Research Article

Synthesis, characterization and biological activity of mixed ligand Co(II) complexes of schiff base 2-amino-4-nitrophenol-n-salicylidene with some amino acids

Ajay R. Patil[1], Kamini J. Donde*[2], Sambhaji S. Raut[1], Vishwanath R. Patil[1] and Rama S. Lokhande[1]

[1]*Department of Chemistry, University of Mumbai, Santacruz (E), Mumbai, India*
[2]*Department of Chemistry, Ramnarain Ruia College, Matunga (E), Mumbai, India*

ABSTRACT

Co(II) complexes of Schiff base 2-amino-4-nitrophenol-N-salicylidene with some amino acids were synthesized. The Schiff base and its mixed ligand complexes, in general, were non-hygroscopic and stable solids. The structural characterization of Schiff base and cobalt complexes were done on the basis of their melting point, solubility, elemental analyses, conductivity measurements, GC-MS, FTIR, NMR, UV-Visible spectroscopy, magnetic susceptibility, and X-ray diffraction studies. The morphology of mixed ligand Co(II) complexes was studied by scanning electron microscopy. The compounds were subjected to simultaneous thermogravimetric analysis to study their decomposition mechanism and thermal stability. The Schiff base and mixed ligand complexes were preliminary scanned against various strains of microbes to study their biological effect.

Keywords: Synthesized, Non-hygroscopic, Microscopy, Thermogravimetric analysis, Biological effect.

INTRODUCTION

Schiff bases are a significant class of ligand in coordination chemistry and find extensive purpose in different field [1]. Schiff bases derived from pyridoxal and amino acids are considered very important ligands from the biological point of view. Transition metal complexes of such ligands are important enzyme models. The rapid development of these ligands resulted in an enhanced research activity in the field of coordination chemistry leading to very interesting conclusions [2, 3]. Mixed ligand complexes have been found to act as an active catalyst in reactions of industrial importance including hydrogenation, hydroformation, and oxidative hydrolysis of olefins and carboxylation of methanol. These complexes have also shown catalytic activity in various oxidation reactions of environmental and biological importance [4-9]. Mixed ligand complexes containing amino acids as a secondary ligand are of significance as they are potential models for enzyme-metal ion substrate complexes [7-10].

The lot of attention is being given to the study of mixed ligand complexes of Schiff bases containing salicylaldehyde and amino acids separately. Main aim of this study was to establish research topics where these two areas could be combined in a novel way. To fulfill this Schiff base 2-amino-4-nitrophenol-N-salicylidene was selected as a starting point and the mixed ligand Co(II) complexes were prepared by introducing the various amino acids (viz. alanine, phenylalanine, valine, cysteine, leucine and glutamic acid) with Co(II) ions.

EXPERIMENTAL SECTION

Chemical

Water used in present investigation was redistilled in a glass distillation assembly. The glass distilled water was having pH of about 6.98 to 7.00. A.R. grade hydrochloric acid used was purchased from s. d. fine Chemical Ltd, Mumbai. All solvents used for synthesis and characterization experiments were high performance liquid chromatography grade and analytical reagent. Solvents such as ethanol, dimethylsulfoxide (DMSO) were purchased from Merck India Ltd. and s. d. fine Chemical Ltd, Mumbai and were used without purification.

Metal salt cobalt sulphate ($CoSO_4.H_2O$) was purchased from s. d. fine Chemical Ltd, Mumbai and used without purification. All Amino acids were purchased from Sigma-Aldrich Ltd and used without further purification. 2-amino-4-nitrophenol of AR grade was purchased from M/S Fluka Chemicals and used without purification. Nutrient broth, nutrient agar, Sabouraud's broth and streptomycin, bacterial and fungal subcultures were obtained Himedia India Ltd.

All glassware used were made of Pyrex or corning glass. The burettes, pipettes, and standard flasks were calibrated by the method described by Vogel [11]. An analytical balance of 100µg sensitivity was used for weighing samples.

Instrumentation

For the determination of metal content, a known amount of metal complex (about 300-500 mg) in 250 ml beaker was decomposed by using the acid mixture (2 ml conc. HCl, 2 ml Conc. HNO_3, 2-3 drops of $HClO_4$). The solution was evaporated almost to dryness repeatedly till the decomposition was complete. The residue was treated with conc. HCl and the solution was evaporated nearly to dryness. It was extracted with dilute HCl and diluted to 100 ml with distilled water. The metal content was estimated by Atomic Absorption Spectrophotometry (AAS) [11]. AAS studies were done on PerkinElmer Analyst 200, Atomic Absorption Spectrophotometer. The elemental microanalyses of C, H, and N were carried out with a Thomas and Coleman Analyser-Carlo Erba 1106. A digital conductometer, model ELICO LI-120 was used for conductance measurement and the measurements were made at the range of 10^{-3} M concentration at room temperature using DMSO as a solvent. Melting points were uncorrected and determined by capillary tube method. A digital pH meter, model ELICO LI-120 was used for pH measurement. Room temperature magnetic susceptibility measurements for all the mixed ligand Co(II) complexes reported in the present study were made by the Guoy's method using $Hg[Co(SCN)_4$ as calibrant. Effective magnetic moments were calculated after applying diamagnetic corrections for the ligand components using Pascal's constants [12]. UV-Vis Spectra in DMSO were measured on Shimadzu UV-2100 spectrophotometer. FTIR spectra were recorded on a Perkin-Elmer 1600 series FTIR spectrophotometer as KBr discs. ^{1}H NMR spectra were recorded on a Bruker AMX-300 spectrometer in $CDCl_3$/DMSO and chemical shifts were reported in δ ppm values relative to the internal standard tetramethyl silane (TMS). The mixed ligand complexes of ANPS with various amino acids were characterized for its crystalline behaviour by X-ray diffraction technique. The experiment was carried out using Rich-Seifert (Model ISO-Debyeflex 2000) diffractometer. The XRD pattern was obtained using CuKα radiation that was monochromatised with a curved Graphite crystal monochromator and nickel filter. Samples were scanned at a scanning speed of 3°/min in the 2θ range 0° to 80°.The crystallite size has been calculated from its relationship with peak width at half maximum. Scanning Electron Microscopy (JEOL 6320FV Field-Emission High Resolution SEM) of the films on Si substrates determined the confirmation of successful pattern transfer. Electron micrographs were obtained on Cameca (France) model SU-30 Scanning Electron Microscope. Thermolysis of compounds was performed with Perkin-Elmer / Pyris Diamond to obtain TG-DTA assembly curves. The crystallite size of complexes was evaluated with X-ray powder diffraction. Diffraction patterns were obtained using an XPERT-PRO diffractometer (PAnalytical) with a radius of 240 mm. Molar mass of ANPS was determined by Shimadzu GC-MS / QP-2010.

Synthesis of the Co(II) complexes

The 2-amino-4-nitrophenol-N-salicylidene [ANPS] was synthesized as per procedure reported [8, 13, 14]. Solution of amino acids (viz. alanine, phenylalanine, valine, cysteine, leucine and glutamic acid) (0.5 gm) prepared in 10 ml 0.1M HCl were mixed with aqueous solution of Cobalt sulphate ($CoSO_4.7H_2O$) (0.5g, 2.0 mmol) and warmed ethanolic solution of Schiff base (2.0 mmol). The mixture was stirred vigorously. The pH of the reaction mass was adjusted to 10.0-11.0 by using 0.1M NaOH solution. The dark brown coloured precipitation occurred. The precipitate was then digested on water bath for 3h. The precipitate obtained was then cooled, filtered and washed with warm water followed by 50 % ethanol and dried in vacuo at 70-80°C.

_____

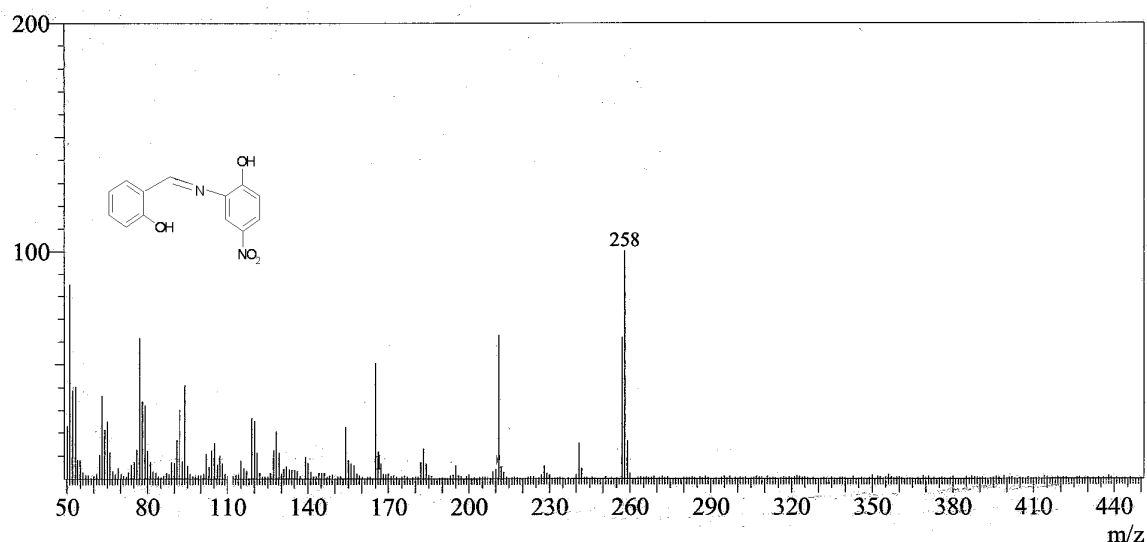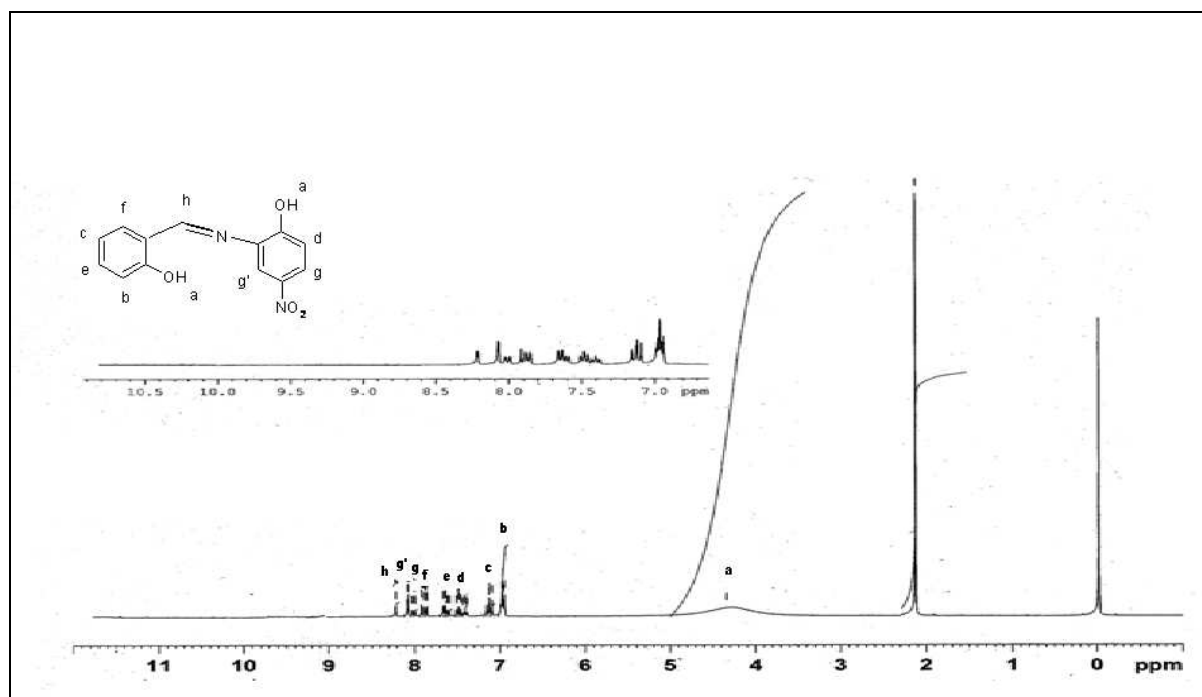**Figure 1: Mass Spectrum of ANPS**

Figure 2: NMR Spectrum of ANPS

RESULTS AND DISCUSSION

Characterization of Metal Complexes

The elemental analyses data indicate that the mixed Co(II) ligand complexes of ANPS with amino acid may be in 1:1:1 stoichiometry. The synthesis of the mixed-ligand Co(II) complexes of ANPS may represented by the general formula L_1ML_2

$$M + L_1 + L_2 \rightleftharpoons L_1ML_2 \qquad \text{----------------------- (I)}$$

In the process of complexation, ANPS form an anionic species by deprotonation of –OH functional group and then form metal complex. Similarly the amino acid also form anionic species by deprotonation of -NH_2 functional group and combine with metal ion by formation of covalent bond. The complex formation was further supported by the insolubility of the complexes in water and dilutes alkali solution.

The ANPS and its mixed Co(II) ligand complexes, in general, were non-hygroscopic and stable solids. The ANPS was easily soluble in many organic solvents, where as the complexes were having limited solubility in organic solvents. The complexes were dark brown in colour and thermally stable (Table 1), indicating a strong metal-ligand bond. The elemental analyses data (Table 1) of the metal complexes were consistent with their general formulation as shown in equation (I).
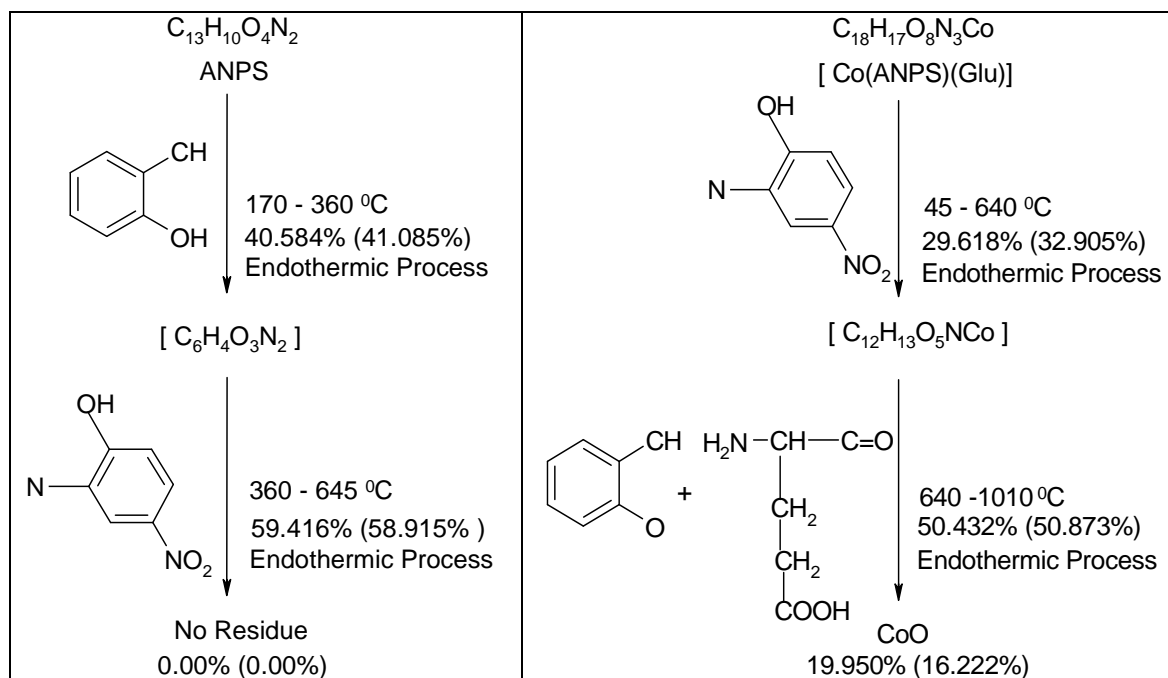

Figure 3: Proposed thermal decomposition patterns of ANPS and [Co(ANPS)(Glu)]
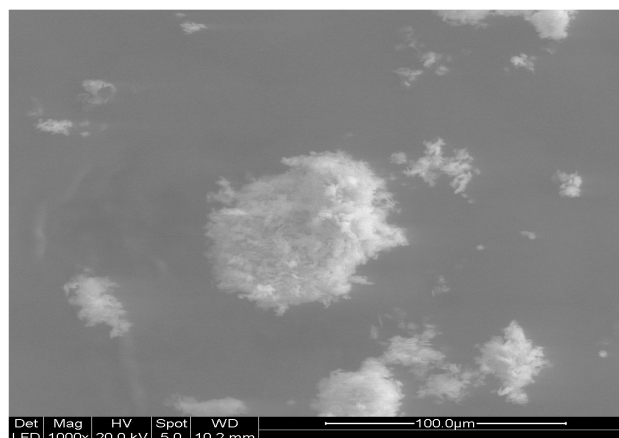

Figure 4: SEM Image of ANPS

The metal content of all the Co(II) complexes was estimated by the AAS methods reported in the literature [11,15]. The low molar conductivity values of the complexes in DMSO at 10^{-3}M concentration in the range 0.062-0.075 Ohm^{-1} cm^2 mol^{-1} indicate their non-electrolytic nature [16]. The weight obtained from the mass spectra was 258, which was in good agreement with the value obtained from its molecular formula. The molecular weights of the Co(II) complexes were calculated by using the molecular weight of the ANPS, amino acid and the structural formula of the complexes suggested by the geometry discussed later in FTIR, UV-Visible, Magnetic Susceptibility, TG-DTA studies. The mass spectrum of ANPS is shown in Figure 1.

Infrared spectra
The FTIR spectra of compounds were quite complex due to presence of numerous bands with varying intensities,

making the task quite difficult. Attempt however had been made to assign some of important bands on the basis of reported spectra of several mixed ligand complexes [8, 14, 17]. The significant feature of the infrared spectra of ANPS was presence of band at 1642.07 cm^{-1} which was assign to frequency of C=N group [18]. This suggested the formation of ANPS by reaction of salicylaldehyde and 2- amino-4-nitrophenol. The broad band observed at 3555.65 cm^{-1}in the spectrum of ANPS was assigned for stretching vibrations of –OH group. The presence of phenolic –OH was also confirmed by another band in the FTIR spectrum at 1377.49 cm^{-1}. The band observed at 1599.30 cm^{-1} was assigned for stretching vibrations of -NO$_2$ functional group in the ligand [17, 18].

[Co(ANPS)(Ala)]   **[Co(ANPS)(Phe)]**

[Co(ANPS)(Val)]   **[Co(ANPS)(Cys)]**

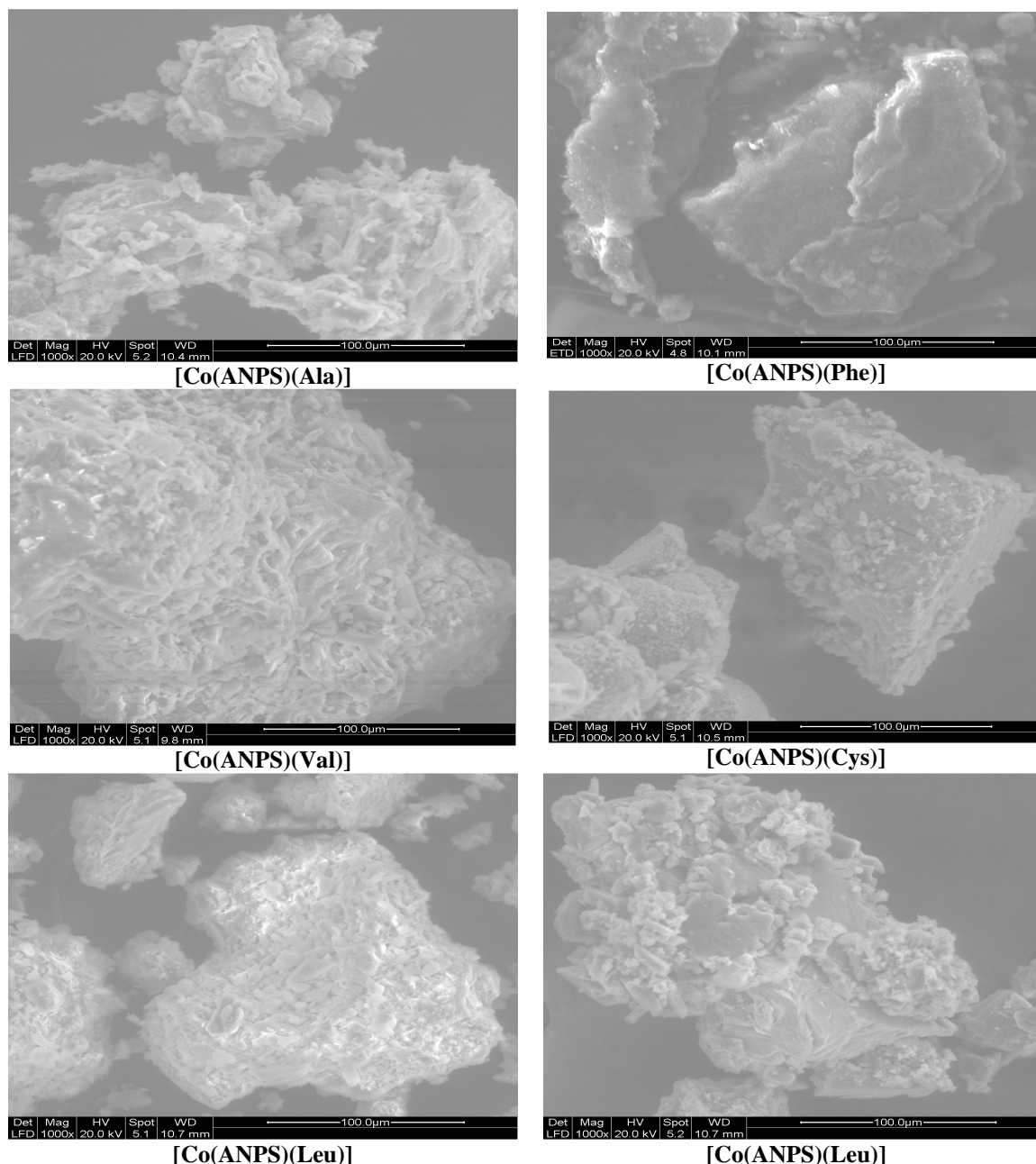**[Co(ANPS)(Leu)]**   **[Co(ANPS)(Leu)]**
Figure 5: SEM Images of Co(II) complexes.

The FTIR spectrum of mixed ligand Co(II) complexes showed the broad and moderate band centered in the range 3428.10-3437.15 cm^{-1} due to –OH vibrations of the phenolic –OH group. These vibrations are reported to occur around 3500 cm^{-1} for the compounds containing free –OH functional group [18,19]. The appearance of –OH vibrations in Co(II) complexes were slightly lower than the vibrations of –OH in the spectrum of ANPS. This may be due to involvement of one of the –OH groups in the metal ligand bonding. The presence of one free phenolic –OH group was confirmed by another band observed in the range 1384.96-1397.31 cm^{-1}. This indicated that the ANPS form covalent bond with metal ion by deprotonation of one –OH group.
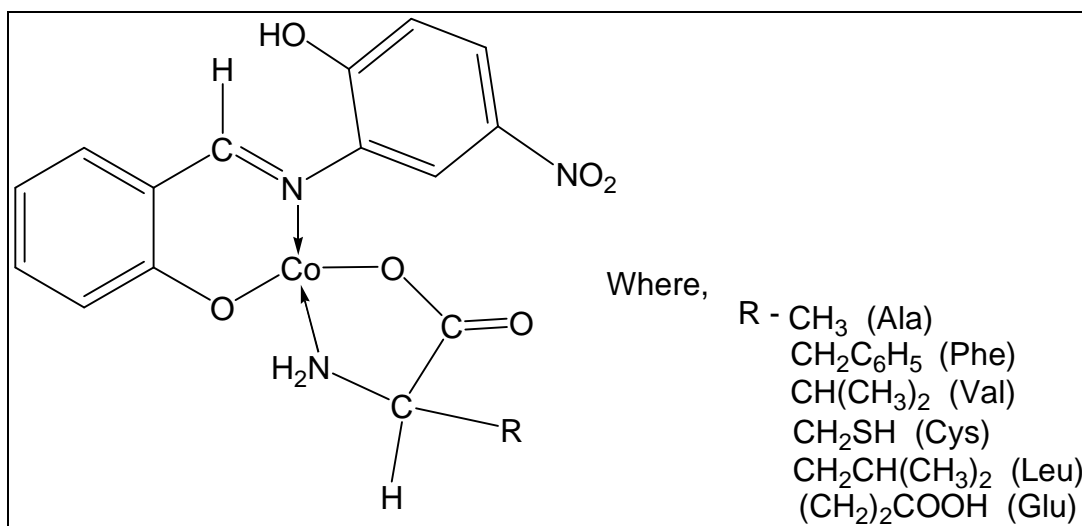
Figure 6: Proposed general structure of mixed ligand Co(II) complexes

The other two bands observed in the range 3069.05-3080.95 cm^{-1} and 3015.02-3069.95 cm^{-1} were assigned for –NH asymmetric and –NH symmetric stretching vibrations of –NH$_2$ group. For free amino acid these values are reported around 3040 cm^{-1} and around 2960 cm^{-1} respectively. These values were shifted to higher wave numbers in the spectra of Co(II) complexes, suggesting coordination of the amino group through nitrogen with the Co(II) ion [14, 17,19].

Literature survey reveals that an asymmetric vibrations and symmetric vibrations due to free amino acid are observed at around 1590 cm^{-1} and 1400 cm^{-1} respectively. These values were shifted to higher wave numbers in the spectra of cobalt complexes i.e. $\upsilon_{asym(COO-)}$ in the range 1732.85-1756.89 cm^{-1} and $\upsilon_{sym}(COO^-)$ in the range 1701.05-1740.25 cm^{-1} indicating the covalent bond formation via deprotonation of the carboxylic acid group during the complexation [19].

The strong band observed in the range 1611.03-1617.47cm^{-1} was assigned for C=N vibrations. These vibrations were observed in the lower wave number as compared to the vibrations in the ANPS. This indicated the coordinate bond formations through the nitrogen atom of C=N group [17,19]. The band due to –NO$_2$ was observed at 1599.30 cm^{-1} in the spectrum of ANPS remain unchanged in the FTIR spectra of mixed Co(II) ligand complexes of Co(II) suggesting non involvment of –NO$_2$ group in the bonding. This band was obtained in the range 1574.74 – 1589.90 cm^{-1} in the IR spectra of Co(II) complexes [17, 19].

The [Co(ANPS)(Cys)] complex show small weak band at 2569.80 cm^{-1} which is assigned for stretching frequency of -SH group in the cystein. The literature value for the -SH vibrations is in the range of 2550-2590 cm^{-1}. Therefore in the [Co(ANPS)(Cys)] complex the band observed in this range was assigned for stretching frequency of -SH group

In FTIR spectra of Co(II) complexes, some new bands of weak intensity observed in the regions around 645.80-649.01 cm^{-1} and 515.18-516.32 cm^{-1} may be ascribed to the M-O and M-N vibrations respectively. It may be noted that these vibrational bands are absent in the infrared spectra of ANPS as well as the amino acids [14, 19, 20]. Some of the important IR bands and their tentative assignments are shown in Table 2

_____

Table 1: Physicochemical studies of compounds

Compound	Empirical Formula (formula wt.)	Yield %	Colour	pH	Mol. Cond. (Ohm^{-1} cm^2 mol^{-1})	M.P (°C)	Elemental Analyses Found (Calc.) %(calculated)						B. M. (μ$_{eff}$)
							C	H	N	S	O	Co	
ANPS	C$_{13}$H$_{10}$O$_4$N$_2$ (258.00)	94	Yellow	1.53	0.071	240	60.10 (60.46)	3.98 (3.87)	10.25 (10.85)	-	25.67 (24.82)	-	-
[Co(ANPS)(Ala)]	C$_{16}$H$_{15}$O$_6$N$_3$Co (403.93)	92	Dark brown	10.56	0.066	215*	47.40 (47.53)	3.98 (3.71)	10.45 (10.40)	-	23.57 (23.77)	14.60 (14.59)	2.22
[Co(ANPS)(Phe)]	C$_{22}$H$_{19}$O$_6$N$_3$Co (479.33)	94	Dark brown	10.62	0.069	250*	55.10 (55.01)	3.90 (3.96)	8.78 (8.75)	-	19.92 (20.00)	12.30 (12.28)	2.30
[Co(ANPS)(Val)]	C$_{18}$H$_{19}$O$_6$N$_3$Co (431.93)	92	Dark brown	10.57	0.062	230	49.78 (50.01)	4.65 (4.40)	9.87 (9.72)	-	22.20 (22.23)	13.50 (13.64)	2.23
[Co(ANPS)(Cys)]	C$_{16}$H$_{15}$O$_6$N$_3$SCo (436.00)	89	Dark brown	10.70	0.075	205	43.85 (44.04)	3.70 (3.44)	9.69 (9.63)	7.45 (7.35)	21.79 (22.02)	13.52 (13.51)	2.25
[Co(ANPS)(Leu)]	C$_{19}$H$_{21}$O$_6$N$_3$Co (445.93)	93	Dark brown	10.60	0.070	215	50.99 (51.13)	4.96 (4.71)	9.47 (9.42)	-	21.33 (21.53)	13.25 (13.21)	2.31
[Co(ANPS)(Glu)]	C$_{18}$H$_{17}$O$_8$N$_3$Co (461.93)	91	Dark brown	10.61	0.065	265*	46.60 (46.76)	3.95 (3.68)	9.10 (9.09)	-	27.68 (27.71)	12.67 (12.76)	2.27

* = Decomposition Temperature, Ala, Phe, Val, Cys, Leu and Glu represent secondary ligands alanine, phenylalanine, valine, cysteine,leucine and glutamic acid, respectively.

Table 2: FTIR absorption data

Compounds	ν$_{OH}$	ν$_{N-H}$ (Asymm) Amino acid	ν$_{N-H}$ (Symm) Amino Acid	ν$_{Coo^-}$ (Asymm) Amino acid	ν$_{Coo^-}$ (Symm) Amino acid	ν$_{C=N}$ (Symm) Schiff Base	ν$_{OH}$ (Phenolic-OH)	ν$_{NO}$ (Stretch)	ν$_{SH}$ (Stretch)	ν$_{M-O}$	ν$_{M-N}$
ANPS	3555.65(b)	-	-	-	-	1642.07(b))	1377.49(s)	1599.30(s)	-	-	-
[Co(ANPS)(Ala)]	3431.93(b)	3080.95(w)	3069.95(w)	1733.15(w)	1712.69(w)	1611.47(s)	1388.85(s)	1589.90(s)	-	648.80(w)	516.20(w)
[Co(ANPS)(Phe)]	3435.49(b)	3069.85(w)	3041.25(w)	1732.85(w)	1701.05(w)	1613.96(s)	1384.96(s)	1580.25(s)	-	648.50(w)	515.90(w)
[Co(ANPS)(Val)]	3428.10(b)	3079.50(w)	3025.89(w)	1752.10(w)	1740.25(w)	1617.47(s)	1390.85(s)	1576.52(s)	-	649.01(w)	516.30(w)
[Co(ANPS)(Cys)]	3437.15(b)	3070.58(w)	3045.20(w)	1756.89(w)	1736.56(w)	1614.89(s)	1392.50(s)	1578.42(s)	2569.80(w)	645.80(w)	515.80(w)
[Co(ANPS)(Leu)]	3432.02(b)	3069.05(w)	3015.02(w)	1748.50(w)	1706.65(w)	1615.10(s)	1397.31(s)	1574.74(s)	-	649.01(w)	515.18(w)
[Co(ANPS)(Glu)]	3432.50(b)	3070.30(w)	3035.10(w)	1750.25(w)	1731.02(w)	1611.03(s)	1391.15(s)	1577.17(s)	-	648.50(w)	516.32(w)

b-broad, s-strong, w-weak

Table 3: UV-visible spectral data of Compounds

Compound	Solution spectra		Assignments
	ν (λ) nm (Cm^{-1})	OD	
ANPS	210 (47,619)	0.758	$\pi \rightarrow \pi^*$
	255 (39,215)	0.393	$\pi \rightarrow \pi^*$
	324 (30,864)	0.215	$n \rightarrow \pi^*$
[Co(ANPS)(Ala)]	212 (47,169)	0.640	$\pi \rightarrow \pi^*$
	257 (38,910)	0.373	$\pi \rightarrow \pi^*$
	325 (30,769)	0.240	$n \rightarrow \pi^*$
	370 (27,027)	0.265	C.T $^2Ag \rightarrow ^3B_3u$ [yz$\rightarrow$L(π^*)]
	402 (24,875)	0.302	C.T $^2Ag \rightarrow ^2B_2u$ [xz$\rightarrow$L(π^*)]
	501 (19,960)	0.298	$^2Ag \rightarrow ^2B_3g$ (xz$\rightarrow$xy)
	660 (15,151)	0.197	$^2Ag \rightarrow ^2B_1g$ (x^2-y$^2 \rightarrow$xy)
[Co(ANPS)(Phe)]	211 (47,393)	0.600	$\pi \rightarrow \pi^*$
	256 (39,062)	0.370	$\pi \rightarrow \pi^*$
	324 (30,864)	0.250	$n \rightarrow \pi^*$
	371 (26,954)	0.270	C.T $^2Ag \rightarrow ^3B_3u$ [yz$\rightarrow$L(π^*)]
	402 (24,875)	0.315	C.T $^2Ag \rightarrow ^2B_2u$ [xz$\rightarrow$L(π^*)]
	500 (20,000)	0.300	$^2Ag \rightarrow ^2B_3g$ (xz$\rightarrow$xy]
	657 (15,221)	0.205	$^2Ag \rightarrow ^2B_1g$ (x^2-y$^2 \rightarrow$xy)
[Co(ANPS)(Val)]	212 (47,169)	0.635	$\pi \rightarrow \pi^*$
	255 (39,215)	0.372	$\pi \rightarrow \pi^*$
	325 (30,769)	0.253	$n \rightarrow \pi^*$
	370 (27,027)	0.284	C.T $^2Ag \rightarrow ^3B_3u$ [yz$\rightarrow$L(π^*)]
	401 (24,937)	0.365	C.T $^2Ag \rightarrow ^2B_2u$ [xz$\rightarrow$L(π^*)]
	500 (20,000)	0.323	$^2Ag \rightarrow ^2B_3g$ (xz$\rightarrow$xy)
	660 (15,151)	0.198	$^2Ag \rightarrow ^2B_1g$ (x^2-y$^2 \rightarrow$xy)
[Co(ANPS)(Cys)]	210 (47,619)	0.650	$\pi \rightarrow \pi^*$
	257 (38,910)	0.375	$\pi \rightarrow \pi^*$
	325 (30,769)	0.241	$n \rightarrow \pi^*$
	370 (27,027)	0.265	C.T $^2Ag \rightarrow ^3B_3u$ [yz$\rightarrow$L(π^*)]
	402 (24,875)	0.315	C.T $^2Ag \rightarrow ^2B_2u$ [xz$\rightarrow$L(π^*)]
	501 (19,960)	0.310	$^2Ag \rightarrow ^2B_3g$ [xz$\rightarrow$xy]
	664 (15,060)	0.205	$^2Ag \rightarrow ^2B_1g$ [x^2-y$^2 \rightarrow$xy]
[Co(ANPS)(Leu)]	211 (47,393)	0.650	$\pi \rightarrow \pi^*$
	255 (39,215)	0.375	$\pi \rightarrow \pi^*$
	326 (30,674)	0.254	$n \rightarrow \pi^*$
	372 (26,881)	0.301	C.T $^2Ag \rightarrow ^3B_3u$ [yz$\rightarrow$L(π^*)]
	400 (25,000)	0.384	C.T $^2Ag \rightarrow ^2B_2u$ [xz$\rightarrow$L(π^*)]
	498 (20,080)	0.310	$^2Ag \rightarrow ^2B_3g$ [xz$\rightarrow$xy]
	660 (15,152)	0.204	$^2Ag \rightarrow ^2B_1g$ [x^2-y$^2 \rightarrow$xy]
[Co(ANPS)(Glu)]	210 (47,619)	0.601	$\pi \rightarrow \pi^*$
	257 (38,910)	0.380	$\pi \rightarrow \pi^*$
	325 (30,769)	0.238	$n \rightarrow \pi^*$
	370 (27,027)	0.264	C.T $^2Ag \rightarrow ^3B_3u$ [yz$\rightarrow$L(π^*)]
	401 (24,937)	0.330	C.T $^2Ag \rightarrow ^2B_2u$ [xz$\rightarrow$L(π^*)]
	500 (20,000)	0.317	$^2Ag \rightarrow ^2B_3g$ [xz$\rightarrow$xy]
	667 (14,992)	0.210	$^2Ag \rightarrow ^2B_1g$ [x^2-y$^2 \rightarrow$xy]

C.T - Charge transfer band

^{1}H NMR spectra

The NMR signals were obtained in the range δ 4.336-8.450 ppm Figure 2 [14, 17, 21]. The Singlet obtained at δ 4.336 ppm was due to two protons of two –OH groups in ANPS [17]. Due to the effect of similar chemical environment around these –OH groups, only one signal was observed for these groups. The signal obtained in range δ 6.951-6.962 ppm was assigned for doublet due one proton of aromatic ring of aldehyde group. The signal obtained in range δ 7.124-7.399 ppm was assigned for triplet due one proton of aromatic ring of aldehyde group. The signal obtained in range δ 7.502-7.508 ppm was assigned for doublet due one proton of aromatic ring of 2-amino-4-nitrophenol in the ligand. The signal obtained in range δ 7.635-7.663 ppm was assigned for triplet due one proton of aromatic ring of aldehyde group in the ligand. The signal obtained in range δ 7.857-7.878 ppm was assigned for doublet due one proton of aromatic ring of aldehyde group in the ligand.

Table 4: Thermal analysis data of the Compounds

Compound/ M.F/ Mol.Wt.	Dissociation stages	DTA peak temp. °C	Temp. range °C	Weight loss found (Calcd) %	Decomposition assignment	Residue remained
ANPS/ $C_{13}H_{10}N_2O_4$/258	Stage I Stage II	265 620	170-360 360-645	40.58 (41.09) 59.42 (58.92)	$-C_7H_6N_2O$ $-C_6H_4O_3N_2$	$[C_6H_4O_3N_2]$ No residue
[Co(ANPS)(Ala)] $C_{16}H_{15}N_3O_6Co$/403.93	Stage I Stage II Stage III	315 600 1000	30-420 420-780 780-1040	23.41 (22.03) 37.37 (37.63) 20.09 (21.79)	$-C_7H_5$ $-C_6H_4N_2O_3$ $- C_3H_6NO_2$	$[C_9H_{10}N_3O_6Co]$ $[C_3H_6NO_3Co]$ CoO
[Co(ANPS)(Phe)] $C_{22}H_{19}N_3O_6Co$/479.33	Stage I Stage II Stage III	375 580 925	235-550 550-750 750-1050	27.11 (28.75) 23.90 (21.88) 29.59 (30.84)	$-C_6H_4NO_3$ $-C_7H_5NO$ $-C_9H_{10}NO$	$[C_{16}H_{15}N_2O_3Co]$ $[C_9H_{10}NO_2Co]$ CoO
Co(ANPS)(Val)] $C_{18}H_{19}N_3O_6Co$/431.93	Stage I Stage II Stage III	360 720 925	60-650 650-820 820-1040	38.24 (38.20) 14.11 (17.59) 24.49 (26.86)	$-C_7H_5N_2O_3$ $-C_6H_4$ $-C_5H_{10}NO_2$	$[C_{11}H_{14}NO_3Co]$ $[C_5H_{10}NO_3Co]$ CoO
[Co(ANPS)(Cys)] $C_{16}H_{15}N_3O_6SCo$/ 436.00	Stage I Stage II	510 720	30-615 615-1030	32.02 (34.86) 48.04 (47.95)	$-C_6H_4N_2O_3$ $-C_{10}H_{11}NO_2S$	$[C_{10}H_{11}NO_3SCo]$ CoO
[Co(ANPS)(Leu)] $C_{19}H_{21}N_3O_6Co$/ 445.93	Stage I Stage II Stage III Stage IV	350 540 725 905	30-420 420-625 625-805 805-1010	18.76 (19.96) 14.94 (12.78) 16.64 (16.37) 32.99 (34.09)	$-C_7H_5$ $-C_4H_9$ $-C_2H_3NO_2$ $-C_6H_4N_2O_3$	$[C_{12}H_{16}N_3O_6Co]$ $[C_8H_7N_3O_6Co]$ $[C_6H_4N_2O_4Co]$ CoO
[Co(ANPS)(Glu)] $C_{18}H_{17}N_3O_8Co$/ 461.93	Stage I	345	45-640	29.62 (32.90)	$-C_6H_4N_2O_3$ $- C_{12}H_{13}NO_4$	$[C_{12}H_{13}NO_5Co]$ CoO

Table 5: Crystallite size of the Compounds

Compound	2θ of max. int. peak (Degrees)	θ Degrees	Cos θ	β	Crystallite size in (Å) $t = \dfrac{0.9\lambda}{\beta\cos\theta}$
ANPS	15.14	5.57	0.991	0.260	5.38
[Co(ANPS)(Ala)]	77.47	38.74	0.780	0.192	9.25
[Co(ANPS)(Phe)]	30.60	15.80	0.962	0.137	10.51
[Co(ANPS)(Val)]	34.07	17.04	0.956	0.040	36.44
[Co(ANPS)(Cys)]	32.28	16.14	0.961	0.040	36.27
[Co(ANPS)(Leu)]	32.06	16.03	0.961	0.168	08.57
[Co(ANPS)(Glu)]	31.90	15.95	0.961	0.101	14.22

Table 6: Results of antimicrobial activities in ppm

Compound	Antibacterial activity			Antifungal activity	
	a	b	c	d	e
ANPS	100	100	200	100	200
[Co(ANPS)(Ala)]	50	50	100	50	50
[Co(ANPS)(Phe)]	12.5	12.5	50	25	25
[Co(ANPS)(Val)]	25	12.5	100	100	50
[Co(ANPS)(Cys)]	25	25	100	100	100
[Co(ANPS)(Leu)]	25	50	50	100	100
[Co(ANPS)(Glu)]	100	25	50	100	100
Standard	25	12.5	12.5	25	12.5

a. S. typhi, b. S.aureus, c. A. Niger, d. C.Albican, e. S. cerevisiae,
Standard: Ampiciline for anti bacterial activities, Streptomycin for antifungal activities.

The signal obtained in range δ 8.016-8.025 ppm was assigned for doublet due one proton of aromatic ring of 2-amino-4-nitrophenol in the ligand whereas another signal obtained at δ 8.068 ppm was assigned for one proton of aromatic ring of 2-amino-4-nitrophenol in the ligand. It was expected that these two signals would observed at same position but due to the effect of electro negativity of neighboring two nitrogen atoms later was observed at higher δ value than previous signals. The signal obtained at δ 8.450 ppm was assigned for singlet due one proton of -CH=N linkage in the ligand. This confirms the formations of imine ligand. This observation was also supported by the FTIR data of the ligand discussed earlier. The NMR spectral data of ANPS was compared with the spectral data for the similar ligands reported in literatures [22, 23]. Due to the limited solubility in the solvent CDCl₃ and DMSO the NMR spectra of mixed ligand Co(II) complexes lead to noisy base line, a broadening of the signals and did not yield ¹H NMR spectra to satisfactory quality.

Magnetic susceptibility

Room temperature magnetic susceptibility measurements indicate that the mixed ligand Co(II) complexes were paramagnetic in nature. The dark brown Co(II) complexes exhibit a paramagnetic moment in the range 2.12-2.31 B.M. as given in Table 1. Kalia et.al has found similar range of magnetic moment values for Co(II) complexes dithiocarbazate derived from isonazid [24]. According to Kalia et al. these values lie in the range corresponding to one unpaired electron for square planar stereochemistry around Co(II), d^7 complexes. Literature survey also reveals that the Co(II) complexes showing the magnetic moment value in the above mentioned range suggested the square planar stereochemistry around Co(II), d^7 complexes [25-27]. Therefore the square planar stereochemistry for mixed ligand Co(II) complexes of ANPS with amino acids was proposed.

Electronic Absorption Spectra

The electronic absorption spectrum of ANPS in DMSO solvent in the showed three peaks. The high intensity band was observed at 210 nm (47,619 cm^{-1}). The second of low intensity band was observed at 255 nm (39,215 cm^{-1}) and the third band was observed at 324 nm (30,864 cm^{-1}). The data obtained was compared with the literature data of several Schiff bases and their mixed ligand complexes [8]. The electronic absorption for ANPS in the DMSO may occur due to the transitions of the electrons from lower molecular orbital to higher molecular orbital. From the nature and position of the transition in the spectra, it was assigned for intra-ligand transitions.

The Shivankar et al has recorded the π→π* at 48,543-38,760 cm^{-1}, for the aromatic chromophore in the ligand 8-hydroxyquinoline. In addition, the band observed in the UV-Visible spectrum of 8-hydroxyquinoline in the range 29,762-29,717 cm^{-1} was reported for n→π* transition [19]. For the present study, the frequency of first two bands was in range of 210-255 nm (47,619-39,215 cm^{-1}). Hence by comparing with the literature values, these transitions observed in the UV spectrum of ANPS were assigned for π→π* transition for the aromatic chromophore in the ligand ANPS [8, 19, 21-23]. Similarly the third band observed at 324 nm (30,864 cm^{-1}) was assigned for n→ π* transition of C=N group in the ligand [19].

In the metal complexes, the inner ligand transitions were common due to presence of C=N and C=C groups in the ligand structures. These bands remain as it is, but new band in the range 370-377 nm (27,027-26,525 cm^{-1}) was observed, supporting the formation of strong metal–ligand bonds. This band is reported for the charge transfer band [22]. In the solution, electronic absorption spectra of Co(II) complexes, the seven signals were observed. Out of which the three signals were indicative of intra ligand transitions of ANPS, while other three signals were indicative of D_{2h} symmetry for the Co(II) d^7 square planar complexes. A well defined strong band occurring in the range 657-667 nm (15,221-14,992 cm^{-1}) was assigned for the transitions $^2Ag→^2B_{1g}$ ($x^2 –y^2→$ xy) and the another band observed in the range 498-501 nm (20,080-19,960 cm^{-1}) was assigned for $^2Ag→^2B_{3g}$, (xz→ xy). Another band around 400 nm (25,000 cm^{-1}) and a shoulder around 370 nm (27,000 cm^{-1}) were assigned for metal to ligand charge transfer bands. These bands were assigned to the $^2Ag→^2B_{2u}$ [xz→ L(π*)] and $^2Ag→^3B_{3u}$ [yz→ L(π*)] transitions respectively. In general, Co(II) in square planer geometry absorb in the regions 25,000 cm^{-1}, 20,000 cm^{-1}, 15,000-14,000 cm^{-1} [24]. The spectral data is shown in Table 3.

Thermal studies

The thermograms (TG) of the compounds have been recorded in flowing nitrogen atmosphere at the heating rate of 10°C/min on approximately 10 mg samples. The Co(II) complexes investigated showed similar behavior in their TG and Differential Thermal Analysis (DTA) studies.

The ANPS undergo two step decomposition processes. The first step was started at 170°C and completed at 360°C showing weight loss of 40.584%. This was attributed to the loss of aldehyde moiety. While the second step occurred in the range 360-645°C corresponds to the weight loss 59.416%. This may be attributed to weight loss due to 2-amino-4-nitrophenol moiety. The thermogram of ANPS showed 100% weight loss.

The TG of Co(II) complexes showed that they were thermally more stable to varying degree. The complexes show the gradual loss in weight due to decomposition by fragmentation with increasing temperature up to certain extent, after that no significant weight loss was observed. This indicated that, expected decomposition product was cobalt oxide Co(II) in all the cases. The similar observations were recorded for all the Co(II) complexes indicating their high thermal stability [28], but for [Co(ANPS)(Ala)] and [Co(ANPS)(Leu)] the expected decomposition product was accompanied by some part of chelate structure. This observation indicated that, the [Co(ANPS)(Ala)] and [Co(ANPS)(Leu)] are thermally more stable than other cobalt complexes.

The [Co(ANPS)(Cys)] and [Co(ANPS)(Glu)] undergo two step decomposition process. The first step of decomposition process was started in the range 30-45°C and completed in the range 615-640°C which was

accompanied by a weight loss in the range 29.618-32.022%. This may be attributed to the loss of some part of ANPS. The second step started in the range 615-640°C and completed in the temperature range 1010-1030°C which was accompanied by a weight loss of 48.040-50.432%. This may correspond to the combined weight loss due to remaining part of ANPS and amino acid moiety [28].

The [Co(ANPS)(Ala)], [Co(ANPS)(Phe)] and [Co(ANPS)(Val)] undergo three step decomposition process. The first step was started in the range 30-235°C and completed in the range 420-650°C which was accompanied by a weight loss in the range 23.408-38.240%. This may be attributed to the loss of some part of ANPS. The second step was observed starts in the range 420-650°C and completed in the range 750-820°C which was accompanied by a weight loss in the range 14.109-37.369%. This may corresponds to the loss of remaining fragment of ANPS. The third step of decomposition process was started in the range 750-820°C and completed in the range 1040-1050°C which was accompanied by weight loss in the range 20.089-29.590%. This may correspond to the loss of amino acid moiety [11].

The [Co(ANPS)(Leu)] undergo four step decomposition process. The first step was started at 30°C and completed at 420°C which was accompanied by a weight loss of 18.757%. This may be attributed to the loss of some part of ANPS. The second step was started at 420°C and completed at 625°C which was accompanied by a weight loss of 14.941%. This may correspond to the loss of some part of amino acid. The third step of decomposition process occurred in the range 625-805°C which was accompanied by weight loss of 16.638%. This may correspond to the loss of remaining fragment of amino acid moiety [11]. The fourth step of decomposition process occurred in the range 805-1010°C which was accompanied by weight loss of 32.985%. This may corresponds to the loss of ANPS moiety.

In general, the final decomposition product in all the Co(II) complexes was found to be their respective cobalt oxide accompanied. Therefore 100% weight loss was not observed for the Co(II) complexes [19]. This indicated that the thermal stability of increases on complex formation. The data of thermal analyse are summarized in Table 4. The proposed decomposition mechanism for ANPS and [Co(ANPS)(Glu)] are illustrated in Figure 3.

X-ray diffraction study
The X-ray diffractogram of the complexes of ANPS recorded in the range 0-80°. Each diffractor was well resolved into sharp reflux suggesting highly crystalline nature for the mixed ligand complexes [29-36]. The d-spacing of the crystalline part of the compounds was calculated using the Bragg equation. The width 'β' at half the maximum of crystalline peak was measured and the crystallite size was calculated using the Deby-Scherrer relation. The result showed that the crystallite size of the mixed ligand Co(II) complexes ranges from 5.38-36.44 A°. From the Table 5, it is clear that, all mixed ligand complexes showed different crystallite size. The X-ray diffractogram of all mixed ligand complexes also showed that, the natures of peaks are well resolved, sharp, with very good intensity. No noticeable broadening area under the peaks was observed. Hence the all mixed ligand Co(II) complexes synthesized in present investigation were crystalline in nature.

Scanning Electron Microscope
The scanning electron micrographs revealed the morphology of Schiff base and Co(II) complexes. The scanning electron micrographs were taken at 20kV accelerating voltage and magnification was fixed according to need from 150x to 1000x. In SEM image macroscopic phase separation in dense layer was noticed. The domain size of about 100μm with void spaces were observed in all the systems. The void spaces are certainly due to the result of macroscopic phase separation [37]. In scanning electron micrograph of ANPS (Figure 4), the morphology showed that the surface wasspongy and soft with large macroscopic phase separation. This phase separation was minimized after formation of mixed ligand complexes. Also the spongy and softness was reduced due to introduction of metal ion. The similar observations were reported for the reduction in phase separation and softness of the surface on complexation [38]. From SEM images (Figure 5), it was clear that there was a strong change in morphology of Schiff base on complexation [39].

Biological Activities
The compound synthesized in the present investigation has been subjected to various antimicrobial screening programs based on their structural features so as to ascertain their activity against different microorganisms. The various screening programs conducted have been in the *in vitro* study by the serial tube dilution technique [9, 14, 40,41]. The solvent used was DMSO, and the sample concentrations were 200, 100, 50, 25, 12.5 ppm. The test results obtained are listed in Table 6. Antibacterial and antifungal data for *ampiciline* and *Streptomycin* are also included in this table for the purpose of comparison.

It has been observed that the compounds showed very good antibacterial and antifungal activity at 12.5, 25, 50, 100 and 200 ppm. The results have been expressed as percentage inhibition. The results of preliminary study on antimicrobial activity indicated that most of the compounds were highly and few were moderate active against both organisms. In comparison with the activity of ANPS, activity of the metal complexes was more. This indicated that activity increases with introduction of metal ion. The activity of mixed ligand complexes with ligand 'phe' showed higher activity as compared to other complexes. The presence of bulky substituents may be responsible for the enhancement of biological activity [14, 42, 43]. Compared to standard compounds [44, 45] *ampiciline* for anti bacterial activities and *Streptomycin* for anti fungal activities, the present compounds were similar effect against the selected strains of microorganisms.

REFERENCES

[1] A.S. Shaker, F. Yang, and A.A. Salleh, *Eur. j. Sci. Res.*, **2009,** 33(4), 702-709.

[2] F.D. Karia and P.H. Parsania, *Asian J. Chem.*, **1999,** 11, 991-995.

[3] P.G. More, R.B. Bhalvankar and S.C. Pattar, *J. Indan Chem. Soc.*, **2001,** 78, 474-475.

[4] S.M. Devidas, S.H. Quadri, S.A. Kamble, F.M. Syed and D.Y. Vyavhare, *J. Chem. Pharm. Res.*, **2011,** 3(2), 489-495.

[5] A.J.M. Xavier, M.A. Raj and J.M. Marie, *J. Chem. Pharm. Res.*, **2012**, 4(1), 669-672.

[6] M. Padmaja, J. Pragathi and C.G. Kumari, *J. Chem. Pharm. Res.*, **2011,** 3(4), 602-613.

[7] B.R. Thorat, R. Jadhav, M. Mustapha, S. Lele, D. Khandekar, P. Kamat, S. Sawant, D. Arakh, R.G. Atram and R. Yamgar, *J. Chem. Pharm. Res.*, **2011,** 3(6), 1045-1054.

[8] N. Raman, R.Y. Pitchaikani and A. Kulandaisamy, *Proc. Indian Acad. Sci. (Chem. Sci.)*, **2001,** 113(3), 183-189.

[9] A.P. Mishra and Rajendra K. Jain, *J. Chem. Pharm. Res.,* **2010,** 2(6), 51-61.

[10] H.C. Freeman, **"**Metal Complexes of amino acids and peptides", In *Inorganic Biochemistry*; G.L. Eichhorn, Ed., Elsevier Scientific: Amsterdam, **1973,** 1, 121-166.

[11] A.I. Vogel, "A Text Book of Quantitative Inorganic Analysis", 3rd Ed.; ELBS, Longman Green: London, **1961,** 415-456.

[12] P.W. Selwood, "Molecular diamagnetism. In Magnetochemistry", 2nd Ed.; Interscience: New York, **1956,** 83-109.

[13] I. Sakiyan, E. Logoglu, S. Arslan, N. Sari and N. Sakiyan, "Biometals. Antimicrobial activities of N-(2-hydroxy-1-naphthalidene)-amino acid(glycine, alanine, phenylalanine, histidine, tryptophane) Schiff bases and their manganese(III) complexes", Kluwer Academic pub.; Netherlands, **2004,** 17(2), 115-120.

[14] A.R. Patil, K.J. Donde, S.S. Raut, V.R Patil and R.S. Lokhande, *J. Pharm. Reser.*, **2011,** 4(7), 2256-2260.

[15] R.D. Beaty and J.D. Kerber, "Control of Analytical Interferences. In: Concepts, Instrumentation and Techniques in Atomic Absorption Spectrophotometry". Ed by R.D. Beaty and J.D. Kerber, The Perkin-Elmer Corporation, Norwalk, CT, USA, **1993,** 3-5.

[16] W.J. Geary, *Coord. Chem. Rev.*, **1971,** 7(1), 81-122.

[17] S. Gaur, and B. Sharma, *J. Indan Chem. Soc.*, **2003,** 8, 841-842.

[18] S. Panda, R. Mishra, A.K. Panda and K.C. Satpathy, *J. Ind. Chem. Soc.,* **1989,** 66, 472-474.

[19] V.S. Shivankar, R.B. Vaidya, S.R. Dharwadkar and N.V. Thakkar, *Syn. Reac. Inorg. Metal-Org. Chem.*, **2003,** 33(9), 1597-1622.

[20] K. Nakamoto, Y. Morimoto and A.E. Martell, *J. Am. Chem. Soc.,* **1961,** 83, 4528-4532.

[21] Z.H. Chohan, M. Arif, M.A. Akhtar and C.T. Supuran, *Hindavi Publishing Corporation*, *Bioinorganic Chemistry and Applications*, **2006,** 1-13.

[22] B.T. Thaker, K.R. Surati, P. Patel and S.D. Parmar, *J. Iran. Chem. Soc.*, **2006,** 3(4), 371-377.

[23] E. Sinn and C.M. Harris, *Coord. Chem. Rev.,* **1969,** 4(4), 391-422.

[24] S.B. Kalia, K. Lumba, G. Kaushal and M. Sharma, *Indian. J. Chem.,* **2007,** 46A, 1233-1239.

[25] R.J. Fitzgerald and G.R. Brubaker, *Inorg. Chem.*, **1969,** 8(11), 2265-2267.

[26] S.A. Carabineiro, L.C. Silva, P.T. Gomes, L.C.J. Pereira, L.F. Veiros, S.I. Pascu, M.T. Duarte, S. Namorado and R.T. Henriques, *Inorg. Chem.*, **2007,** 46(17), 6880-6890.

[27] T. Esref, A. Mehmet, K. Ahmet and K. Zeliha, *J. Coord. Chem.,* **2006,** 59(8), 861-872.

[28] A.P. Mishra and M. Khare, *J.Indian Chem. Soc.,* **2000,** 77(8), 367-370.

[29] F. Tuna, G. Clarkson, N.W. Alcock and M.J. Hannon, *Dalton Trans.*, **2003,** 2149-2155.

[30] D.J. Szalda, C. Creutz, D. Mahajan and N. Sutin, *Inorg. Chem.*, **1983,** 22(17), 2372-2379.

[31] S.G. Telfer, G. Bernardinelli and A.F. Williams, *Chem. Commun.*, **2001,** 1498-1499.

[32] S.G. Telfer, R. Kuroda and T. Sato, *Chem. Commun.*, **2003,** 1064-1065.

[33] S.G. Telfer, T. Sato, T. Harada, R. Kuroda, J. Lefebvre and D.B. Leznoff, *Inorg. Chem.*, **2004,** 43(20), 6168-6176.

[34] J.M. Rowland, M.M. Olmstead and P.K. Mascharak, *Inorg. Chem.*, **2002,** 41(6), 1545-1549.

[35] C. Provent, G. Bernardinelli, A.F. Williams and N. Vulliermet, *Eur. J. Inorg. Chem.*, **2001,** 1963-1967.

[36] M.A. Masood, E.J. Enemark and T.D.P. Stack, *Angew Chem Int Ed Engl.,* **1998,** 37, 928-932.

[37] B. Massimilano, L. Vincenzo, P. Andrea, P. Piero, G. Narducci and Ruggeri, *Macromolecular Symposia*, **2006,** 235, 143-151.

[38] Y. Aydogdu, F. Yakuphanoglu, A. Aydogdu, C. Alkan and I. Aksoy, *Materials Letters*, **2002,** 54, 352-358.

[39] L.A. Saghatforoush, R. Mehdizadeha and F. Chalabian, *J. Chem. Pharm. Res*., **2011,** 3(2), 691-702.

[40] I. Sakyan, E. Logoglu, S. Arslan, N. Sari and N. Akiyan, *Biometals.,* **2004,** 17(2), 115-120.

[41] J.L. McLauglin, C.J. Chang and D.L. Smith, "Bench top' bioassay for the discovery of bioactive natural products: an update. In: Atta-ur-Rahman", Ed. I Amsterdam, The Netherlands: Elsevier Science., **1991,** 9, 383-409.

[42] V.K. Patel, A.M. Vasanwala and C.N. Jejurkar, *Indian J. Chem.,* **1989,** 28A, 719-721.

[43] Z.H. Chohan, M. Praveen and A. Ghaffar, *Metal-Based Drugs*., **1997,** 4(5), 267-272.

[44] Z.H. Chohan, A.K. Misbahul and M. Moazzam, *Indian J. Chem*., **1988,** 27(A), 1102-1104.

[45] M.T. Madigan, J.M. Martinko and J. Parker, "Microbial growth control. In Biology of Microorganisms", 8th Ed.; Prentice-Hall: New Jersey, **1997,** 397-429.