CHELATION, PHYSICOCHEMICAL AND ANTIMICROBIAL ACTIVITIES OF SOME MIXED CLOXACILLIN-VITAMIN C METAL COMPLEXES

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Abstract: A new series of Ni(II), Co(II) and Mn(II) chloride complexes of mixed Cloxacillin and Vitamin C were synthesized using Refluxing method. Characterization of the ligands and their complexes were carried out by Molar conductivity, Elemental analysis and FT-Infrared spectroscopy. Antibacterial activity of the ligands and their metal complexes were investigated against selected test bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). The stoichiometry of the reaction between the ligands and the metal ions was determined following standard procedures. Based on the mobility of ions, the complexes were non-electrolytic in nature. The percentage elemental composition of the complexes and ligands indicated that they are in good agreement with each other. The FT-IR data showed a bathochromic shift in the band of the complexes observed at 1670 cm^{-1} which is attributed to nitrogen of azomethine and oxygen of carbonyl group in cloxacillin. In vitamin C, Coordination occurred through the oxygen of hydroxyl and carbonyl group. The complexes were found to be in octahedral geometry. Evaluation of antibacterial activity revealed the metal complexes to be higher in activity compared to their parent ligands implying that metal complexes possess a better affinity for bacterial plasma membrane of than their ligands. The relationship between ligands and metals confirmed a 1:1 metal to ligand ratio in all the complexes.

Keywords: Cloxacillin; Vitamin C; synthesis; complexes; bacteria; ligands

1. Introduction:

Studies of coordination on biological activities is becoming an area of interest [1]. Some synthetic compounds has been investigated in which their chemistry has great attention for drug design because of the successful synthesis of some metal drug complexes which are in market for sales. Example are Auranofin, Flammazine and Cisplatin [2]. The role of metals in the biological system is well recognized as they show necessary chemistry in different physico-chemical processes [3]. Importance of metal drug complexes has been increased in the design of drug through coordination [4]. In recent years, compounds containing donor groups were used widely in coordination chemistry [5]. It is very important to synthesize some cloxacillin complexes due to their therapeutic properties. The use of mixed chemotherapy is well recognized in the treatment of malaria [6]. Combination of drugs are more effective against sensitive organisms. Incorporation of metals into the body systems is either for therapeutic or diagnostic purpose [7, 8]. Coordination of metals to ligands has call a great attention in achieving a design of new active drugs. It has been observed that complexes has a remarkable antimicrobial properties [9]. Synthesis of new metal complexes has become a great interest in the area of bioinorganic chemistry [10]. From previous research, it has been known that complexes can be involved in reactions within the body cell which is impossible for some organic substances [11,12]. In this research, we report here the synthesis, characterization and biological activities of some mixed cloxacillin- vitamin C complexes.

2. Materials and methods:

All chemicals and reagents used for this research were of analytical grade and obtained from sigma-aldrich without further purification.

Cloxacillin and vitamin c were obtained from Rajrab pharmaceutical company, Ilorin Kwara state Nigeria.
Evaluation of the antimicrobial activity were done on seeded nutrient agar with different concentrations against selected test bacteria: *Escherichia coli*, *pseudosomonas aureginosa*, *staphylococcus aureus* and *Klebsiella pneumonia*e which were collected and the antibacterial assay carried out at the Department of Microbiology,

University of Ilorin, Ilorin Kwara state Nigeria. The activity was determined based on the size of zone of inhibition.

2.1. Synthesis of complexes:

The Procedure described by Lawal *et al.* (2007) [4] was adopted. An aqueous solution of metal chloride (1 mmol) was added to a solution of Cloxacillin (1mmol) in 10ml of distilled H_2O and 10ml solution of Vitamin C (1mmol) in distilled water was added drop wisely and stirred. It was refluxed for 4 hours and allowed to cool and left to stand for a week to crystallize at room temperature. The residue formed was filtered and washed with cold distilled water and thereafter dried in vacuum with the use of silica gel.

2.2 Characterization of complexes:

The elemental analysis was carried out at Medac limited, Brunnel science centre, Eghan, United Kingdom. The FT-IR spectroscopy were performed as KBr pellet using Buck- scientific M 500 model at Redeemer University. The conductivity measurements of the complexes were performed on WTW Conductometer bridge at cell constant $0.65cm^{-1}$.

2.3. Job's method of continuous variation:

Solution (1 mmole) of each of the ligands and complexes were prepared. Solution of the metal ions were measured into 9 different test tube with the use of a burette. Solution of the ligands were also measured into the test tube containing the metal ions solution to make up to 9 ml. This is to get the total number of mole of the reactant stable throughout the reaction but the mole fraction is different for each of the reactant during mixture. The solutions prepared were mixed thoroughly in a small beaker for about 45 minutes and was left to stand 24 hours. Each mixed solution were transferred from the test tubes to cuvette of UV-Visible spectrophotometer to determine the absorbance. The absorbance were determined and noted. Mole fraction of the metal ions against absorbance were plotted on a graph to observed mole fraction of metal ions when their significant change in the absorbance [14].

2.3.1. Aqueous solubility:

Solution (10 ml) of the complexes and ligands were prepared at a specific temperature. They were dried in an evaporating dish. The mass of the residues remain in each case were determined. The solubilities were determined using the equation below. The solubility values of the complexes were compared with the parent free ligands [14]. $S = \frac{\text{Mass}}{\text{Volume}}$ X 1000

2.3.2. Thermal and acid stabilities:

The thermal and acid stabilities of the metal drug complexes were determined spectrophotometrically. The solutions (0.1 mg/ml) of the complexes were diluted. The absorption spectra were obtained and the wavelength at maximum absorption band (λmax) were determined. Six 0.1 mg/ml solutions of each of the complexes were prepared and the temperature were adjusted to 20 ^{o}C, 40 ^{o}C, 60 ^{o}C, 80 ^{o}C, 100 ^{o}C and 120 ^{o}C respectively. They were left to stand for 24 hrs and the absorbance in each case were observed. The solution were also prepared at the same concentration at the pH of 1-7. The absorbance were reported and noted. Changes in absorbance with pH and temperature determine the stability of the complexes following Beer Lambert's law**.** [14]

2.4. Antibacterial screening assay:

This was done following the method of Mohammed *et al* (2014) [13]. About 7 gram of nutrient agar was added to 250ml of distilled water. The mixture was heated for about 15minutes to form a homogenous solution and sterilized in an autoclave at 121^{0}C for 15 minutes. The molten agar was poured into petri plates and after solidifying was seeded with each test bacterium. A well measuring 5mm in diameter was bored at the centre of the agar plate and 1ml each of varying concentrations of the complexes and ligands were separately introduced. Plates were incubated at 37^{0}C for a period of 24hours and zones of clearance measured to the nearest millimeter.

3. Results and discussion:

Table 1: Analytical data of the complexes.

Ligands/complexes	Yield %	Mpt (decompose)	μ_{eff} (B.M)	Molar Conductance Ω^{-1} cm^2 mol^{-1}	C	H	N	O
Cloxacillin	-	>164	-	-	-	-	-	-
Vitamin C	-	190	-	-	-	-	-	-
Ni (Clox)(Vit C)Cl_2	75	219	4.53	25	35.89 (35.47)	3.11 (3.14)	5.02 (4.99)	21.05 (21.42)
Co(Clox)(Vit.C)Cl_2	60	236	2.46	37	42.47 (42.39)	3.68 (3.43)	5.95 (5.64)	24.92 (24.35)
Mn (Clox)(Vit. C)Cl_2	55	220	1.59	28	40.66 (40.00)	3.52 (3.67)	5.69 (5.42)	23.85 (23.12)

Table 2: IR Spectra of ligands and complexes.

Ligand/ complexes	υ(C=N)	υ(C=O)	υ(N-H)	υ(C-N)	υ(O-H)	υ (M-O)	υ (M-N)
Cloxacillin	1670	1723	3304	1361	3301	-	
Vitamin C	-	1757	-	-	3346	-	
Ni(Clox)(Vit C)Cl_2	1651	1779	3372	1365	3409	577	468
Co(Clox)(Vit C)Cl_2	1648	1780	3369	1367	3411	536	440
Mn(Clox)(Vit C)Cl_2	1636	1783	3358	1359	3404	529	526

Physicochemical properties of the metal complexes and their ligands:

Aqueous Stability:

The aqueous solubility of the metal drug complexes and their parent free ligands at a determined temperature are presented in the Table 3. Changes in the absorbance at different temperature are measured of the relative thermal stability of the complexes.

Table 3: Absorbance of complexes and their parent ligands at different temperatures.

Ligand/ complexes	20 ^oC	40 ^oC	60 ^oC	80 ^oC	100 ^oC	120 ^oC	λ max
Cloxacillin	0.14	0.17	0.13	0.19	0.12	0.16	318
Vitamin C	0.43	0.40	0.38	0.32	0.31	0.45	357
Ni(Clox)(Vit C)Cl_2	0.16	0.11	0.25	0.29	0.17	0.14	481
Co(Clox)(Vit C)Cl_2	0.09	0.17	0.13	0.19	0.12	0.15	452
Mn(Clox)(Vit C)Cl_2	0.32	0.29	0.21	0.38	0.36	0.24	417

Relative acid stability of the complexes and their parent ligands at different temperature:

The absorbance of the solution of the parent free ligands and their complexes at different pH are presented in Table 4. Changes in the absorbance at different pH are measurement of relative acid stability of the complexes.

Table 4: Absorbance of the complexes and their parent free ligands at different pH.

Ligand/ complexes	pH 1	pH 2	pH 3	pH 4	pH 5	pH 6	pH 7	λ max
Cloxacillin	0.37	0.39	0.41	0.45	0.47	0.49	0.52	317
Vitamin C	0.41	0.47	0.48	0.52	0.54	0.55	0.56	354
Ni(Clox)(Vit C)Cl_2	0.52	0.54	0.61	0.58	0.60	0.71	0.68	481
Co(Clox)(Vit C)Cl_2	0.53	0.56	0.61	0.67	0.71	0.74	0.79	451
Mn(Clox)(Vit C)Cl_2	0.62	0.68	0.69	0.73	0.76	0.78	0.82	418

Table 5: Job's method of continuous variation for Nickel complexes.

Ligand – Metal ratio	Mole fraction	Absorbance

1:9	0.1	0.47
2:8	0.2	0.59
3:7	0.3	0.71
4:6	0.4	0.78
5:5	0.5	0.83
6:4	0.6	0.77
7:3	0.7	0.61
8:2	0.8	0.58
9:1	0.9	0.47

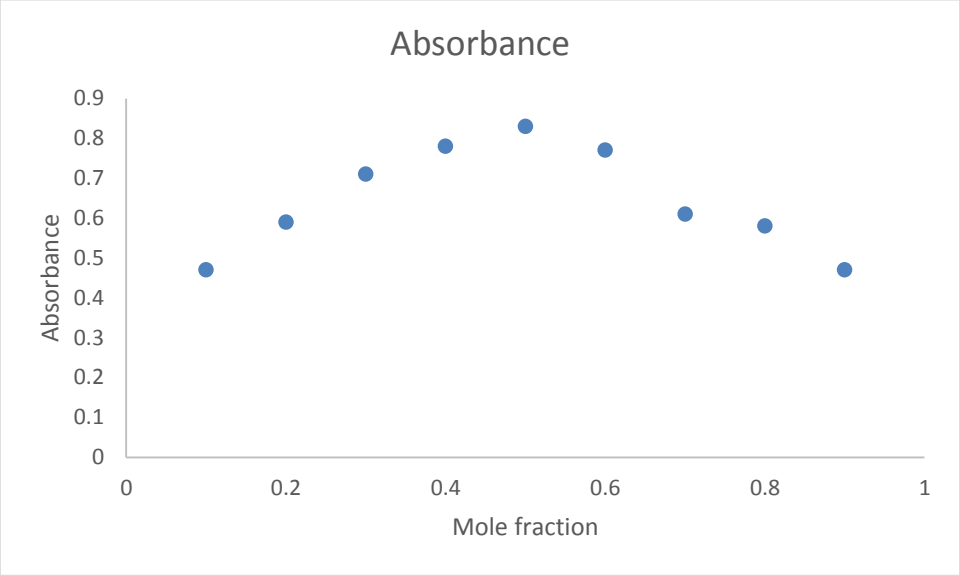


Figure 1: Graph plot of Job's method of continuous variation for nickel complexes.

Table 6: Job's method of continuous variation for cobalt complexes.

Ligand – Metal ratio	**Mole fraction**	**Absorbance**
1:9	0.1	0.67
2:8	0.2	0.73
3:7	0.3	0.87
4:6	0.4	0.91
5:5	0.5	0.89
6:4	0.6	0.81
7:3	0.7	0.76
8:2	0.8	0.64
9:1	0.9	0.56

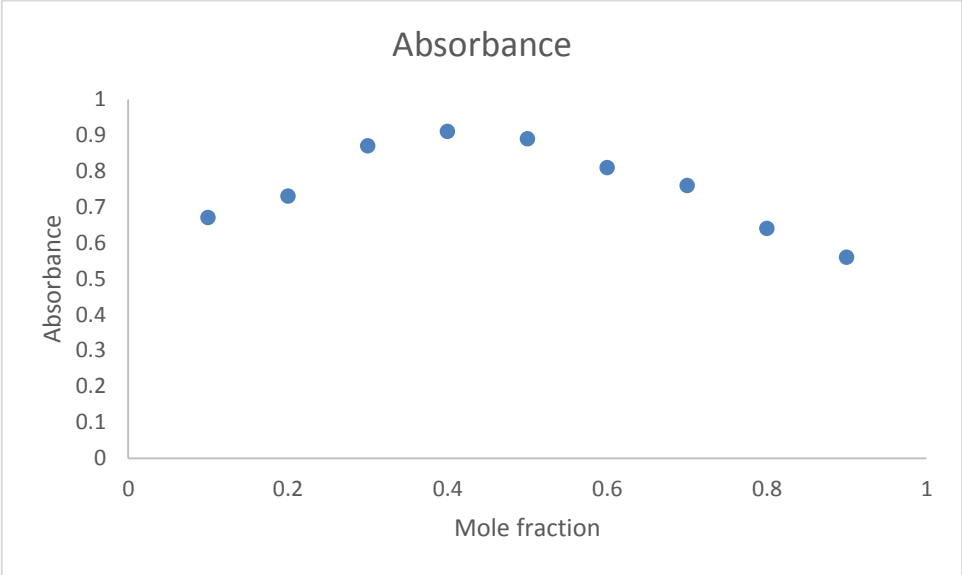


Figure 2: Graph plot of Job's method of continuous variation for cobalt complexes.

Table 7: Job's method of continuous variation for manganese complexes.

Ligand – Metal ratio	Mole fraction	Absorbance
1:9	0.1	0.17
2:8	0.2	0.25
3:7	0.3	0.28
4:6	0.4	0.35
5:5	0.5	0.39
6:4	0.6	0.21
7:3	0.7	0.17
8:2	0.8	0.13
9:1	0.9	0.10

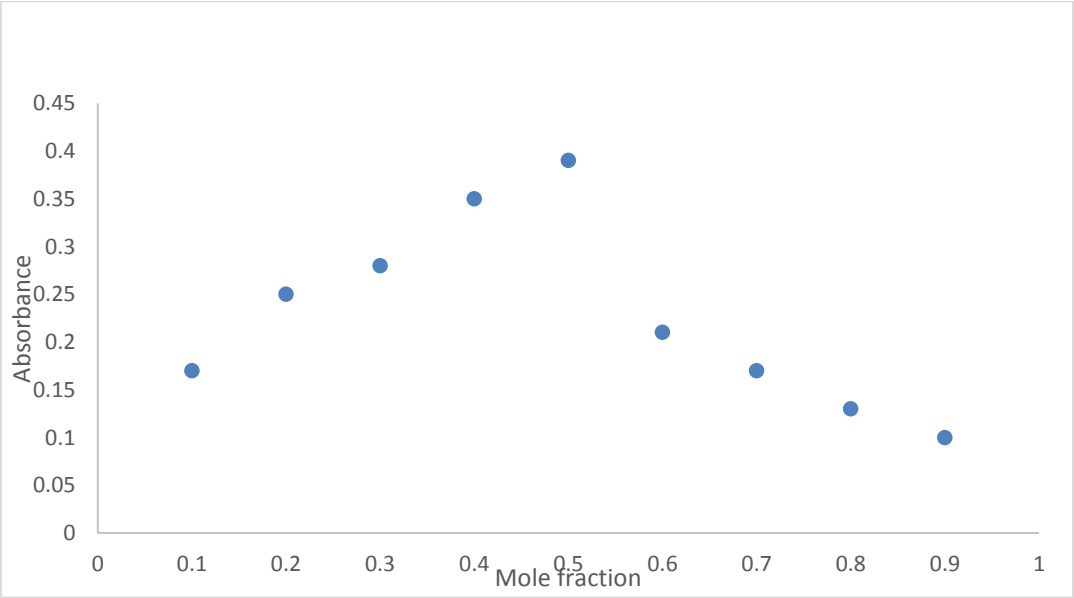


Figure 3: Graph plot of Job's method of continuous variation for Nickel complexes.

Table 8: Antibacterial activities of ligands and complexes:

Ligand/ complexes	***Escherichia coli***				***Staphylococcus. aureus***				***Pseudomonas aeruginosa***				***Klebsiella pneumoniae***			
	20	40	60	80	20	40	60	80	20	40	60	80	20	40	60	80
Cloxacillin	6	3	9	6	8	6	9	4	7	15	3	9	3	2	-	3
Vitamin C	10	7	5	8	5	8	3	5	1	9	4	5	9	6	4	7
Ni(Clox)(Vit C)Cl_2	26	15	22	17	6	10	12	16	18	15	20	22	14	19	25	11
Co(Clox)(Vit C)Cl_2	5	-	7	3	5	6	5	3	7	1	4	6	3	2	4	3
Mn(Clox)(Vit C)Cl_2	23	20	12	19	21	18	16	8	13	17	28	16	19	24	22	29

Discussion:

The analytical data of the parent ligands and their complexes are presented in Table 1. The complexes obtained a good yield within the range of 60-80%. In the synthesis of the complexes, coordination of 6 (octahedral geometry) has been suggested. This is as a result of more yield of the complexes. The complexes decomposed within 215-240 ^{o}C indicating that they are very stable in air and are in good agreement with some similar metal (II) complexes according to Ashraf (2011) [15].Vitamin C melted at 190^{0}C. Cloxacillin decomposed at >164^{0}C. Based on the mobility of ions of the complexes, it was observed that the complexes were non-electrolytic in nature due to water values in the range of 25-28 Ω^{-1} cm^2 mol^{-1} [16]. From the elemental analysis, it was observed that the complexes are in good agreement with each other when compared. The magnetic susceptibility of the complexes indicated that they are paramagnetic within the range of 1.59 - 4.53 B.M. This is in good agreement with some similar compounds [17].

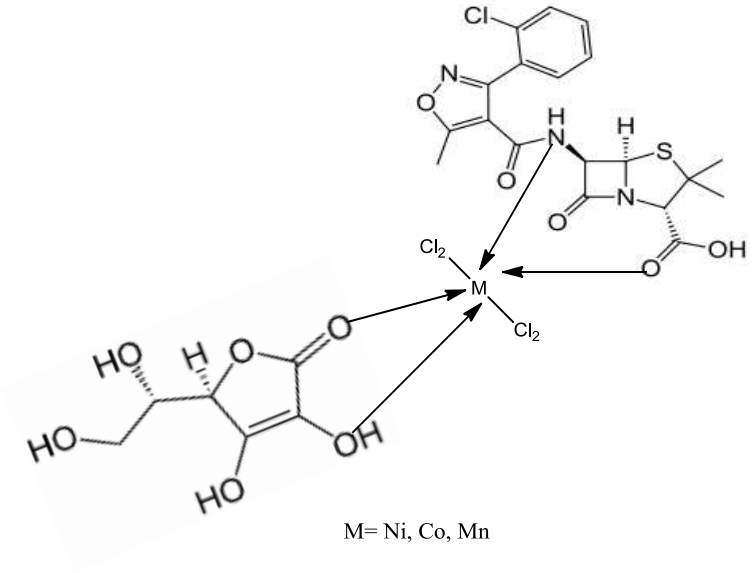
The result of IR Spectra of the free ligands and their complexes are shown in Table 2 and compared. Based on the spectra, the absorption band at 3304 cm^{-1} in cloxacillin is attributed to N-H stretching which is shifted to high frequency in all the complexes. This is in good agreement with coordination through the nitrogen of the amine group and oxygen of the carbonyl group in cloxacillin and oxygen of the carbonyl and hydroxyl group in Vitamin C [18]. A sharp band at 1780 cm^{-1} is assigned to υ(C=O) vibrations. The shift of bands in the complexes when compared with the parent ligand confirmed changes in the vibration of the ligands upon coordination [19]. The complexes indicated new bands within 529-577 cm^{-1} and 440-526 cm^{-1} region. They are assigned to υ (M-O) and υ (M-N) respectively [20]. It can be observed that these bands are absent in the spectra of the ligands.

The observed result in the thermal acid stabilities. The differences in absorbance of the parent ligands are significant but not significant in the complexes. This indicated high concentration of the complexes at certain condition due to ability of the synthesized complexes to withstand the acidity medium. The complexes are very stable in aqueous and acid media [23].

The use of Job's method of continuous variation is the study of complexes in solution. This is to determine the metal to ligand ratio. Graph plot of absorbance against mole fraction in each of the complexes showed a curve with maximum absorbance due to the ligand mole fractions used in estimating the number of coordinated ligands confirming 1:1 metal -ligand ratio in all the complexes [17].

The antibacterial activities of the parent ligands and complexes are presented in Table 8. The complexes have more antibacterial activity against all the selected organisms: *E.coli, S. aureus, Klebsiella pnemonia* and *Pseudomonas aeruginosa*. Based on the result, it was observed that the complexes exhibited more antibacterial activity on coordination when compared with the parent ligands. It has been confirmed that the complexes having nitrogen and oxygen atoms inhibit the activity of microbial enzymes. Coordination to synthesize complexes helps to lower the polarity of metals due to sharing of positive charges by donating atom in complex ring systems [20,21]. It was also observed that complexes affect the antimicrobial behavior of ligands [16]. Cobalt complex had lower antibacterial activity when compared with the ligands. This could be due to loss of some necessary pharmacophoric moieties due to coordination to the central metal [21]. There are some antibacterial drugs that form complexes in the presence of transition metal thereby affecting microbial enzyme function in the organisms [22].

4. Proposed structure:



M= Ni, Co, Mn

5. Conclusion:

According to the analytical data of the ligands and their complexes, it has been suggested that the complexes are in octahedral geometry. Decomposition value at a specific temperature showed high value confirming high stability. The free ligand acts as bidentate. It has been confirmed that the complexes are antibacterially more active than their parent ligands. It has also been suggested that the complexes are non –electrolytic in nature.

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