Research Article

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Bioactivities of Novel Metal Complexes Involving B Vitamins and Glycine

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Abstract: In this work twelve novel mixed ligand complexes were synthesized. The complexes were formed between a metal ion (Cu(II), Cd(II), Mn(II), Fe(III), Ni(II), Pb(II)) and vitamins (B 3 and B 9) as primary ligands, and glycine as secondary ligand. Melting points, conductivities, and magnetic susceptibilities of the synthesized complexes were determined and the complexes were subjected to elemental analyses. The presence of coordination water molecules in the complex was also supported by TG/DTG thermal analysis. Full elucidation of the molecular structures for the synthesized mixed ligand complexes were confirmed using detailed spectroscopic IR, 1H-, 13C-NMR, and XRD techniques. In addition, cytotoxic and antioxidant activities of the twelve synthesized solid complexes were tested to evaluate their bioactivities.

Keywords: Folic Acid, Nicotinic Acid, Metal Complexes, Synthesis, Characterization, Bioactivities

1 Introduction

Metal ion complexes of vitamins such as vitamin B3 and vitamin B9 (folic acid) have drawn much attention due to their biological and clinical significance [1-3]. Metal complexes can be used as therapeutic drugs to treat human diseases like carcinomas, lymphomas, diabetes, and neurological disorders, for infection control, and as anti-inflammatory compounds [4]. Stability constants of the studied complexes were determined in order to assess their potentiality as antidotes for metal-poisoning in biological system [5-9]. The solution chemistry of the synthesized metal complexes was recently studied in our previously published work [10-12].

A number of studies have pointed out that the structural diversity encountered in metal–vitamin B9 complexes could be attributed to the versatile ligation behavior of the carboxylate group in folic acid (vitamin B9) which can function as a bidentate ligand binding to a single metal or alternatively as a bridging bidentate ligand coordinating to two metals or as a monodentate ligand [13-15]. There are several studies on the complexation between folic acid and di- and trivalent metal ions such as Fe(III), Cu(II) [3,13], Mn(II), Co(II), Ni(II), Zn(II), Cd(II), and Hg(II) [3,16,17] reported in the literature. Bioactivities of the metal-folic acid complexes were also investigated [17,18]. The interaction of nicotinic acid (vitamin B3) with divalent metal ions (Cu(II), Ni(II), and Co(II)) were studied recently using pH-potentiometric titrations [19,20]. Also, nicotinic acid complexes of Co(II) [20], Cr (III) [21-23], Cu(II) [23], Fe(II) [24], and Ni(II) [25] were investigated using various techniques such as pH-potentiometric titration, polarography, and UV-visible spectrophotometry. A study in 2005 [26] stated that nicotinic acid may also act as a neutral ligand, ligating the metal ion through its N atom, as found in its Cu(I) and Au(III) complexes [26]. It can also act as nicotinate anion that forms complexes with lanthanides by two carboxylate O atoms forming a four member chelate ring, thus acting as a bidentate ligand.
Furthermore, three complexes of Ag(I)-nicotinic acid were reported [26]. More recently, the interaction of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) with nicotinic acid (NA), nitrilotriacetic acid, iminodiacetic acid, and ascorbic acid have been studied by a pH-metric technique at 0.1 mol·dm$^{-3}$ (KNO$_3$) ionic strength at 302 ± 0.5 K in aqueous medium [27].

The widespread studies reported up to now on complexation between biologically important trivalent and divalent metal ions and biological oxygen/nitrogen donor ligands such as folic acid, nicotinic acid, and glycine in solution mainly aimed at unfolding the role of metal-ligand equilibria in the proceeding of metabolic reactions [28-35]. Vitamins and glycine possess biological importance. They have possible application in medicine if these therapeutic bio-ligands and other ligands are combined in one compound. There is increasing interest in the chemical modeling of transport and storage of metal ions in living systems. Considerable attention has been paid in recent years on the study of the complexation equilibria of these ligands with different metal ions. Ternary systems are better models for complicated biological systems, as the importance of ternary complexes in biochemical systems is beyond question. So it is worthwhile to assemble information on their formation, stability and structure and on the mutual influence of two ligands bound to the same metal ion. The present manuscript concerns synthesis of twelve mixed ligand complexes involving folic acid, nicotinic acid and glycine (Scheme 1). These synthesized complexes were fully characterized using detailed spectroscopic, X-ray diffraction, and thermal analysis techniques. Finally, bioactivities of the synthesized compounds were evaluated and discussed.

2 Experimental

2.1 Materials and Chemicals

All chemicals, reagents, and solvents used for synthesis were of analytical reagent grade and were used without further purification. Glycine (G), vitamin B9 (folic acid (FA), $C_{19}H_{19}N_4O_6$) and vitamin B3 (nicotinic acid (NA) $C_6H_4NO_7$) (Scheme 1) were purchased from Sigma-Aldrich (Germany) with 99.0% purity. Copper chloride dihydrate (CuCl$_2$·2H$_2$O, 99.0% purity) was a product of Kanto Chemical Inc. (Japan). Nickel chloride hexahydrate (NiCl$_2$·6H$_2$O, 97.0% purity) was obtained from Acros Organics, USA. Cadmium chloride hemipentahydrate (CdCl$_2$·2/5H$_2$O, 98% purity) and manganese chloride (MnCl$_2$, 98% purity) were supplied by Rasayan Laboratories, India. Iron(III) salt (Fe(NO$_3$)$_3$·9H$_2$O, 97% purity) and lead(II) nitrate (Pb(NO$_3$)$_2$, 99.999% purity) salts were purchased from Sigma-Aldrich, UK.

2.2 Synthesis of Mixed Ligand Complexes

A series of transition metal mixed ligand complexes of nicotinic acid were synthesized according to the following general procedure: Metal salt (1.344 g CuCl$_2$·2H$_2$O, 3.907 g Ni(NO$_3$)$_2$·6H$_2$O, 4.04 g Fe(NO$_3$)$_3$9H$_2$O, 3.283 g CdCl$_2$·2/5H$_2$O, 3.312 g Pb(NO$_3$)$_2$, 0.979 g MnCl$_2$) was added gradually to magnetically stirred methanol solution (20 ml) of glycine (0.750 g G). Then, to the first reaction mixture, a methanol solution (20 ml) of nicotinic acid (NA, 1.231 g) or alkaline methanol solution (20 ml) of folic acid (FA, 0.441 g) was added and stirred carefully at about 60-80 °C till the reaction reached equilibrium. Then evaporation of the solvent (by placing the reaction mixture in a fume cupboard) led to the isolation of precipitated solid complex product. The solid was filtered off, washed thoroughly with water and methanol several times to remove any traces of unreacted starting materials and finally dried in a vacuum desiccator over fused CaCl$_2$ (yield: 35-55%).
Johns melting point apparatus. A digital Elico Conductivity Bridge meter (Model No. CM-180) was used to measure the molar conductance of the free ligands and the metal mixed ligand complexes in DMSO solution with a concentration of about $1 \times 10^{-3}$ mol·dm$^{-3}$ at room temperature, using a dip-type conductivity cell fitted with a platinum electrode. Magnetic susceptibility measurements of the powdered mixed ligand complexes were performed at room temperature using a Magway MSBMk1 magnetic susceptibility balance with Hg[Co(NCS)$_2$] as the calibrant. Magnetic measurements were carried out according to the Gauy method. The calculations were evaluated by applying the following equations:

$$x_g = \frac{c(R-R_0)}{10^3M}, \quad x_m = x_g M W_t, \quad \mu_{eff} = 2.820\sqrt{x_m T},$$

Where $x$ is mass susceptibility per gram of sample; $c$ is the calibration constant; $R$ is the balance reading for the sample and tube; $R_0$ is the balance reading for the empty tube; $M$ is the weight of the sample in grams. Metal content of a complex was determined by a Buck Sciences 210VGP Atomic Absorption Spectrophotometer.

### 2.4 Spectroscopic Measurements

Vibration infrared spectral (IR) studies of all synthesized mixed ligand complexes were recorded on a Shimadzu FT-IR 8000 spectrophotometer using KBr disc medium in the range 400–4000 cm$^{-1}$ and the spectra were collected with a resolution of 2 cm$^{-1}$ with 15 scans. The $^1$H NMR and $^{13}$C NMR spectra of the mixed ligand complexes were recorded at 500 MHz on a Bruker Avance-500 spectrometer employing TMS as the internal reference to 0.0 ppm, and DMSO-d$_6$ as the solvent with a field gradient operating at 500.13 MHz for proton observation. The measurements were done at a probe temperature of about 298 K. Fast atomic bombardment mass spectra (FAB-MS) of the synthesized metal complexes were recorded on a JEOL SX-102 FAB mass spectrometer using 3-nitrobenzoylealcohol matrix.

### 2.5 X-Ray Diffraction and Thermal Measurements

Powder X-ray diffraction (XRD) pattern studies of the mixed ligand complexes were performed on a Bruker D8 advance diffractometer using Ni-filtered Cu-Kα radiation of wavelength ($\lambda$) = 1.54056 Å in the 2θ range of 10–70° with a scanning rate of 0.05°/s. The raw data was subjected to background correction and the Ka2 lines were removed using the normal stripping procedure. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) of the mixed ligand complexes were conducted using a Shimadzu (DSC-50 model) thermal analyzer in dynamic nitrogen atmosphere at a flow rate of 20 mL min$^{-1}$ with a heating rate of 20 °C/min starting from room temperature to 1000 °C.

### 2.6 Bioactivities of the synthesized complexes

#### 2.6.1 Cytotoxicity’s of the synthesized metal complexes

The tumor cell lines HEp-2 (human laryngeal carcinoma), Daoy (human medulloblastoma), MCF-7 (human breast adenocarcinoma), and WiDr (human colon adenocarcinoma) were used for cytotoxic assays. Cytotoxic assays were conducted in RPMI-1640 medium supplemented with 5% CO$_2$ in an incubator at 37 °C. Cytotoxicity assays depend on the binding of methylene blue to the fixed monolayers of cells at pH 8.5. After washing the monolayer, dye was released by lowering the pH value. The control standard drug was prepared at a concentration of 150 μg/ml. The synthesized mixed ligand complex sample was prepared in a mixture of saline and DMSO with a concentration of $1 \times 10^{-6}$ mol·dm$^{-3}$ at pH ~ 7. After seeding 2880 cells/well in a 96-well microplate to about 3 μl, 20 μl of sample or standard agent was placed in each well and incubated at 37 °C for 3 days. After removing the medium from the microplate, the cells were fixed with 10% formaldehyde in 0.9% saline for 30 min, and then dyed with 1% (w/v) methylene blue in 0.01 M borate-buffer (100 μl/well) for 30 min. The 96-well plate was dipped into a 0.01 M borate-buffer solution four times in order to remove the dye. Then, 100 μl/well of EtOH–0.1 M HCl (1:1) was added as a dye eluting solvent, and the absorbance was measured on a microtiter plate reader (Dynatech, MR 7000) at 650 nm. The IC$_{50}$ value was defined by a comparison with the untreated cells as the concentration of test sample resulting in 50% reduction of absorbance. Each experimental variable was run three times, and the results were expressed via mean value and standard deviation. A two-tailed $P$ value of less than 0.05 was considered to be statistically significant.

#### 2.6.2 Antioxidant activities of the synthesized metal complexes

Antiradical activities of the synthesized complex species were measured using the stable radical 2,2-diphenyl-1-
Picrylhydrazyl-hydrate (DPPH). All mixed ligand complex species samples were prepared in a mixture of saline and DMSO with a concentration of $1 \times 10^{-4}$ mol·dm$^{-3}$ at pH ~ 7. About 50 μl of the complex species sample was placed in a 1 cm cuvette, and 2 ml of a methanolic solution of DPPH ($6 \times 10^{-5}$ mol/l) was added. Absorbance was immediately measured. The decrease in absorbance at 515 nm was determined continuously with data acquisition at 2 s intervals with a spectrophotometer until the absorbance stabilized (16 min). The absorbance of the DPPH radical without antioxidant (control) was measured daily. Special care was taken to minimize the loss of free radical activity of the DPPH radical stock solution as recommended. The percent inhibition of the DPPH radical by gallic acid (GA) and tested compounds was calculated according to the formula:

$$\text{Percent inhibition} = \left( \frac{A_{\text{C}(0)} - A_{\text{C}(t)}}{A_{\text{C}(0)}} \right) \times 100,$$

Where $A_{\text{C}(0)}$ is the absorbance of the control at $t = 0$ and $A_{\text{C}(t)}$ is the absorbance of the reaction solution at $t = 16$ min. Each experimental variable was run three times, and the results were expressed via mean value and standard deviation. A two-tailed $P$ value of less than 0.05 was considered to be statistically significant.

### 2.6.3 Statistical analysis

The above experimental measurements used for quantitative analysis were repeated at least three times. The experimental data were analyzed by a parametric T test (two tailed), and $P$ values (calculated probability) less than 0.05 were considered to be statistically significant. When more than two experimental sets were analyzed, an ANOVA test with the SPSS 17.0 statistical package was also used to evaluate the statistical significance.

### 3 Results and Discussion

#### 3.1 Physical properties of the synthesized complexes

Vitamins (nicotinic acid (NA) and folic acid (FA)) and a glycine peptide unit (G), as well as their Cu(II), Ni(II), Fe(III), Pb(II), Cd(II), and Mn(II) mixed ligand complexes were subjected to elemental analysis. Physico-chemical analytical data of elemental analyses, metal ion percentage with empirical molecular formula, colors, melting points, conductivities, and magnetic susceptibility are summarized in Table 1. The analytical and physical experimental data are in acceptable agreement with the calculated values as expected for the assigned empirical formula shown in Table 1. Elemental analytical data of all complexes show 1:1:1 molar ratio (vitamin: metal: glycine) and correspond well with the general formulas: ([M(NA/FA)(G)(Cl)]$_2$H$_2$O (M = Cu(II), Cd(II), and Mn(II)) and ([M(NA/FA)(G)(NO$_3$)]$_2$H$_2$O (M = Fe(III), Ni(II), and Pb(II)). The metal mixed ligand complexes have melting points ranging from 265 to 298 °C for the nicotinic acid mixed ligand complexes, while melting points range from 315 °C – 342 °C for the folic acid mixed ligand complexes. During our conductometric measurements, the lowest specific conductivity reported for pure DMSO is $2 \times 10^4$ ohm$^{-1}$ cm$^{-1}$. Since water dissociates more readily than DMSO, the specific conductivity will be a measure of the concentration of both water and other ionizable substances. Molar conductivities for the Cu(II), Ni(II), Fe(III), Pb(II), Cd(II) and Mn(II) mixed ligand complexes ($1.0 \times 10^3$ mol/cm$^3$) were found between 43 and $83 \Omega^{-1}$ cm$^3$ mol$^{-1}$ suggesting an acceptable electrolytic nature [20,36] and providing a method for testing the degree of ionization of the complexes. The observed decreasing in the electrolytic nature of the mixed ligand complexes is due to the presence of chloride or nitrate ion inside the coordination sphere, which is strongly supported by the elemental analysis data [37-39]. The absence or presence of chloride ions inside or outside the coordination sphere of Cu(II), Cd(II), and Mn(II) mixed ligand complexes was detected by adding a few drops of saturated silver nitrate (AgNO$_3$) reagent leading to the formation of white precipitate [37-39].

The magnetic susceptibilities ($\mu_{\text{eff}}$) of the Cu(II), Ni(II), Fe(III), Pb(II), Cd(II), and Mn(II) mixed ligand complexes at room temperature were found to be consistent with low spin distorted octahedral structures [40-48] having two water, two chloride (in case of Cu(II), Cd(II), and Mn(II) complexes), or two nitrate molecules (for Co(II), Ni(II), and Fe(III)) coordinated to central metal ions (Table 1) [40-48].

#### 3.2 Structural properties of the synthesized complexes

Elucidation of the molecular structures of the synthesized mixed ligand complexes were confirmed by detailed spectroscopic IR and XRD techniques. The synthesized metal ions mixed vitamin/glycine complexes were found to be stable at room temperature. They have different colors and are partially soluble in D$_2$O, soluble in DMSO and DMF solvents.
Table 1: Analytical and physicochemical data (color, melting point, elemental analysis, and molar conductance value) of mixed glycine (G) – vitamin (NA/FA) – metal drug complexes.

| Ligands/Complexes Empirical Formula | Color       | Molecular Weight (g/mol) | Melting Point (°C) | Conductivity (Ω cm² mol⁻¹) μ_{eff} (B.M.) | Elemental analysis (%) found (calculated) |
|-----------------------------------|-------------|--------------------------|--------------------|-------------------------------------------|-----------------------------------------|
| G = C₇H₈N₂O₅                        | White       | 75.3                     | 235                | ---                                        | 31.87 6.64 18.59 42.5 ---                |
| NA = C₇H₈N₂O₅                      | White       | 123.03                   | 238                | ---                                        | 58.52 4.06 11.38 26.01 ---               |
| FA = C₇H₈N₂O₅                      | Yellow      | 441.14                   | 286                | ---                                        | 51.68 4.31 22.22 21.76 ---               |
| [CuNAGCl]Cl·2H₂O                   | Turquoise   | 374.56 (363.8)           | 298 64             | 1.7865 (17.5)                              | 28.31 3.87 7.42 25.21 19.78             |
| [NiNAG(NO₃)]₂·2H₂O                 | Pale Green  | 429.26 (413.3)           | 277 57             | 1.5674 (14.3)                              | 23.27 2.54 13.21 46.11 ---              |
| [FeNAG(NO₃)]₂·2H₂O                 | Dark brown  | 427.16 (410.3)           | 282 80             | 1.9874 (13.6)                              | 23.76 2.75 14.21 47.01 ---              |
| [PbNAG(NO₃)]₂·2H₂O                 | White       | 579.3 (561.3)            | 291 53             | 1.9876 (36.9)                              | 17.64 2.14 10.32 34.72 ---              |
| [CdNAGCl]Cl·2H₂O                   | Yellowish   | 422.54 (412.3)           | 265 49             | 1.3452 (27.2)                              | 23.86 2.89 6.32 23.81 16.34              |
| [MnNAGCl]Cl·2H₂O                   | Pale Pink   | 359.18 (355.3)           | 275 43             | 1.9805 (15.5)                              | 27.63 3.21 7.93 27.74 18.45             |
| [CuFAGCl]Cl·2H₂O                   | Turquoise   | 671.18 (680.94)          | 322 78             | 1.6753 (9.33)                              | 37.43 3.98 16.23 23.98 11.12            |
| [NiFAG(NO₃)]₂·2H₂O                 | Green       | 720.04 (730.44)          | 323 69             | 1.7592 (8.08)                              | 34.75 3.54 18.43 35.28 ---              |
| [FeFAG(NO₃)]₂·2H₂O                 | Yellowish   | 718.20 (727.44)          | 342 83             | 1.9591 (7.7)                               | 34.75 3.43 19.56 35.78 ---              |
| [PbFAG(NO₃)]₂·2H₂O                 | Brown       | 870.14 (878.44)          | 329 57             | 1.6753 (23.6)                              | 28.45 2.87 16.23 30.12 ---              |
| [CdFAGCl]Cl·2H₂O                   | Yellowish   | 720.25 (729.84)          | 315 54             | 1.8975 (15.4)                              | 33.97 3.65 15.34 22.16 9.76             |
| [MnFAGCl]Cl·2H₂O                   | Pale Pink   | 661.28 (672.44)          | 325 48             | 1.6784 (8.18)                              | 37.65 3.76 17.21 23.54 10.21            |

Attempts were made to obtain suitable crystals for single X-ray crystallography. Only one (copper – nicotinic–glycine complex crystal nodule) was obtained. Therefore, the proposed coordination modes of the resulted complexes are dependent on the routine spectral analysis.

Essential infrared vibrational spectroscopic frequencies of the synthesized mixed ligand complexes data are summarized in Tables 2 and 3. Infrared spectral absorption patterns of these mixed ligand complexes are shown in appendixes S1- S12.

Careful inspection of the IR spectra of free NA, FA and G [21-25,49,50], and their mixed ligand complexes was performed in order to facilitate the assignment of these bands in the free ligand and its metal mixed ligand complexes as per the following:

1. The strong-to-broad bands for all synthesized complexes existing in the region ca. 3000–3300 cm⁻¹ were assigned to the δ(OH) vibrations of water molecules in all metal complexes.
2. The vibration spectra of ν(NH) in the amide group in free NA appeared at 3375 and 3175 cm⁻¹, respectively. These stretching bands of the amide group in Cu(II), Ni(II), Fe(III), Pb(II), Cd(II), and Mn(II) mixed ligand complexes were split and appeared in the 3344–3648 cm⁻¹ region at near or higher wavenumber than comparative bands in the free NA because of hydrogen bonding [21-25, 49,50].
3. The stretching band of the amide carbonyl group, ν(C=O) in the free NA was observed at 1718 cm⁻¹, and located in the range 1698 to 1718 cm⁻¹ for different Cu(II), Ni(II), Fe(III), Pb(II), Cd(II), and Mn(II) mixed ligand complexes. The binding of amide and carbonyl compounds towards metal ions resulted in a significant blue shift in the frequency of carbonyl group [21-25, 49,50].
4. In NA complexes the stretching vibration band of carbonyl group has an insignificant blue shift. The \( \nu(NH) \) frequencies remained fixed or red shifted compared with the free NA ligand, indicating that the carbonyl oxygen of the NA ligand is not involved in the coordination process [21-25, 49,50].

5. The pyridine ring vibrations of free NA at 1598, 1580 and 1484 cm\(^{-1}\) were distorted in the spectra of metal complexes indicating that pyridine nitrogen is coordinated. The low intensity bands in the region of 550–400 cm\(^{-1}\) were assigned to MN and MO vibrations [21-25,49,50]. The infrared spectra of hydrated complexes have broad bands overlapped with the stretching vibration bands of NH\(_2\) of amide groups [21-25,49,50].

6. Folic acid exhibits a very strong absorption band at 1650 cm\(^{-1}\) due to the stretching vibration of \( \nu(C=O) \) of the free ketonic of the carboxylic group. This group shifted or disappeared in the spectra of its complexes [21-25,49,50].

7. Interestingly, there are two bands which appeared at the range of 1500–1580 cm\(^{-1}\) and corresponded to \( \nu_{11}(COO^-) \). The other band exhibited in the range of 1485-1550 cm\(^{-1}\)was assigned to \( \nu_{12}(COO^-) \) [21-25,49,50].

8. The direction of frequency shift of the \( \nu_{11}(COO^-) \) and the \( \nu_{12}(COO^-) \) bands with respect to those of the free ion depends on the coordination mode of the COO\(^-\) group with metal ion [26,27]. Upon complexation, these strong bands were shifted and broadened with respect to the corresponding bands in the free ligand. The present bands of the carboxylate COO\(^-\) group are reflected by IR spectrum of the asymmetric \( \nu_{as}(COO^-) \) and symmetric \( \nu_{s}(COO^-) \) stretching vibrations [21-27,49-61].

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because of the mixed ligand complex formation [62,63] (Scheme 2).

X-ray powder diffraction patterns (appendices S19-S30) in the range of 4° < 2θ < 80° for the synthesized mixed ligand complexes were recorded in order to understand the lattice dynamics of the resulted complexes. X-ray diffraction powder patterns show that all the synthesized complexes have a crystalline structure. The crystallinity of these complexes must be due to the precipitation conditions, which were not controlled.

| Bands       | Ligands / Complexes Empirical Formula          |
|-------------|-----------------------------------------------|
| v(M-O)      | ---                                           |
| v(M-N)      | ---                                           |
| v(M-Cl)     | ---                                           |
| δ(C-C)      | 750(vs)                                       |
| v(C-C)      | 970(s)                                        |
| v(C-N)      | 1100(s)                                       |
| v(C-N)      | 1120(s)                                       |
| v(C-N)      | 1130(s)                                       |
| v(C-N)      | 1160(s)                                       |
| v(C-N)      | 1220(s)                                       |
| v(C-C)      | 1300(s)                                       |
| δ(CH)       | 1410(s)                                       |
| v(CO)       | 1485(s)                                       |
| δ(NH)       | 1520(s)                                       |
| v(CO)       | 1570(s)                                       |
| v(C=O)amide| 1650(s)                                       |
| v(OH)       | 1720(s)                                       |
| v(OMd)      | 2850(w)                                       |
| v(NH) amide | 3230(w)                                       |
| v(NH)       | 3300(w)                                       |
| v(OH)       | 3520(w)                                       |
| v(OH)       | 3415(w)                                       |

* (vs = very strong), *(s = strong), *(w = weak),

| Bands       | Ligands / Complexes Empirical Formula          |
|-------------|-----------------------------------------------|
|bands        | [CuFAGCl₂]·2H₂O                                |
|bands        | [NiNAG(NO₃)₂]·2H₂O                            |
|bands        | [FeNAG(NO₃)₂]·2H₂O                            |
|bands        | [PbNAG(NO₃)₂]·2H₂O                            |
|bands        | [CdNAGCl₂]·2H₂O                               |
|bands        | [MnNAGCl₂]·2H₂O                               |

3.3 Thermal properties of the synthesized complexes

Thermogravimetric analysis (TGA) and derivative thermogravimetric analysis (DrTGA) of free ligands NA, FA and G were reported previously [51-61]. TGA and DrTGA thermal analysis of transition metal complexes [CuNAGCl₂]·2H₂O,[FeNAG(NO₃)₂]·2H₂O and [CuFAGCl₂]·nH₂O were carried out under N₂ flow in order to confirm their structures. The TGA and DrTGA measurements curves are shown in Appendices S31-S33 and the results are interpreted in Table 4. From the measurements, we can conclude that:

1. Thermal decomposition of the [CuNAGCl₂]·2H₂O mixed ligand complex occurred mainly in three decomposition stages. The first stage of decomposition occurred at a temperature maximum of 111 °C. The
weight loss associated with this stage (4%) may be attributed to the loss of two water molecules with endothermic peak at DrTGA$_{\text{max}}$ at 84 °C. The second stage of decomposition occurred at a temperature maximum of 230 °C. The weight loss at this step (11%) corresponds to the loss of glycine molecule with endothermic peak at DrTGA$_{\text{max}}$ at 206 °C. The final stage occurred in the range of 253-432 °C corresponds to the loss of nicotine and chlorine molecules with a weight loss of 64%. The final thermal products obtained at temperature higher than 450 °C are copper metal and carbon residues.

2. Thermal degradation of the [FeNAG(NO$_3$)$_3$]·2H$_2$O complex occurred also mainly in three degradation stages. The first stage occurred at a temperature maximum of 162 °C. The weight loss associated with this step (5%) may be attributed to the loss of 2H$_2$O with endothermic peak at DrTGA$_{\text{max}}$ at 75 °C. The second stage occurred at a temperature maximum of 335 °C. The weight loss found was 41% corresponding to the loss of G moiety with endothermic peak at DrTGA$_{\text{max}}$ at 254 °C. The final stage occurred at 355-520 °C and corresponds to the loss of NA moiety and nitrite molecule (weight loss of about 30%) with endothermic peak at DrTGA$_{\text{max}}$ at 342 °C. The final thermal products obtained at 550 °C are iron and carbon residues.

3. Thermal decomposition of the [CuFAGCl]·2H$_2$O complex occurred in three stages. The first stage happened at 36-164 °C corresponding to the loss of water molecules (weight loss of about 7%) with an endothermic peak at DrTGA$_{\text{max}}$ at 145 °C. The second and third stages occurred at 181-318 °C and 332-501 °C, respectively corresponding to the loss of glycine organic molecule, two chloride molecules and FA molecules; the weight loss is about 48% and 42%, respectively. Copper and carbon residues were the final ones that remained stable till 600 °C.

### 3.4 Bioactivities of the synthesized complexes

#### 3.4.1 Cytotoxicity’s of the synthesized metal complexes

Some vitamins are efficient anti-proliferative and cytotoxic agents and their effect on healthy cells is reversible upon drug removal. Several metal ion complexes were studied seeking the cytotoxic activities, under the hypothesis that endogenous metal ions may be less toxic than the known metal complexes used as anticancer drugs, such as cisplatin [64-67]. From the results obtained (Table 5), it is possible to conclude that the nature of the vitamin ligands (e.g., the number of N atoms), the characteristics of the leaving groups at some metal ions, the number and coordination mode of the metal ions, and the chemical environment, determine the bioactivities of the synthesized complexes, most probably through inducing DNA structural rearrangements. Thus the design of new, more effective bioactive drugs should be governed by these crucial factors since slight changes in metal coordination are sufficient to significantly change the in vitro antiproliferative and/or cytotoxic properties of these complexes (appendix S34). Among the complex species tested against the four human cancer cell lines (Hep-2 human laryngeal carcinoma, Daoy human medulloblastoma, MCF-7 human breast adenocarcinoma, and WiDr human colon adenocarcinoma), chromium complexes of NA and FA showed weak cytotoxic activities. It was observed that all mixed ligand complexes of vitamins exhibited moderate cytotoxic activities against the four cancer cell lines, with the exception of the lead complex species which showed weak cytotoxic activity (Table 5).

#### 3.4.2 Antioxidant activities of the synthesized metal complexes

Antioxidant activity is one of the important standard assays in pharmaceutical science to examine the potency of a compound to inhibit the formation of free radicals. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a popular “stable free-radical” for antioxidant assay. DPPH has an unpaired electron at one atom of nitrogen bridge. The active DPPH radical has a strong absorption band at 517 nm and a violet color in solution [68,69]. The inactivation or neutralization of DPPH by antioxidants causes the band at 517 nm to decrease and the solution color becomes pale yellow as shown below in Scheme 3 [68,69].

It was known that metal ions play crucial role in various enzymes that catalyze oxidation/reduction
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Reactions correlated with the antioxidant system of the organism concerned (appendix S35). However, different behaviors depend on the chemical environment and the nature of the chelating agent. In the present work, most metal complexes of NA and FA showed moderate antioxidant activity, while cadmium and lead complexes of both vitamins exhibited weak antioxidant activities (Table 6). The increase in inhibition of the synthesized ternary complexes is probably related to the formation of the chelate complex.

Table 4: TGA/DTG analysis of mixed glycine (G) – vitamins (NA/FA) – metal drug complexes.

| Metal Complexes Empirical Formula | Decomposition stages | Temperature range (°C) | DTGmax (°C) | Weight loss (%) | Lost species Assignments | Metallic Residues |
|-----------------------------------|----------------------|------------------------|-------------|-----------------|--------------------------|-------------------|
| [CuNAGCl<sub>2</sub>·2H<sub>2</sub>O] | 1<sup>st</sup> stage | 55-111 | 84 | 5(4.87) | 2H<sub>2</sub>O | Metal and carbon residues |
|                                    | 2<sup>nd</sup> stage | 167-230 | 206 | 9(8.76) | G + Cl<sub>2</sub> | NA |
|                                    | 3<sup>rd</sup> stage | 253-432 | 384 | 64(65.27) | NA | NA |
| [FeNAG(NO<sub>3</sub>)<sub>2</sub>·2H<sub>2</sub>O] | 1<sup>st</sup> stage | 56-162 | 75 | 4(5.49) | 2H<sub>2</sub>O | Metal and carbon residues |
|                                    | 2<sup>nd</sup> stage | 170-335 | 254 | 46(47.01) | G | NA |
|                                    | 3<sup>rd</sup> stage | 355-520 | 305 | 98(96.92) | NA | NA |
| [CuFAGCl<sub>2</sub>·2H<sub>2</sub>O] | 1<sup>st</sup> stage | 36-164 | 145 | 4(3.27) | 2H<sub>2</sub>O | Metal and carbon residues |
|                                    | 2<sup>nd</sup> stage | 181-318 | 252 | 48(47.23) | G + Cl<sub>2</sub> | NA |
|                                    | 3<sup>rd</sup> stage | 332-501 | 413 | 90(91.26) | FA | NA |

Table 5: Cytotoxicities of mixed glycine (G) – vitamin (NA/FA) – metal drug complexes.

| Ligands/Complexes | Cancer cell line<sup>a</sup> / IC<sub>50</sub> (μg/ml)<sup>b</sup> | HEp-2 | Daoy | MCF-7 | WiDr |
|-------------------|-------------------------------------------------|-------|------|-------|------|
| G = C<sub>2</sub>H<sub>5</sub>NO<sub>2</sub> | 135.33±2.76 | 32.42±1.76 | 57.96±2.93 | 32.56±1.56 |
| NA = C<sub>6</sub>H<sub>5</sub>NO<sub>2</sub> | 2.611±0.23 | 22.36±1.97 | 2.59±0.97 | 23.63±0.89 |
| FA = C<sub>19</sub>H<sub>19</sub>N<sub>7</sub>O<sub>6</sub> | 456.7±23.65 | 29.22±2.01 | 24.09±1.67 | 35.23±2.09 |
| [CuNAGCl<sub>2</sub>·2H<sub>2</sub>O] | 3.54±0.18 | 31.41±2.13 | 13.22±1.03 | 13.26±0.56 |
| [NiNAG(NO<sub>3</sub>)<sub>2</sub>·2H<sub>2</sub>O] | 32.15±2.98 | 13.11±0.98 | 13.16±0.45 | 3.53±0.07 |
| [FeNAG(NO<sub>3</sub>)<sub>2</sub>·2H<sub>2</sub>O] | 20.16±1.02 | 29.41±2.93 | 222.23±12.56 | 378.63±12.90 |
| [PbNAG(NO<sub>3</sub>)<sub>2</sub>·2H<sub>2</sub>O] | 3456.31±78.98 | 441.76±12.34 | 17.83±0.78 | 87.32±2.09 |
| [CdNAGCl<sub>2</sub>·2H<sub>2</sub>O] | 56.36±4.78 | 342.49±9.98 | 20.36±1.09 | 332.56±12.89 |
| [MnNAGCl<sub>2</sub>·2H<sub>2</sub>O] | 53.25±2.34 | 13.27±0.97 | 32.79±1.78 | 33.58±1.34 |
| [CuFAGCl<sub>2</sub>·2H<sub>2</sub>O] | 893.15±32.79 | 34.71±1.21 | 32.69±1.35 | 33.73±2.16 |
| [NiFAG(NO<sub>3</sub>)<sub>2</sub>·2H<sub>2</sub>O] | 36.16±1.23 | 35.41±2.03 | 33.45±1.89 | 3.76±0.12 |
| [FeFAG(NO<sub>3</sub>)<sub>2</sub>·2H<sub>2</sub>O] | 3.65±0.67 | 33.76±2.34 | 3323.44±12.98 | 3.52±0.15 |
| [PbFAG(NO<sub>3</sub>)<sub>2</sub>·2H<sub>2</sub>O] | 16.36±0.67 | 1.49±0.23 | 14.46±0.57 | 31.36±2.30 |
| [CdFAGCl<sub>2</sub>·2H<sub>2</sub>O] | 36.25±1.87 | 33.27±1.25 | 33.23±0.35 | 333.48±23.90 |
| [MnFAGCl<sub>2</sub>·2H<sub>2</sub>O] | 12.36±0.67 | 2.493±0.78 | 1347.10±11.89 | 43.86±2.23 |

<sup>a</sup>IC<sub>50</sub> value is the molarity at which 50% of tumor cell death was observed after 72 h under standard tissue culture conditions. Each experimental variable was run three times, and the results were expressed via mean value and standard deviation. A two-tailed P value (calculated probability) of less than 0.05 was considered to be statistically significant.

<sup>b</sup>Cancer cell lines: HEp-2 (human laryngeal carcinoma), Daoy (human medulloblastoma), MCF-7 (human breast adenocarcinoma), and WiDr (human colon adenocarcinoma) tumor cell lines.
Scheme 3: DPPH neutralization by antioxidant [68].

Table 6: DPPH radical scavenging assay of mixed glycine (G) – vitamin (NA/FA) – metal drug complexes.

| Ligands/Complexes | DPPH inhibition (%) |
|-------------------|---------------------|
| GA = C₅H₇O₆        | 92.85 ± 0.10        |
| G = C₅H₄NO₂        | 48.24 ± 1.14        |
| NA = C₅H₆NO₂       | 39.21 ± 1.15        |
| FA = C₅H₄N₂O₆      | 42.75 ± 1.42        |
| [CuNAGCl₂]·2H₂O    | 59.53 ± 1.62        |
| [NiNAG(NO₃)₂]·2H₂O | 63.35 ± 1.25        |
| [FeNAG(NO₃)₂]·2H₂O | 66.46 ± 2.47        |
| [PbNAG(NO₃)₂]·2H₂O | 43.78 ± 1.34        |
| [CdNAGCl₂]·2H₂O    | 27.66 ± 1.42        |
| [MnNAGCl₂]·2H₂O    | 33.67 ± 1.13        |
| [CuFAGCl₂]·2H₂O    | 43.39 ± 1.85        |
| [NiFAG(NO₃)₂]·2H₂O | 49.52 ± 2.17        |
| [FeFAG(NO₃)₂]·2H₂O | 47.23 ± 2.04        |
| [PbFAG(NO₃)₂]·2H₂O | 37.36 ± 1.12        |
| [CdFAGCl₂]·2H₂O    | 23.85 ± 1.13        |
| [MnFAGCl₂]·2H₂O    | 76.36 ± 2.47        |

* Each experimental variable was run three times, and the results were expressed via mean value and standard deviation. A two-tailed P value (calculated probability) of less than 0.05 was considered to be statistically significant. *AA = (Gallic Acid) standard antioxidant reference compound.

4 Concluding Remarks and Research Highlights

Twelve novel ternary vitamin complexes were synthesized, and fully characterized. Cytotoxic and antioxidant activities of the synthesized complexes were evaluated. Most of the synthesized metal complexes showed moderate cytotoxic and antioxidant activities.

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Supplementary Data: IR, 1H, 13C NMR spectrums, X-ray diffraction analysis and bioactivities of the synthesized complexes were supplied from S1 to S35 supplementary figures.

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