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Synthesis and Spectroscopic Characterization of Calcium, Iron and Zinc Bisglycinates: Application for Nutrient Fixation in Maize Plant

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ABSTRACT
Bisglycinate complexes of calcium, iron and zinc were synthesized in a 1:2 (M-L) ratio and characterized using FTIR, UV-vis, XRD and XRF spectroscopic methods. The metal complexes were found to be slightly soluble in water, ethanol and methanol, but insoluble in diethyl ether, acetone and chloroform. They were very soluble in DMSO studied attemperature of 25^oC. The melting point of the complexes ranges from 290 – 295^0C, 285 – 288^0C and 240 – 244^0C for calcium, iron, zinc bisglycinate and the ligand respectively. A bathochromic shift was observed for the UV-Vis absorption results, and indication of possible coordination of the ligand to the metal ion. The XRD result revealed the crystalline nature because of the sharp peaks observed. Distinct peaks position were observed at 2-theta for calcium bisglycinate (25.1, 31.30, 35.00, 37.72), iron bisglycinate (20.31, 25.01, 30.32, 32.22)and zinc bisglycinate (20.00, 23.00, 28.21, 38.01) as in the spectra. The crystallographic examinations indicate that the complexes exhibit a square planar geometry for calcium complex and octahedral geometries for iron and zinc complexes.The XRF results showed zinc (36.23%), iron (15.04%) and calcium (27.77%),a confirmation that the complexes are that of those of the three elements, calcium, zinc and iron.The synthesized complexes were compared with conventional inorganic fertilizers (NPK) on maize growth and discovered to all have positive effects on the growth compared to the ones without fertilizers. This, if optimised can go a long way in tackling stunted growth chlorosisin plants.

KEYWORDS*: Synthesis; zinc bisglycinate; calcium bisglycinate; iron bisglycinate;*

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I. INTRODUCTION

Metal chelate compounds are common components of fertilizers to provide micronutrients. These micronutrients (manganese, iron, zinc, calcium and copper) are required for the health of the plants. Their deficiencies result in yellowing of leaves, retarded growth and general low quality crops. Chelated compounds are more stable than non-chelated compounds. Therefore, metallic chelates are widely used in agriculture as micronutrient fertilizers to supply plants with Iron, Manganese, Zinc, Calcium and Copper (Clemens, 2001).

Plants require a range of transition metals as essential micronutrients for normal growth and development. These metals are essential for most redox reactions which, in turn, are fundamental to cellular function. Iron (Fe) is a key component of haem proteins (e.g. cytochromes, catalase, and Fe-S proteins such as ferredoxin) and a range of other enzymes. Cu is an integral component of certain electron transfer proteins in photosynthesis (e.g. plastocyanin) and respiration (e.g. cytochrome *c* oxidase) and is involved in lignification (laccase), while Mn is less redox active but is also involved in photosynthesis (e.g. O_2 evolution). Zn is non-redox-active but has a key structural and/or catalytic role in many proteins and enzymes. Other transition metals such as Ni and Mo are also essential micronutrients for plant function. When any of these metals are present in short supply, a range of deficiency symptoms can appear and growth is reduced (Marschner,1995). However, although essential, these metals can also be toxic when present in excess with the production of reactive oxygen species and oxidative injury being particularly important (Schützendübel and Polle, 2002). Thus their concentrations within cells must be carefully controlled, and so plants and other organisms possess a range of potential mechanisms for metal ion homeostasis and tolerance, including membrane transport processes (Clemens, 2001; Hall, 2002). Thus for healthy plant growth and development, a range of transition metals must be acquired from the soil, transported around the plant, and distributed and compartmentalized within different tissues and cells. Clearly membrane transport systems are likely to play a key role in these events.

Mineral deficiency is the situation where a plant cannot get enough of a particular mineral from the soil for healthy growth. Whether you're growing in soil or you're using a soilless medium like a hydroponic system, the metals in your feeding program all help your plants taking in nutrients, producing chlorophyll, and helping to regulate your plants' metabolism. While it can be tricky for plants to have a deficiency in their metals, too much or too little of vital metals means plants won't be able to create and process chlorophyll which means your plants won't eat (Hall, 2002). In this research, calcium bisglycinate, iron bisglycinate and zinc bisglycinate were synthesised and applied onto a maize plant for growth improvement and comparison with conventional fertilizers.

II. MATERIALS AND METHODS

The materials/apparatus used for this study were in good condition and the reagents/chemicals employed were of analytical grade.A slight modification of the method of Chen *et al*., 2011was employed for the synthesis of the complexes.

Synthesis of Calcium Bisglycinate

Exactly 5.0g of glycine was dissolved into 25.26 grams of water, once the glycine was significantly dissolved, about 1.84 grams of calcium oxide was added. The ratio of the glycine to the calcium oxide is 3:1. The solution was continually stirred for 15 minute until all of the calcium is dissolved. The reaction mixture was refluxed gently at 50^oC for 2hours and sprays dried, providing a calcium bisglycinate powder. The synthesized Calcium Bisglycinate was stored in a corked reagent bottle and characterized using UV-Vis, FT-IR, Crystallography, XRF and XRD spectrophotometric analysis.

$$CaO + \text{glycine} \longrightarrow Ca(\text{glycine})_2 + H_2O$$

3.2.2 Synthesis of Iron Bisglycinate

Exactly 14.0g of water was added to 4 grams of ferrous sulfate containing 20% Fe (II) by weight. The solution was stirred until the ferrous sulfate dissolved.Glycine(2.2 grams) was poured into the solution and stirred continuously for 30 minutes. To the aqueous solution was added 1 gram of calcium oxide. Again the solution was continually stirred until all of the CaOwas dissolved. As the calcium oxide went into solution, a white precipitate of calcium sulfate as well as about 3 grams of a ferrous glycine chelate having a ligand to metal molar ratio of 2: 1 was formed. The synthesized Iron Bisglycinate was stored in a corked reagent bottle and characterized using UV-Vis, FT-IR, Crystallography, XRF and XRD spectrophotometric methods.

$$FeSO_4 + \text{glycine} + CaO \longrightarrow Fe(\text{glycine})_2 + CaSO_4 + H_2O$$

3.2.3 Synthesis of Zinc Bisglycinate

Exactly 9.0g of glycine, 3.42 grams of calcium oxide, and 10.80 grams of zinc sulfate (35.5% zinc by weight) were simultaneously dissolved into 33.76 grams of water. Under no added heat, the solution was continually stirred for about 30 minutes. As the zinc sulfate and the calcium oxide went into solution, a white precipitate of calcium sulfate and 12.82 grams of a zinc glycine chelate having a ligand to metal molar ratio of 2: 1 was formed. The synthesized Zinc Bisglycinate was stored in a corked reagent bottle and characterised using UV-Vis, FT-IR, Crystallography, XRF and XRD spectrophotometric analysis.

$$ZnSO_4 + \text{glycine} + CaO \longrightarrow Zn(\text{glycine})_2 + CaSO_4 + H_2O$$

Stoichiometry of the reactions

Calcium Bisglycinate

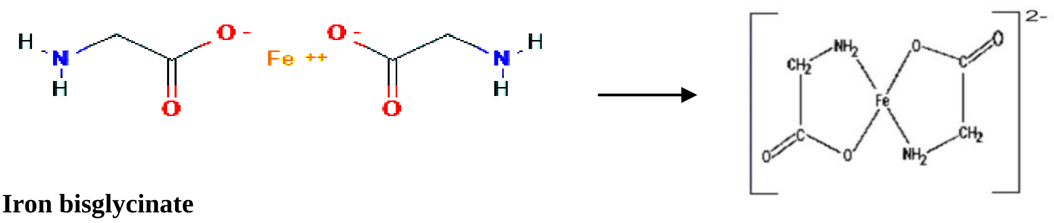
$$CaO_{(s)} + 2NH_2\text{-}CH_2\text{-}COOH_{(s)} \longrightarrow Ca(NH_2\text{-}CH_2\text{-}COO)_{2(s)} + H_2O_{(l)}$$

Ferrous Bisglycinate

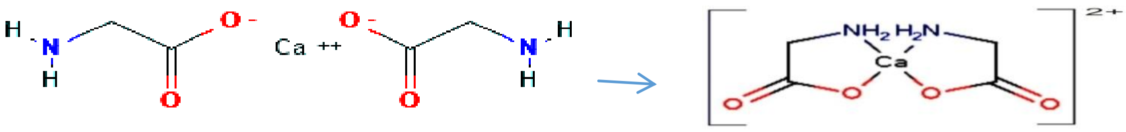
$$FeSO_{4(s)} + 2NH_2\text{-}CH_2\text{-}COOH_{(s)} + CaO_{(s)} \longrightarrow Fe(NH_2\text{-}CH_2\text{-}COO)_{2(s)} + CaSO_{4(s)} + H_2O_{(l)}$$

Zinc Bisglycinate

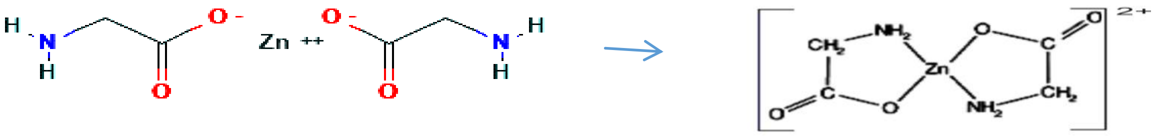
$$ZnSO_{4(s)} + 2NH_2\text{-}CH_2\text{-}COOH_{(s)} + CaO_{(s)} \longrightarrow Zn(NH_2\text{-}CH_2\text{-}COO)_{2(s)} + CaSO_{4(s)} + H_2O_{(s)}$$



Iron bisglycinate



Calcium bisglycinate



Zinc bisglycinate

Characterization of complexes

Instrumental methods were applied to characterize the complexes, the ligands and the metal salts. FT-IR,UV-vis, X-ray fluorescent (XRF), XRD and crystallographic techniques were used to characterise the reactants and products accordingly.Other physicochemical characteristics measured included conductivity, colour, solubility, pH, melting point and boiling point.

III. Results And Discussion

Table 1: Physical properties for glycine and its metal complexes

Compound	Colour	Melting point, range (℃)
$C_2H_5NO_2$	White	240 - 245
$C_4H_4CaN_2O_4$	White	290 - 295
$C_4H_8FeN_2O_4$	Light brown	285 - 288
$C_4H_8N_2O_4Zn$	White	240 - 250

Table 2: Solubility Tests for Glycine and its Metal complexes

Compound	Water	Ethanol	Methanol	Diethyl ether	Acetone	Chloroform	DMSO
$C_2H_5NO_2$	SLS	SLS	SLS	SLS	S	S	VS
$C_4H_4CaN_2O_4$	SLS	I	SLS	I	SLS	SLS	VS
$C4H_8FeN_2O_4$	I	SLS	SLS	I	SLS	I	VS
$C_4H_8N_2O_4Zn$	SLS	I	SLS	I	VS	VS	VS

KEY: SLS= Slightly soluble, S= Soluble, I= Insoluble, VS= Very soluble

From the physical properties of glycine and its metal complexes in Table 1, showed that the ligand is white in colour with melting point ranging from 240 – 245 (^{0}C). The three complexes (calcium, iron and zinc bisglycinate) has a melting point rang of 290 – 295, 285 – 288, 240 – 250 (^{0}C) with the corresponding colours white, light and white respectivelyThis confirms the completely filled d-orbital of zinc complexes. This clearly shows the ease of absorption of metals when introduced to the plants.

Table 2 showed the solubility tests for glycine and its metal complexes. All the glycine with the corresponding metal complexes were shown to be slightly soluble in water except for iron bisglycinate which appears to be totally insoluble and all were slightly soluble in methanol and very soluble in dimethyl sulphur oxide (DMSO). All the complexes appear to be insoluble in Diethyl ether except for the ligand which appears to be slightly soluble. All other description is shown in the table.

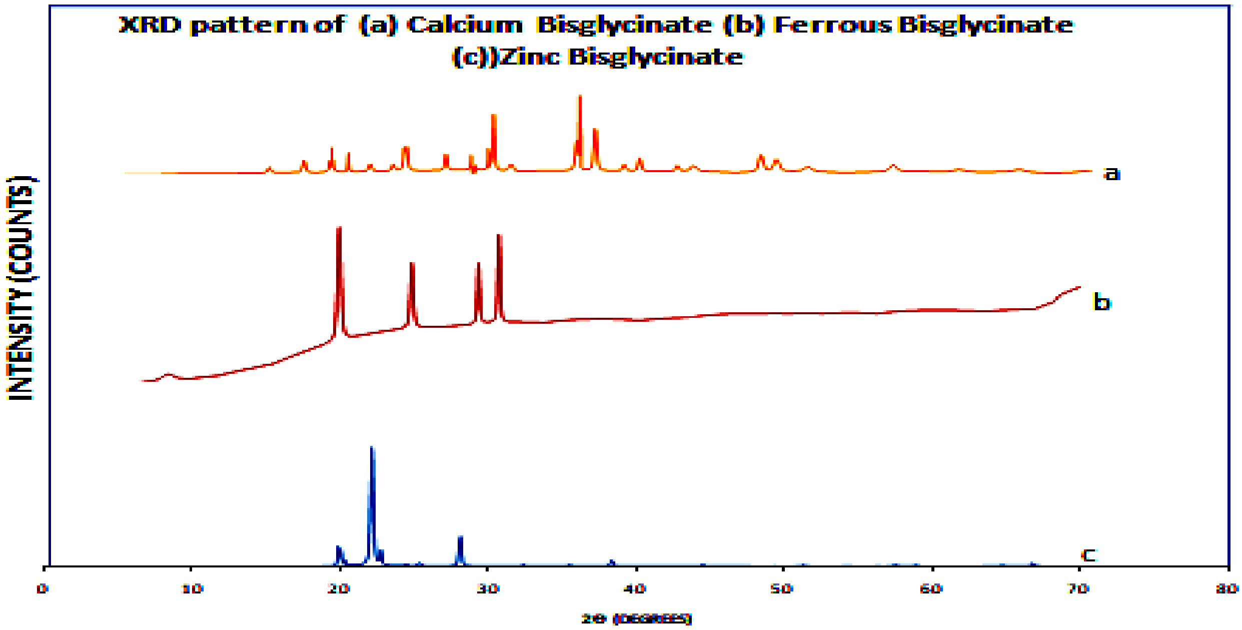
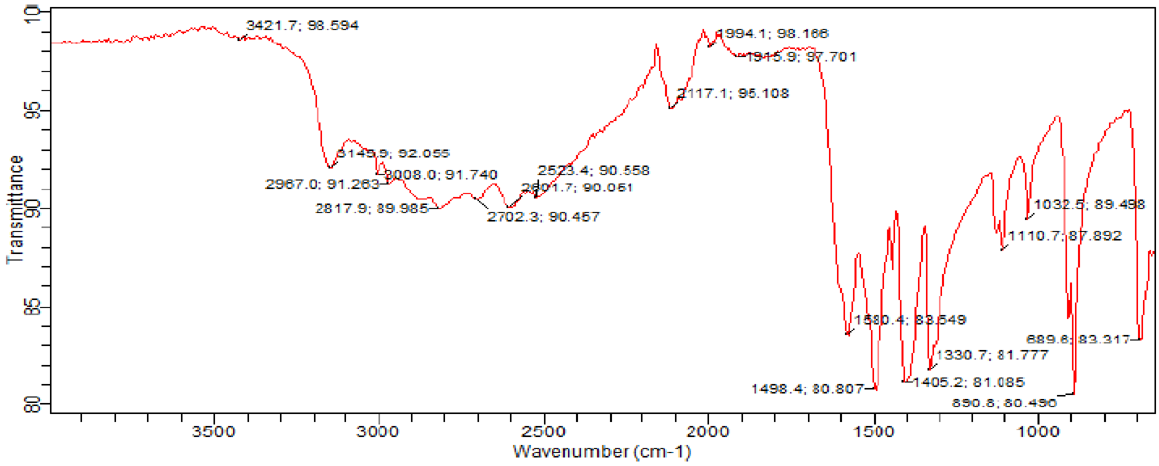


Figure 1: CombinedXRD patterns of calcium bisglycinate, ferrous bisglycinate and zinc bisglycinate

From Figure 1 above, shows the XRD spectrum of Calcium, Iron and Zinc bisglycinate made with the help of X-ray diffractometer. The Powdered XRD patterns of the metal complexes shows a sharp crystalline peaks indicating their crystalline nature. The Scherrer Equation (D = K Λ/B.cosΘ, where D = Crystalline size, Λ =wavelength of X-ray radiation) was used to relate the size of the crystallites in a solid to the broadening of a peak in a diffraction pattern, (Cu Kα – 1.54060 Å), k = constant taken as 0.94, Θ = diffraction angle, B = Full width at half maximum height. The distinct peaks at position 2-theta for the XRD pattern of calcium bisglycinate (a), iron bisglycinate (b) and zinc bisglycinate (c) as in the spectra, results to the following respective peaks at position2-theta (25.1, 31.30, 35.00, 37.72), (20.31, 25.01, 30.32, 32.22) and (20.00, 23.00, 28.21, 38.01). The full-width half maximum of the characteristic peaks estimated for the crystallites sizes of the complexes to a, b, and c is as shown in the spectra in Figure 1 above. Most of the important peaks indicate high degree of sharpness confirming the Crystallinity of the complexes.



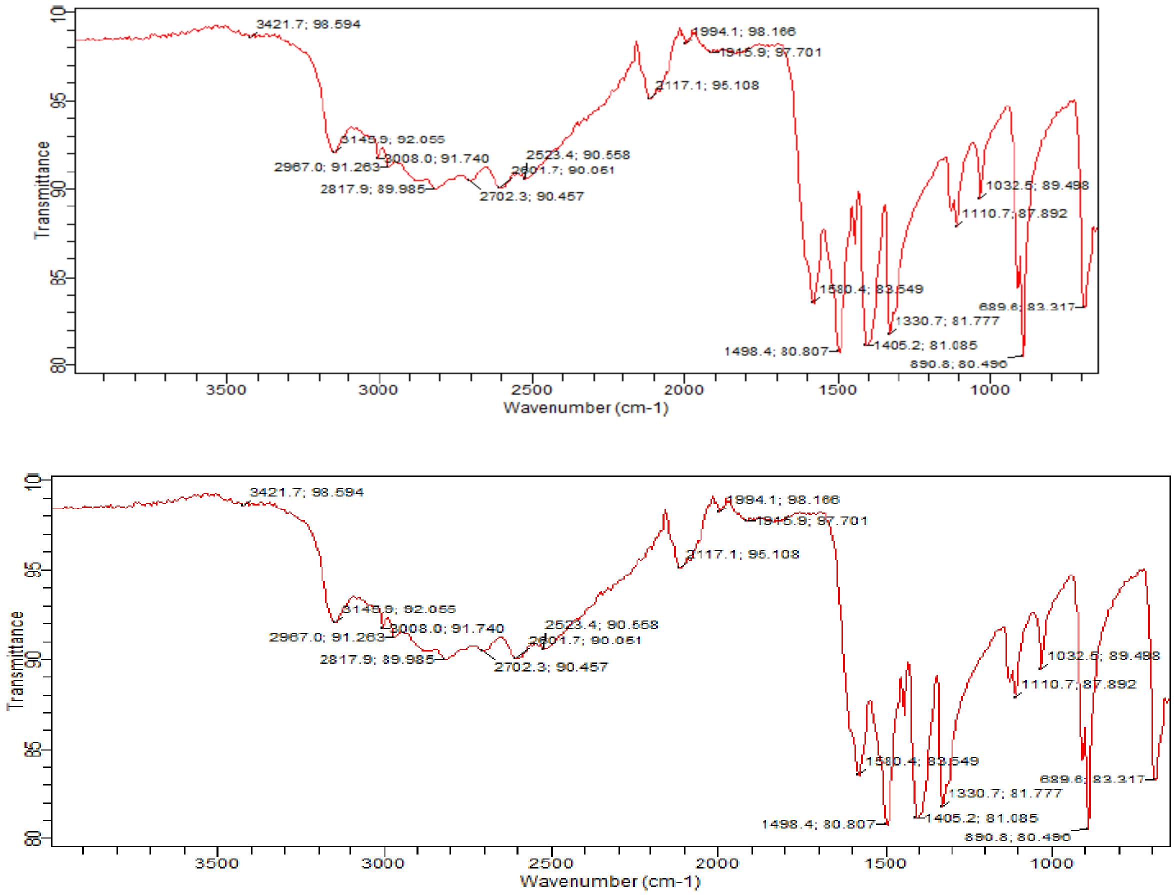


Figure 2: FT-IR Spectra of Glycine

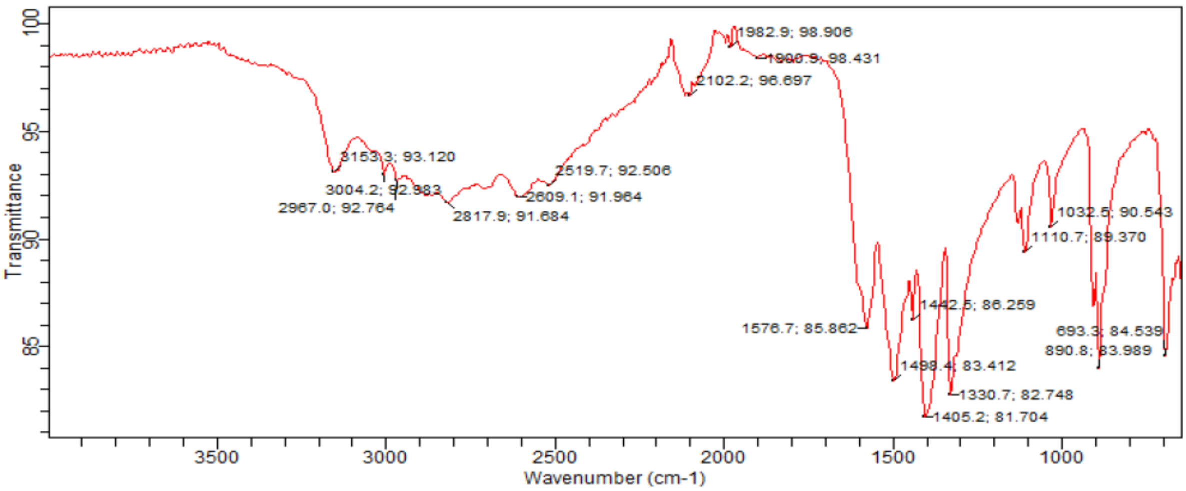


Figure 3: FT-IR Spectra of Calcium bisglycinate complex

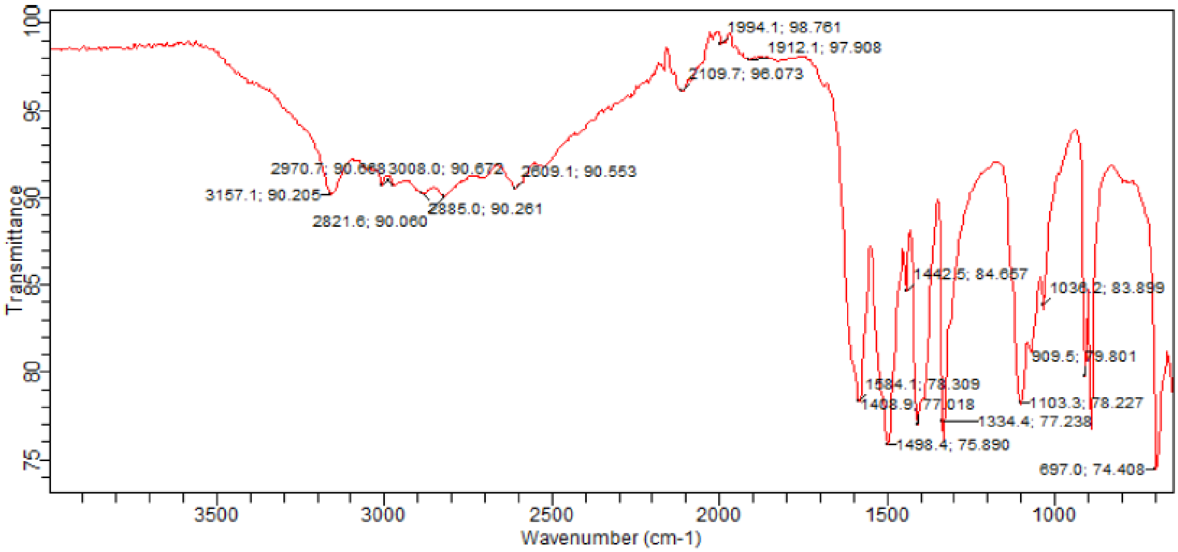


Figure 4: FT-IR Spectra of ferrous bisglycinate complex

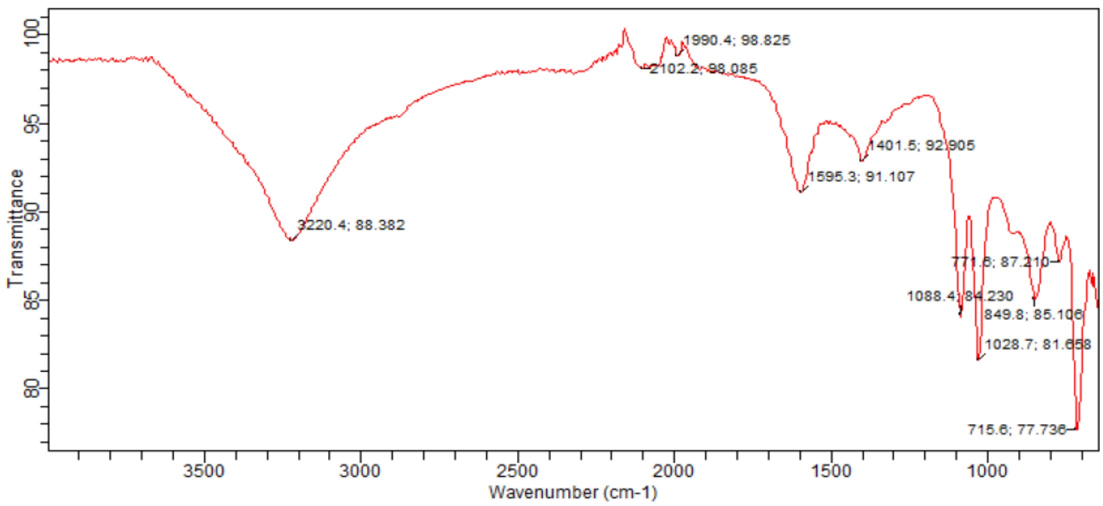


Figure 5: FT-IR Spectra of Zinc Bisglycinate

Table 3: Characteristic bands of the ligand and the Complexes

Compound	O-H cm^{-1}	N - H cm^{-1}	C - O cm^{-1}	N –C cm^{-1}	C =O cm^{-1}
$C_2H_5NO_2$	3421.7	3149.9	1110.7	2267.8	1680.4
$C_4H_4CaN_2O_4$	3153.3	3004.7	1032.5	2278.9	1576.7
$C_4H_8FeN_2O_4$	3157.1	1498.4 bend	1103.3	2240.1	1684.1
$C_4H_8N_2O_4Zn$	3220.4	1595.3	1088.4	2242.5	1990.4 wagging

From Table 3above, extracted from the spectra's in Figures 2, 3, 4, and 5, the FT-IR showed the presence of these main functional groups: O-H at 3422cm^{-1}, C=O at 1680cm^{-1}, N-H at 3150cm^{-1}, C-O at 1111cm^{-1} of the ligand were shifted to lower the wavelengths in the complexes with over 200cm^{-1} confirming the formation of the complexes. The functional groups that formed the ligand, glycine and the three complexes, calcium bisglycinate, iron bisglycinate and the zinc bisglycinate were revealed. It was observed that for glycine gave the IR band of 3421.7cm-1, 3149.9cm-1, 1110.7cm-1, and 2267.8cm-1. The band which appeared at 3149.9cm-1 correspond to N-H stretch, the band at 3421.7cm-1 is due to O-H Stretch, at 1680.4cm-1 may be due to C=O Stretch, the band at 1110.7 could be attributed to C-O Stretch.The FTIR of the synthesised complexes appeared to be sharper than that of the corresponding ligand. The IR bands of calcium bisglycinate ($C_4H_4CaN_2O_4$) were observed to be 3153.3cm-1, 3004.7cm-1, 1032.5cm-1, 1576.7cm-1 reveals the functional groups O-H Stretch, N-H Stretch, C-O Stretch and C=O Stretch respectively. Similarly, the sharp absorption bands at 3157.1cm-1 Stretch, 1498.4cm-1 bend, 1103.3cm-1 Stretch, 1684.1cm-1 Stretch reveals for iron bisglycinate. The last complex produces the sharp absorption bands because of the change in dipole moment of the molecule 3220.4cm-1 stretching, 1595.3cm-1, 1088.4 cm-1, 1990.4cm-1bend corresponding to O-H, N-H, C-O, and C=O respectively. Stretching vibration always have higher energy than bending as well as asymmetric and symmetric.

No vibration was recorded around the finger print region so it calls for easy interpretation. We studied the infrared spectrum of chelated metal glycinate and glycine. The formation of NH_2–M bond and COO–M bond and the disappearance of NH_3-glycine bond and COO- bond were the indications of the formation of five-membered ring structure of chelated metal glycinates. There were NH3 and COO- groups in the molecules. In the infrared spectrum of chelated metal glycinate, NH_3 peaks (1111cm^{-1}, 1131cm, 2120cm1 and COO-characteristic peaks (502cm1, 607cm1, 697cm1) all disappeared (Note COO- and other base groups combined). The NH_2 peaks (3342cm1, 3450cm1 also inferred that the three chelated metal glycinate have generated M–NH_2 group and COO–M groups).

Table 4:XRF Elemental composition of iron, calcium and zinc bisglycinate

Element	**Content (%)**		
	Iron Bisglycinate	**Calcium Bisglycinate**	**Zinc Bisglycinate**
Si	8.212±0.100	7.945±0.098	11.119±0.2300
K	0.040±0.008	4.026±0.006	0.098±0.0080
Ca	0.000+0.000	**27.776±0.172**	0.000+0.000
Fe	**15.036±0.031**	0.214±0.004	5.012±0.0308
Nb	0.014±0.000	0.017±0.000	6.41±0.0001
S	0.592± 0.029	0.000+0.000	0.918± 0.0291
P	0.204±0.004	0.000+0.000	0.402±0.0041
Zn	0.049 ±0.001	0.000+0.000	**36.234 ±0.0010**

The XRF results for the complexes clearly showed the dominancy of the three elements, calcium, zinc and iron. A confirmation of the fact that the products are those of the respective elements. Zinc is 36.23% with other elements in little percentages. For iron, 15.04% was recorded while 27.77% was obtained for calcium.

Application

(a) Synthesized complexes (b) non-fertilizer (c) inorganic, NPK fertilizer

Plate 1: Effects of the zinc, calcium and iron bisglycinates and inorganic fertilizer on maize growth

As shown above in plate 1, the effects of the synthesized metal bisglycinates can be seen. Though the inorganic fertilizer (nitrogen-phosphorus-potassium, NPK) performed a little better (c) than the metal bisglycinates (a), it can be seen that the metal bisglycinates produced much better results than when left with only manure (b). It can also be observed that the growth of the metal complexes gave better results in times of fast growth compared to that of the inorganic fertilizer, an indication that it improves growth.

IV. Conclusion

Zinc, iron and calcium bisglycinates were synthesized, characterised and applied onto a maize farmland and the results compared with that of convention fertilizer. The XRD result revealed the crystalline nature because of the sharp peaks observed. Distinct sharp peaks positions were observed at 2-theta for calcium bisglycinate, iron bisglycinate and zinc bisglycinate, indicating crystallinity. The crystallographic examinations indicate that the complexes exhibit a square planar geometry for calcium complex and octahedral geometries for iron and zinc complexes.The XRF results showed zinc (36.23%), iron (15.04%) and calcium (27.77%), a confirmation that the complexes are that of those of the three elements, calcium, zinc and iron . The synthesized complexes were compared with conventional inorganic fertilizers (NPK) on maize growth and discovered to all have positive effects on the growth compared to the ones without fertilizers. This, if optimised can go a long way in tackling stunted growth chlorosis in plants.

Based on the practical results obtained, it can be said that the research is a step in the right direction towards improving plant growth and saving cost of acquiring inorganic fertilizers especially in rural communities. The metal amino acid complexes are of extreme importance from nutritive point of view for animals and plants particularly iron, zinc and calcium complexes because of their easy absorption due to their smaller size. Their bonding strengths are strong enough for the molecules to remain intact through application and absorption, but not as strong as to resist breakdown for metabolic usage to the metal atoms because they are not synthetic or foreign to living system. If optimized, it can go a long way in solving most of the problems and challenges faced by rural farmers.

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