard. The spectrum of the ligand (AAT) exhibited a signal related to one proton appeared at (δ=11 ppm) which was assigned to carboxylic proton of side chain for L-Tryptophan moiety. The signal observed at (δ=9.421 ppm) was attributed to (NH) proton of indol ring. The multiple signals observed in the region (δ=7.89 – 7.41 ppm, 9H) were assigned to chemical shifts of aromatic protons of naphthyl and indol moieties. The signals assigned to chemical shifts at (δ=8.131 ppm, 2H) and (δ=4.35 ppm, 1H) were attributed to (NH_2) and (CH –NH_2) moieties. While the signals referred to chemical shifts of aliphatic (CH) and (CH_2) protons appeared as doublets at (δ=3.402 and 3.294 ppm, 2H) and triplet at (δ=4.352, 4.074 and 4.037 ppm, H) respectively. On the other hand, the signal of (N-CH_3) and (C-CH_3) for L-Tryptophan moiety were observed at (δ=3.148 ppm, 3H) and (δ=2.765 ppm, 3H). the spectrum of [La(AAT)$_2$Cl$_3$] complex was exhibited slight shifts in positions of the protons signals assigned to aromatic (δ =7.495-7.297 ppm, 9H) and aliphatic protons(δ=3.53 and 3.42 ppm, 2H) due to complex fromation ,while no shifts was observed in the position of (NH) signal of indol ring proton (δ=9.422 ppm) which exclude indol (NH) group from coordination to the La(III) ion [21,27].

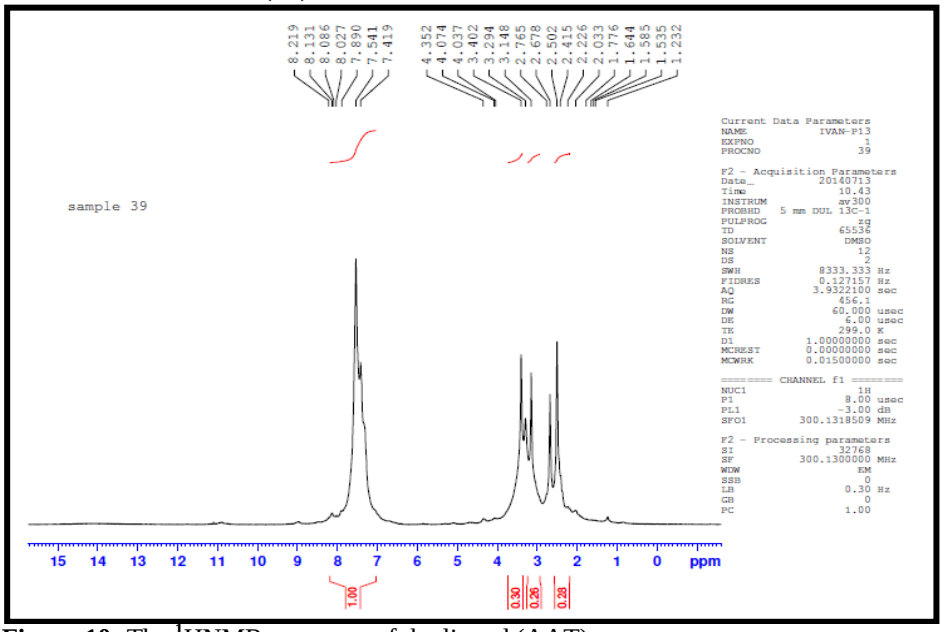


Figure 10- The ^{1}HNMR spectrum of the ligand (AAT)

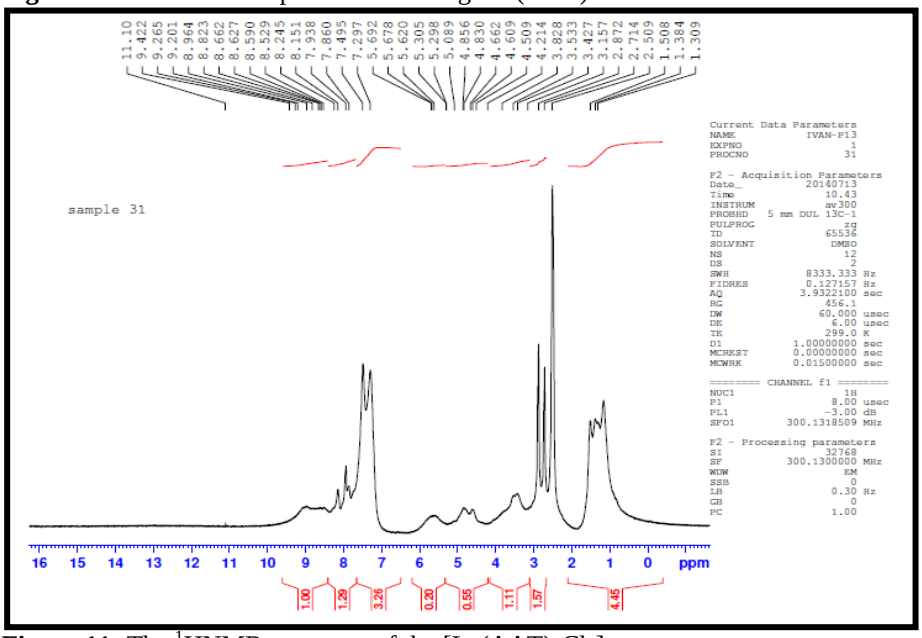


Figure 11- The ^{1}HNMR spectrum of the [La(AAT)$_2$Cl$_3$]

Antimicrobial Assay

Many azo ligands and their complexes show antimicrobial activity [28]. The prepared ligand (AAT) and it's Ln-complexes (1mg/ml) were tested invitro against the following microorganisms: *E.coli* and *Staph aureus* as Bactria species and two fungal species, *Candida albicans* and *Aspergillus niger*. The results are given in Table-5 showed that all prepared complexes exhibit high antimicrobial activities than the ligand (AAT).The higher activity of the complexes may be attributed to the different properties of metal ions upon chelation, in which the metal ion adsorbed on the cell wall of the microorganisms with blocking the portein synthesis that is an important for further growth of the microorganism. Therefore metal ions are essential for the growth-inhibitory influence [29]. Basis on the tweedy's chelation theory and overtone's concept of cell permeability, the lipid membrane favors the passage of only lipid-soluble substance, so lipophilicity is essential factor controlling the antibacterial and antifungal activity. The chelation process reduces the polarity of the metal atom mainly due to partial partnership of the positive charge of metal ion with donor groups consequently this will enhance the delocalization of-π-electrons over the entire ring and supports the lipophilicity of the complexes [25]. Furthermore the metal ions discourages of one or more cellular proteins, causing the normal cellular processes to be single [30].

Table 5- Influence of azo ligand (AAT) and it's Ln-complexes on the growth of tested bacteria and fungal at(1mg/ ml)

Compound	Bacteria		Fungal	
	E. coli **gram (-)**	***Staphloccus aureus*** **gram (+)**	***Candida albicans*** **yeast**	***Aspergillus niger*** **Filamentous**
Ligand (AAT)	+	+	+	+
$[La(AAT)_2Cl_3]$	++	+	++	+++
$[Ce(AAT)_2Cl_3]$	++	++	+++	++
$[Nd(AAT)_2Cl_3]$	+++	++	+++	++
$[Eu(AAT)_2Cl_3]$	++	+++	++	+++
$[Gd(AAT)_2Cl_3]$	++	+	++	+++

Slightly active = + (inhibition zone 6-9 mm)
Moderately active = ++ (inhibition zone 9-12 mm)
Highly active = +++ (inhibition zone >12 mm)

Conclusion:

The neutral N,O-bidentate ligand (AAT) was obtained by the coupling of diazotized 4-aminoantipyrine with L-Tryptophan. This ligand was found to be bonded with lanthanide through carbonyl oxygen and azoimine nitrogen, which formed series of Ln(III)-complexes with (1:2) (metal:ligand) stoichiometry at optimum pH, yielded a series of neutral complexes of the general formula $[M(L)_2Cl_3]$ to give a pentagonal bipyramidal for all prepared complexes. The characteristic of sample have been performed by different physicochemical techniques, UV-Vis, FTIR and ^{1}HNMR spectra. Finally all the prepared compounds were appeared a good biological activity against the studied bacteria and fungi.

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