Synthesis, Growth, Structural Elucidation and Pharmacological Activities of *o*–Aminobenzamide and L–Asparagine derived Ni(II), Cu(II) and Zn(II) Compounds

M. Chitra, M. Revathi, J. Dharmaraja, S. Shobana, M. Anusuya

Abstract: ***The present study mainly investigates the synthesis and growth of some novel bioactive Ni(II), Cu(II) and Zn(II) compounds (1–3) derived from 2–aminobenzamide (o–aminobenzamide: 2AB: L) and L–asparagine (asn: B). The synthesised compounds were structurally characterized by various physico–chemical and spectral (FTIR, UV–vis., TGA/DTA, XRD and SEM) studies. The micro elemental (C, H and N) analysis suggests that stoichiometry of the metal(II) compounds to be 1:1:1 (Metal: 2AB: asn). The observed low molar conductance values reveal their non–electrolytic nature and the observed electronic spectra coupled with magnetic moment values clearly indicate that the ligands 2AB (L) and asn (B) coordinate with metal(II) ions in tetradentate manner through amino–N & amido–O of (L) and deprotonated carboxylato–O & amino–N atoms of (B) to form a stable 6, 5 membered chelate ring. Powder X–ray diffractogram and SEM pictograph implies that all the compounds have well–defined nanocrystallinity with homogeneous morphology. All the derived compounds (1–3) show significant in vitro biological and antioxidant activities than the 2AB(L) and asn(B) in their free state and the activities go behind the order as Control >> CuLB >> NiLB ≈ ZnLB > 2AB(L) > asn(B).***

Keywords: 2–Aminobenzamide, L–Asparagine, Spectral, XRD, SEM, Pharmacological study.

Abbreviations:
2AB (L) = 2–Aminobenzamide or *o*–Aminobenzamide;
Asn (B) = L–Asparagine, AA = Ascorbic acid,
DPPH = 2, 2–Diphenyl–1–picrylhydrazyl,
SEM = Scanning Electron Micrography

I. INTRODUCTION

Generally, the amide functional groups are the most dynamic sub–unit present in the huge number of natural and man–made drug products and it plays an essential building component in peptides and proteins synthesis. In modern coordination chemistry, the formation of metal compounds with amide moieties have been received considerable attention due to its multifarious coordinating sites via amide–O and / or amide–N atoms and it also shows a wide range of biological, medicinal and clinical activities [1]. Among the heterocyclic amide compounds, the benzamide and its associated derivatives (Metoclopramide, Cisapride, Sulpiride, Remoxipride, Nemonapride, Prucalopride, Mosapride, etc.) have been viewed as a privileged and proficient drug scaffolds in many areas such as antagonistic, anticoagulant, analgesic, anti–inflammatory, antimalarial, anti–hypertensive, antifouling, anti–proliferative, anti–helmintic, anti–nociceptive, anticancer, antiemetic, antiarrhythmic, anti–HSV, antipsychotic [2–5] etc. Owing to their pivotal role in pharmacological properties, these benzamides have been approved as effective receptors, inhibitors and activators like dopamine D2 and D3, 5–hydroxytryptamine (5–HT2, 5–HT3 and 5–HT4), gastrointestinal prokinetic receptors, positive modulators of AMPA in mammalian brain, DNA methyltransferase (DNMT), poly(ADP–ribose)polymerase (PARP), phosphatidylinositol3 kinase (PI3K), Histone deacetylases (HDACs or HDIs) enzyme for suppressing the growth of tumor cells in the human body, Heat Shock Protein 90 (HSP90) inhibitors and glucokinase potassium channel activators [6–8] respectively in modern clinical and bio–medicinal fields. 2–Aminobenzamide (2AB; CAS No. 88–68–6; Anthranilamide) and their derivatives are occurred in nature and isolated from Streptomyces species [9]. They are selective and excellent fluorescent labelling agents (analysis of N–glycan in oligosaccharides) [10–11], scavenging agents, inhibitors for Pseudomonas aeruginosa infections, orally active antithrombotic agents and highly selective potent for serine protease factor *Xa* (*FXa*) inhibitors in the blood coagulation cascade [12–13] as well as efficient chemo–sensors. Transition M(II) compounds play a crucial role in modern clinical, medicinal and pharmacological fields since they show significant therapeutic applications [14]. Generally, metals are essential trace elements to human, higher animals and plants [15].

It stabilizes RNA and DNA against thermal denaturation and also found in highest concentrations in the human body organs and tissues such as serum, bone, blood, nucleic acids, hair, kidney and lung [15, 16].

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Amino acids are the major metabolic intermediates and the basic structural units of proteins and peptides and also acting as potent modulators and precursors for the synthesis of various non–protein nitrogen contacting metabolites such as heme, creatine, purines, pyrimidines etc., many hormones, antibiotics and signaling molecules [17]. Generally, L–amino acids are widely used in mammalian metabolism and they are exclusively used in protein synthesis, also involved in number of intracellular metabolic functions, energy productions, hormone synthesis, transport nutrients and oxygen [18, 19]. As part of our ongoing research [20–22], the major goal of the present work is to synthesize and grow some novel Ni(II), Cu(II) and Zn(II) compounds derived from 2–aminobenzamide (2AB: L) and L–asparagine (asn: B). The synthesised compounds (**1–3**) were structurally characterized by means of analytical and spectral techniques. In addition, the *in vitro* biological and antioxidant activities of free 2AB (L) & asn (B) ligands and its metal(II) compounds (**1–3**) have investigated and the results were compared with the standard controls.

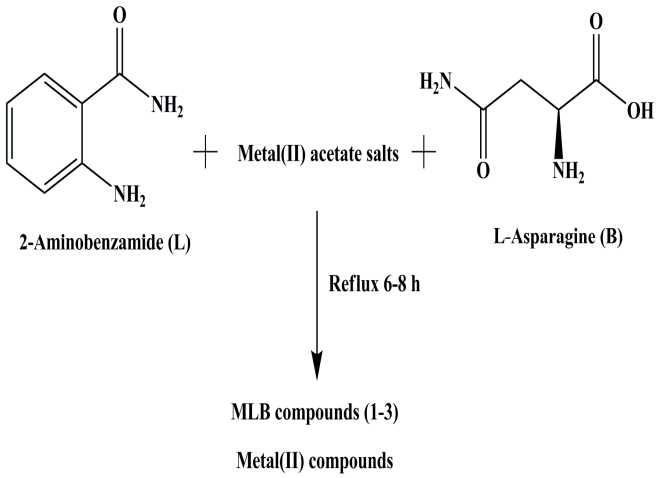
II. EXPERIMENTAL METHOD

A. Materials and Methods

All the chemicals and solvents used in this work were of extra pure analytical grade and purchased from Sigma Aldrich and Fluka (Puriss) without further purification. Further, 2,2–diphenyl–1–picrylhydrazyl (DPPH: $C_{18}H_{12}N_5O_6$) and ascorbic acid (AA: $C_6H_8O_6$) were purchased from Sigma Aldrich. The solvents used for the preparation and physical measurements were purified according to the literature methods [23]. Triply distilled CO_2 free water with specific conductance equal to ($1.81 \pm 0.1\ \Lambda^{-1}\ cm^{-1}$) was used for the preparation of all solutions. Melting point (m.p.) of all the compounds was determined on Gallenkamp apparatus in an open glass capillary tube and is uncorrected. Micro analytical data were performed on Elementar Vario EL III CHNS analyzer and the metal(II) content in the compounds was estimated gravimetrically by the standard ammonium oxalate method. Molar conductance (Λ_m) of the compounds (1×10^{-3} mol solution in DMSO) was measured using an Elico CM 180 conductivity bridge by using a KCl solution as calibrant. Fast atomic bombardment mass spectra (FAB–MS) were recorded using a VGZAB–HS spectrometer in a 3–nitrobenzylalcohol matrix. Magnetic susceptibility measurements were carried out on a Gouy balance at room temperature using mercuric tetra(thiocyanato)cobaltate(II) as the calibrant and the diamagnetic corrections were applied in compliance with Pascal's constant [24]. Electronic spectra (200–1100 nm) were measured using a Hitachi U–2000 double beam spectrophotometer. The IR spectra were recorded on KBr discs on a JASCO FT/IR–410 spectrometer (400–4000 cm^{-1}). Thermal analyses (TGA/DTA) were recorded on a Perkin Elmer (TGS–2 model) thermal analyzer with a heating rate of 10 K/min in the dynamic N_2 atmosphere (flow rate 20 ml/min). Powder X–ray diffraction (PXRD) patterns were recorded on a Bruker AXS D8 advance powder X–ray diffractometer (Detector: Si(Li)PSD, X–ray source: Cu, Wavelength: 1.5406 A°). Scanning Electron Micrography with Energy Dispersive Spectrometry associated (SEM / EDS) using JSM–5610 scanning electron microscope was used for morphological evaluation.

B. General procedure for synthesis of compounds (1–3)

2–Aminobenzamide (0.136 g, 1 mmol) was completely dissolved in hot 50 % (v/v) water–ethanol mixture (10 ml) and added drop by drop to the metal(II) acetate salts [Ni $(CH_3COO)_2 \cdot 4\ H_2O$ (0.250 g, 1 mmol) or $Cu(CH_3COO)_2 \cdot H_2O$ (0.200 g, 1 mmol) or $Zn(CH_3COO)_2 \cdot 2\ H_2O$ (0.220 g, 1 mmol)] in aqueous (10 ml) medium and stirred well at room temperature for 2–3 h. To this resulting solution, aqueous solution (10 ml) of L– asparagine (0.132 g, 1 mmol) was added so that the overall ratio of metal: 2AB: asn was 1:1:1 and refluxed for 6–8 h on a water bath (**Scheme 1**). The reaction mixture was maintained at pH = 7.4 by adding few drops of aqueous Na_2CO_3 solution. The resulting solution was reduced to 1/3 of its original volume by water bath and kept aside. On standing, the characteristic colored solid compounds were collected by vacuum filtration and then washed several times with cold and hot water, ethanol and anhydrous ether.



Scheme I: General synthetic route of metal (II) compounds (1–3)

C. General procedure to grow crystals of compounds

The saturated solution of (30 ml, 1 mol) of above synthesized compounds (**1–3**) were prepared (50 % v/v water–ethanol mixture) at 36 ± 0.1 °C maintained in a thermostat of Toshniwal GL 15.01 constant temperature bath. By slow evaporation, the seeds were obtained from the mother solution and the obtained seeds were collected, and then employed for the bulk growth of the crystals. After 20–25 days of period, the good quality of high purity transparent crystals was grown from the saturated solution by slow evaporation method by repeated recrystallizations (Fig. 1) and the obtained crystal compounds were air dried in air and stored *in vacuo* over anhydrous $CaCl_2$ at room temperature (Yield: 65–75 %).

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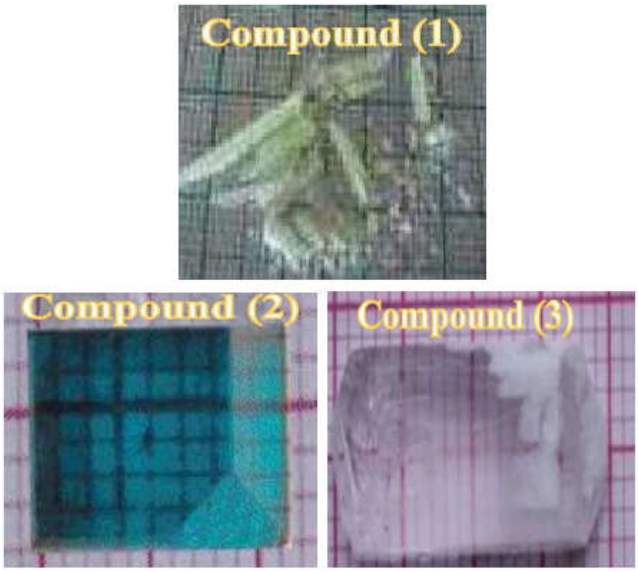

Fig. 1: Photograph of crystal grown of Ni(II), Cu(II) and Zn(II) compounds (1–3)

D. In vitro biological activities

In vitro biological activities of free 2–aminobenzamide (L), L– asparagine (B) and its metal(II) compounds (**1–3**) were tested against some pathogenic bacterial species such as *Escherichia coli*, *Staphylococcus saphyphiticus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* using Muller Hinton nutrient agar (NA) and fungal species such as *Aspergillus niger*, *Enterobacter* species and *Candida albicans* using potato dextrose agar (PDA) as the medium by modified well diffusion technique [25]. The detailed procedure for measuring the zone of inhibition values (in mm) as described earlier [20, 22] and all the analyses were made in three replicates for each. The obtained zone of inhibition values (in mm) was compared with the standard tetracycline (for antibacterial) and nystatin (for antifungal) control drugs (3×10^{-3} mol) respectively. The zone of inhibition is given as the average of three independent determinations.

E. In vitro antioxidant studies

In vitro antioxidant activities of free 2–aminobenzamide (L), L– asparagine (B) and their metal(II) compounds (**1–3**) have been investigated by DPPH (2, 2–diphenyl–1–picrylhydrazyl) free radical scavenging model according to the Blois method [26]. The detailed procedure for measuring the free radical scavenging activity (in %) were as described earlier [20, 22]. A blank DPPH solution (without sample) was used for the baseline correction because the odd electron in the DPPH free radical gives a strong absorption maximum (λ_{max}) value at 517 nm (purple colour with $\varepsilon = 8.32 \times 10^3$ mol^{-1} cm^{-1}) by using a Hitachi U–2000 double beam spectrophotometer. After incubation, there was a change (decrease) in the absorbance values which indicate that the compounds have significant free radical scavenging activity. Ascorbic acid was used as the reference or positive control. All the analyses were made in three replicate and the results were compared with the control.

III. RESULTS AND DISCUSSION

A. Physico–chemical studies

The synthesized grown crystal compounds (**1–3**) are more air stable and non–hygroscopic in nature. The synthesised compounds show the stoichiometry ratio of 1:1:1 (metal:2AB:asn) and they are found to be soluble in methanol, ethanol, DMSO, DMF and dioxan but insoluble in common organic solvents such as benzene, acetone, *etc*. The obtained analytical data and various physico–chemical properties such as color, yield and melting point of the synthesized compounds are given in Table 1. The micro elemental analytical (C, H and N) data are in good agreement with the calculated values which shows 1:1:1 (metal:2AB:asn) ratio and the observed low molar conductance (Λ_m) values in DMSO solution (10^{-3} M) reveals that the compounds behave as non–electrolytes [27]. The mass spectrum (FAB–MS) of compounds (**1–3**) show fragmentation pattern along with *m/z* value at 402.07, 407.05 and 408.05 [*M+1*] respectively which indicate the stoichiometry of metal compounds as [M(II)–2AB(L)–asn(B) (OH_2) (OAc)].

B. FTIR spectral study

The vibrational spectral study helps to investigate the mode of coordination between the free ligands (L and B) with M(II) ions in the metal compounds (**1–3**) by comparing the spectral data of the free ligands with the respective spectra of the compounds and their representative FTIR spectra is shown in Fig. 2. From the vibrational spectral studies, the ligand 2AB (L) acts as a bidentate and binds with the metal(II) compounds through N–amino and O–amido groups [28, 29] to form a stable six membered chelates. Similarly, the amino acid L–asparagine (B) binds to the M(II) ions in a bidentate manner through amino–N and carboxylato–O atoms. This is further supported by the appearance of two new bands obtained in the far infrared region, 420–445 cm^{-1} corresponding to (M–O) and 574–592 cm^{-1} to (M–N) bonds stretching frequency respectively [28, 29]. This ν(M–O) and ν (M–N) peak values follow Irving–William order of stability as: Cu(II) > Ni(II) > Zn(II) and also comparable with the crystal field stabilization energies. In all the compounds, two bands in the regions 1588–1624 cm^{-1} and 1362–1382 cm^{-1} were ascribed to the asymmetric and symmetric vibration of the coordinated carboxylate acetate group [28, 29]. The magnitude of $\Delta\nu$ value falls in the range 226–242 cm^{-1} suggests the coordination of the acetate group in the unidentate fashion. Furthermore, the presence of coordinated water molecules in all the compounds (**1–3**) are indicated by a broad band in the region 3342–3480 cm^{-1} and two weaker bands in the region 819–844 cm^{-1} and 714–728 cm^{-1} due to ν(OH) rocking and wagging mode of coordinated water (H_2O) molecules respectively [28, 29]. This is consistent with the results of micro elemental analysis.

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Table1: Various physico–chemical parameters, molar conductivity and melting point of metal(II) compounds (1–3)

Compounds	Colour	Empirical Formula	Molecular weight	*m/z*	Yield (%)	M.pt (°C)	Elemental analysis, found (*Calc.*) (%) C	H	N	M	Λ_m (Ω^{-1} cm^{-1} mol^{-1})
Compound (**1**)	Pale green	$NiC_{13}H_{20}N_4O_7$	403.01	402.07	65	265	38.68 (38.74)	4.98 (5.01)	13.87 (13.90)	14.48 (14.56)	18.25
Compound (**2**)	Deep blue	$CuC_{13}H_{20}N_4O_7$	407.87	407.05	68	270	38.16 (38.28)	4.89 (4.94)	13.68 (13.74)	15.51 (15.58)	20.16
Compound (**3**)	Pale yellow	$ZnC_{13}H_{20}N_4O_7$	409.74	408.05	75	> 280	38.04 (38.11)	4.88 (4.92)	13.59 (13.67)	15.90 (15.96)	24.51

Table 2. Electronic absorption spectral data, ligand field parameters and magnetic measurement values of M(II)–2AB(L)–asn(B) compounds (1–3) in DMSO medium at 37 °C.

Compounds	λ_{max} (cm^{-1}) ε (dm^{3}mol^{-1}cm^{-1})	Band assignments	Geometry	μ_{eff} (BM)	Lunde's factor (*g*)	Ligand field parameter Dq (cm^{-1})	B (cm^{-1})	β	β (%)	LFSE (kJ mol^{-1})	ν_2/ν_1
Compound (**1**)	10345 (74) 17025 (156) 25332 (250) 34865 (485)	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ LMCT (L → M)	Octahedral	3.09	2.279 (2.250 for hexaaquo Ni(II) complexes)	1034.5	754.80 (1030 for free ion)	0.73	26.72	148.60	1.65
Compound (**2**)	14561 (135)	$^2E_g(D) \rightarrow {}^2T_{2g}(D)$	Distorted octahedral	1.85	–	–	828 (for free ion)	–	–	–	–
Compound (**3**)	26429 (152)	LMCT (L → M)	Octahedral	Dia.	–	–	–	–	–	–	–

Table 3. *In vitro* biological activities of free ligands and their metal(II) compounds (1–3) by modified well diffusion method at 24 h (Zone formation in mm)

Compound	Diameter of inhibition zone (in mm) for different microorganisms at 24 h						
	E. coli	*S. saphyphiticus*	*S. aureus*	*P. aeruginosa*	*A. niger*	*E. species*	*C. albicans*
Control	22	30	27	32	32	24	33
2AB (L)	–	14	11	–	12	–	10
Asn (B)	15	14	16	–	14	13	–
Compound (**1**)	16	19	–	27	23	–	26
Compound (**2**)	18	21	24	–	26	21	29
Compound (**3**)	14	–	21	23	–	18	25

[–] = Less active, error limit: ± 2–3 %.

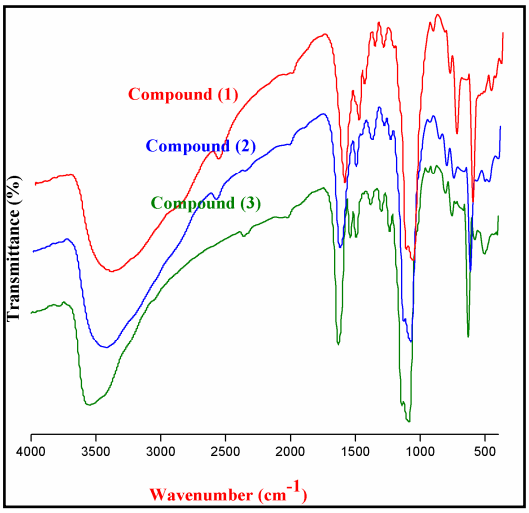

Fig. 2: FTIR spectra of metal(II) compounds (1–3)

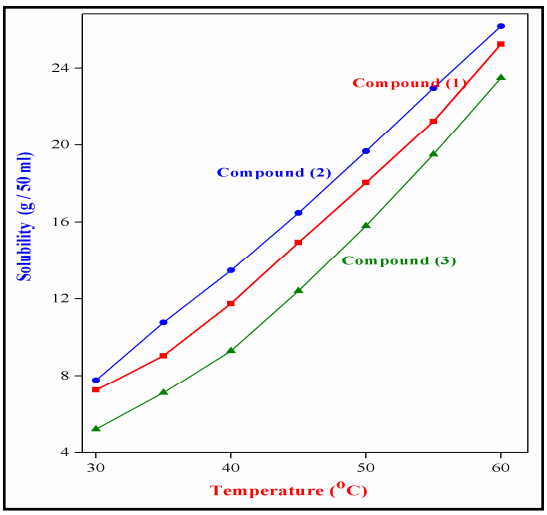

Fig. 3: Solubility nature of synthesised metal(II) compounds (1–3)

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C. Magnetic measurements and Electronic absorption spectra

The observed magnetic moment (μ_{eff}) values 3.09 and 1.86 BM for Ni(II) and Cu(II) compounds (**1–2**) respectively offers the possibility of a distorted octahedral geometry [30]. The Zn(II) ion being diamagnetic and it does not show any d–d transitions in the visible region. These observed magnetic moment values also support the absence of any considerable metal–metal (M–M) interaction in the solid phase *i.e.*, ruled out bimetallic complexes. Absorption regions (λ_{max}), band assignments, proposed geometry, ligand field parameters and magnetic moment values of metal(II) compounds (**1–3**) are given in Table 2. Furthermore, the observed magnetic measurement values and absorption values (Table 2) are in good agreement for the octahedral environment around the metal(II) ions [30].

D. Solubility studies

The solubility nature of synthesised crystal compounds (**1–3**) was determined by a hot plate with magnetic stirrer and the temperature was maintained in a thermostat of Toshniwal GL 15.01 constant temperature bath. The synthesised crystal compounds (**1–3**) was dissolved in 50 ml of triply distilled CO_2 free water in an air tight container and stirred continuously in a hot plate magnetic stirrer. The constant stirring of the solution consequently leads to some precipitate formation as a result supersaturation. Then, 20 ml of the mother solution was pipetted out into a clean petri dish and warmed up at 40–50 ^oC till the solvent water was evaporated completely and the solubility was checked by gravimetric method [31]. The same procedure was followed for different temperatures and also the variation of solubility with temperature is represented in Fig. 3. From Fig. 3, it is clearly indicating that the solubility nature of the compounds increases with increase of temperature.

E. Microhardness analysis

Generally, the hardness of the compounds is defined in terms of the resistance which offers to the motion of dislocations, deformations or damage under as applied stress. The microhardness measurements were made on the synthesised crystal compounds (thickness of 3–4 mm) for the applied loads of different magnitudes (25 – 100 g) were applied. Vickers hardness number (H_v) was calculated from the equation: H_v = 1.8544 P / l^2 kg / mm^2, where P is the applied load in kg and l is the mean diagonal length in mm. The calculated H_v values are 10.24, 11.45 and 12.29 kg / mm^2 for compound (**1**), 8.45, 10.86 and 11.56 kg / mm^2 for compound (**2**) and 8.16, 9.97 and 11.01 kg / mm^2 for compound (**3**) at 25, 50 and 100 g for applied loads respectively. The results clearly indicate that, the hardness value (H_v) increases when the load increases up to 100 g but beyond the limit (> 100 g), significant cracks will appear in the crystalline site which is due to the release of internal stress generated by molecular indentation [32].

F. Thermal analysis (TGA/DTA)

Thermogravimetric (TGA/DTA) analyses of metal(II) compounds (**1–3**) have been studied in the temperature range of ambient to 900 ^oC temperature under stable air condition. Thermogravimetric pictogram of Ni(II) compound (**1**) is shown in Fig. 4. In all the compounds, the thermal decomposition occurs in three steps and initially, there was a loss in weight of 4.39–4.45 % (*cal.* 4.52–4.60 %) in the temperature which ranges between 40–210 °C, revealing that one coordinated water (OH_2) molecule present in the inner coordination sphere of the compound [20, 29]. The results are explained according to the analytical suggestions. In the second stage, the compound decomposes took place in the range of 230–290 ^oC with a mass loss of 29.15–29.22 % (*cal.* 29.59–29.65 %) corresponding to removal of one mole of acetate (OAc) moiety from the compound. In all cases, after 310 °C the decomposition of the ligand molecule continues leading to formation of the air stable metal oxide as the end product in the range of 600–810 ^oC and also there is no phase transition and colour change till the compounds melts. The results are in accord with the composition of the metal compounds. Moreover, the kinetic order of decomposition of the metal(II) compounds (**1–3**) was calculated by the modified Horowitz and Metzger equation as: $[C_s = (W_s - W_f) / (W_o - W_f)]$. The observed C_s value (0.64–0.70) indicates that the decomposition follows first order kinetics. From TGA / DTA studies, all the compounds are more stable up to 210 –240 ^oC and this enhances the temperature range for further utilization of the crystal for NLO applications in various fields.

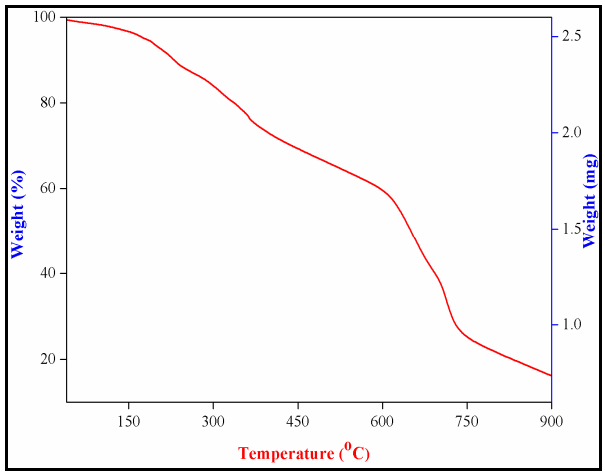

Fig. 4: Thermogravimetric pictogram of Ni(II) compound

G. X–ray diffraction analysis and SEM pictogram

Powder X–ray diffraction (PXRD) study is mainly used to determine the phase identification of newly synthesised crystalline materials and to measure the sample purity and particle size of the sample materials [33, 34]. In this paper, the powder X–ray diffractogram of Ni(II)/Cu(II)/Zn(II)–2AB(L)–asn(B) compounds (**1–3**) were recorded in the range of 0–80^o (2 θ) and the representative diffractogram are given in figure 5.

From the XRD patterns, all the compounds show a well resolved sharp peak with maximum intensities which demonstrate that the compounds have uniform crystalline in nature [33, 34] and also this performance is due to the presence of coordinated water (H_2O) and acetate (CH_3COO^-) moieties inside the coordination sphere [33, 34]. Powder X–ray diffractogram shows a number of weak peaks which imply uniform phase with absence of any impurities in the compounds [33].

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The crystallite sizes (*D*) of metal(II) compounds (**1–3**) were calculated from the main XRD peaks using the Debye–Scherrer's equation ($D = 0.9\lambda/\beta\cos\theta$) and the observed average crystallite size values were found to be 37.54, 45.02 and 30.41 nm respectively for Ni(II), Cu(II) and Zn(II) compounds (**1–3**). These obtained values clearly indicate that the synthesised compounds have micro crystalline nature with different crystallite size.

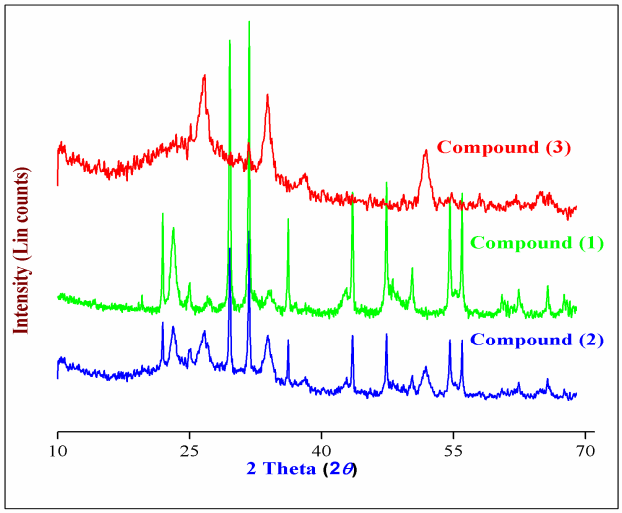

Fig. 5: Powder X–ray diffraction patterns of metal(II) compounds (1–3)

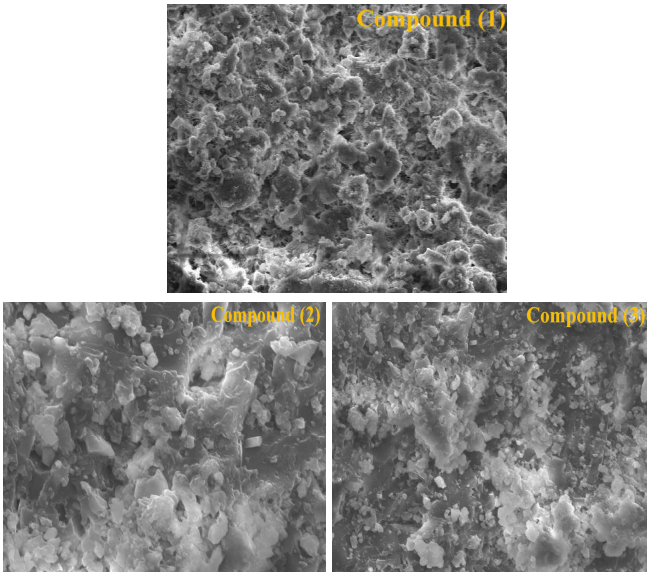

Fig. 6: SEM photographs of (a) Ni(II) and (b) Cu(II) and (c) Zn(II)–2AB(L)–asn(B) compounds (1–3)

The surface morphology and particle size of synthesised Ni(II), Cu(II) and Zn(II) compounds (**1–3**) are illustrated in the Scanning Electron Micrography (SEM) (figure 6**)**. All the synthesised compounds are uniform matrix with homogeneous surface phase materials in the pictogram [33, 34]. Particle size of Ni(II)–2AB(L)–asn(B) compound (**1**) is found to be 27 μm with an irregularly broken granular–like shape. The SEM pictogram of Cu(II) compound (**2**) shows massive frozen crumpled rock like morphology with tiny mist like structure morphology with particle size found to be 30 μm. An irregularly shattered small ice cubic bar like shape is observed for Zn(II) compound with particle size found to be 24 μm.

Based on the observation from micro elemental, conductivity measurement, magnetic susceptibility with UV–visible absorption, various spectral and thermal studies, it has been confirmed that the synthesised metal(II) compounds are octahedral geometry with monomeric nature.

H. In vitro biological activity

In vitro biological activities of free 2AB(L) and asn(B) ligands along with their metal(II) compounds (**1–3**) were tested against few bacterial and fungal strains by modified well diffusion method using agar as nutrient. Commercially available standard drugs tetracycline (for antibacterial) and nystatin (for antifungal) are used as controls. Measurement of zone of inhibition against the growth of bacteria and fungi for the ligands and their compounds is given in Table 3. All the metal(II) compounds show remarkable biological activities against different microorganisms than their free ligands and the higher activities depend upon the metal(II) ions *i.e.*, size, charge distribution, shape and redox potential of the metal chelates. The highest activities can be explained on the basis of Overtone's concept and Tweedy's chelation theory [35–37]. Also, it increases the delocalization of π–electrons over the whole chelate ring resulting in high lipid solubility within the chelate ring system. All the compounds show more significant antibacterial and less pronounced antifungal activities. Biological activities of the metal(II) compounds (**1–3**) are found to be increased as the stability of the compounds increased and the order of activities is given as:

Control > Compound (2) > Compound (1) ≈ Compound (3) > 2AB (L) > asn (B).

I. In vitro antioxidant activity

In vitro antioxidant activities of free 2AB(L) and asn(B) ligands along with their metal(II) compounds (**1–3**) were tested against DPPH free radical scavenging method and ascorbic acid was used as the reference or positive control. All the analyses were done in three replicates and their representative graph is shown in Fig. 7. Reduction capability of free radical (DPPH) is determined by the decrease in its absorbance value at 517 nm (blank) which can be induced by antioxidant.

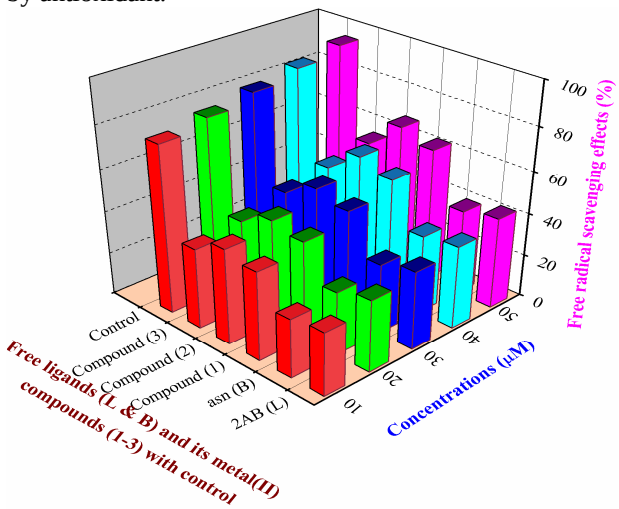

Fig. 7: Free radical scavenging activities of free 2AB (L) and asn (B) ligands along with their metal(II) compounds (1–3)

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From Fig. 7, it is clearly seen that metal(II) compounds have higher activities than the free ligands and this may be due to the presence of positively charged M(II) ions which favors the release of hydrogen to reduce the DPPH radical [38, 39] and easy chelation of ligand moieties with metal(II) ions.

IV. CONCLUSION

In the present paper, we synthesised the novel bioactive MLB type of Ni(II)/Cu(II)/Zn(II)–2AB(L)–asn(B) compounds (**1–3**) and they were characterized by various analytical and spectral techniques. The powder XRD and SEM analyses show that the metal(II) compounds display sharp crystalline peaks with well–defined microcrystalline nature with an average grain size of 30–45 nm and homogeneous particle nature. The measured Vickers hardness (H_v) values clearly demonstrate that when the load increases up to 100 g the hardness value also increases but > 100 g (beyond limit) significant cracks will appear in the crystals. All the compounds under investigation show octahedral environment around the central M(II) ions. *In vitro* biological and antioxidant activities of Cu(II) compounds show more potent activities than other compounds and also the free ligands. Moreover, the synthesised crystalline compounds may be used as promising NLO materials in various fields.

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