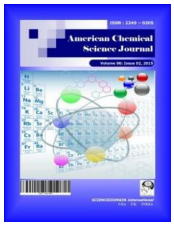

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Synthesis, Characterization and Antimicrobial Studies of Ampicillin Complexes

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Authors' contributions

This work was carried out in collaboration between all authors. Author ASA designed the study. Author AD performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author ATM managed the analyses of the study. Author MAA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The complexes obtained were characterized by solubility in methanol, acetone, ethanol and distilled water in which only the cobalt complex is soluble in hot acetone and methanol. Melting points of the complexes were 270℃ and 251℃ for the Cu and Co comp lexes, respectively. The copper complex was found to have a lower conductance (5.46 x 10^{-6} Ω^{-1} mol^{-1} cm^2) than the cobalt complex (5.58 x10^{-6} Ω^{-1} mol^{-1} cm^2). Infrared spectroscopic analysis shows a hypsochromic shift in v(C=O) band of the spectra from 1396.79 cm^{-1} for the ampicillin ligand to 1268.55 cm^{-1} and 1156.61 cm^{-1} for the complexes of copper and cobalt, respectively, while insignificant shifts for the v(C-N), v(C=N) and v(N-H) bands were noted. These band shifts indicate that the coordination occurs through carbonyl oxygen of the complexes. Investigation of antimicrobial activity was also carried out on gram negative and positive microorganisms of *Escherichia coli, Staphylococcus aureus and Klebsiella pneumonia*. The complexes were found to be active on *Escherichia coli* showing a high zone of inhibition, followed by the *Klebsiella* isolates, and insignificant action on *Staphylococcus aureus*. Based on the inhibition zone findings, the synthesized complexes exhibit higher activities for these microorganisms than the parent ligand.

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1. INTRODUCTION

Few reports regarding metal complexes of antibiotics are known. It is in line with this observation that we investigate the metal chelating ability of ampicillin ligand and to report the antimicrobial activities for possibility regarding their usage as metal- chelator therapy .While the short half-life of antibiotics has proved to provide the benefits of therapy in rapidly clearing bacterial disease, it also results in the release of harmful metals into blood [1]. Ampicillin is an antibiotic and it plays an important role in maintaining the level of bacterial causing disease in the body, [2]. Key Pour et al. [3] reported the use of ascorbic acid as a possible antidote for iron overload in reaction of ferric iron with ascorbic acid. Bessman et al. [4] reported the use of calcium-EDTA as an effective remedy for lead poisoining.Previous reports have shown that efficacy of the therapeutic agent ampicillin increased upon coordination to transition metals [5].

2. MATERIALS AND METHODS

All reagents and chemicals were of analytical grade and used as obtained from Aldrich. Ampicillin was obtained as a gift from Jodhpur Medical College Hospital pharmaceutical store, India. Metal salts used include Copper chloride dihydrate [$CuCl_2.2H_2O$], Cobalt chloride hexahydrate [$CoCl_2.6H_2O$]. IR spectra of the samples in KBr pellets were obtained in the ranges of 4000-400 cm^{-1} using FTIR spectrometer.

2.1 Experimental Procedure

2.1.1 Synthesis of [$Cu(Amp)_2Cl_2$] complex

Ampicillin (8.06 g, 2.0 mmol) was dissolved in 10ml each of acetone and methanol in a conical flask. [$CuCl_2.2H_2O$] (2.68 g, 1.0 mmol) was dissolved in 10ml of ethanol in another flask. The solutions were combined and allowed to react for 5 min. The resulting clear solution was concentrated at 50℃. The precipitate formed was allowed to cool, was filtered, washed and dried to give 4.0 g (48% yield) of a dark brown solid, mp. 270℃, See Tables 1-3 for analytical and characterization data [6].

2.1.2 Synthesis of [$Co(Amp)_2Cl_2$] complex

Ampicillin (8.06, 2.0 mmol) was dissolved in 10ml each of acetone and methanol in a conical flask. [$CoCl_2.6H_2O$] (2.68, 1.0 mmol) was dissolved in 10ml of ethanol in another flask. The solutions were combined and allowed to react for 5 min. The resulting clear solution was concentrated at 50℃. The precipitate formed was allowed to cool, was filtered, washed and dried to give 3.3 g (67% yield) of a yellow color solid, mp. 251℃, See Tables 1-3 for analytical and characterization data [6].

2.2 Antimicrobial Test

2.2.1 Preparation of sensitivity disc

Paper discs were made from whatman No.1 filter paper using a paper puncher and 50 disc each were placed in three screw –capped bottles and sterilized by autoclaved at 121℃ for about 15 minute as demonstrated by [7]. The bottles were then removed and allowed to cool at room temperature.

2.2.2 Preparation of sub culture

2 g of nutrient agar was dissolved in 60 cm^3 of distilled water and then autoclaved at 121℃ for about 15 minutes. It was removed and allowed to cool to room temperature. The media was poured into plates (petridishes) and allowed to cool and solidify. The plates were inoculated singly with the organisms which are *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia,* Incubation was carried out at 37℃ for 24 hours as demonstrated [7].

Table 1. Ampicillin ligand and metal complex solubility

Ligand and complexes	Ethanol		Acetone		Methanol		Distilled H_2O	
	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold
Ampicillin ligand	NS	NS	NS	NS	NS	NS	NS	NS
[$Cu(Amp)_2Cl_2$]	NS	NS	NS	NS	NS	NS	NS	NS
[$Co(Amp)_2Cl_2$]	NS	NS	S	NS	S	NS	NS	NS

KEY; NS=insoluble, S=soluble

Table 2. Physical constants for ampicillin ligand and metal complex

Ligand and complex	Melting point °C	Colour	Conductivity Ω-1 mol^{-1} cm^2	TLC Rf
Ampicillin ligand	237-238	White	2.6x10-6	0.32
[Cu(Amp)2Cl2]	270	Blue	5.46x10-6	0.30
[Co(Amp)2Cl2]	251	Pink	5.58x 10-6	0.29

Table 3. IR spectral data for ampicillin ligand and metal complex solubility

Ligand/complex	v(N-H)	v(C=O)	v(C-S)	v(C-O)	v(C-N)	v(C=N)
Ampicillin ligand	3525.80	1396.79	-----	874.19	1373.60	1684.29
$[Cu(Amp)_2Cl_2]$	3538.68	1268.55	687.45	1126.52	1368.67	1629.94
$[Co(Amp)_2Cl_2]$	3413.14	1156.61	689.10	----	1387.76	1652.17

2.2.3 Preparation of solution/serial dilution

The stock solution was prepared by dissolving 0.002 g of both the ligand and the complex in 2 cm^3 each of DMSO to obtain concentration of 1000 µg/cm^3. Two different concentrations were prepared from the stock solution-500 µg/cm^3 and 250 µg/cm^3. These were obtained by Making stock solution (0.5 cm^3) DMSO was subsequently added to the stock after removal of 0.5 cm^3. The solutions were introduced singly into each bottle containing 50 discs and allowed to stay for some time at room temperature to ensure maximum absorption of solution by the discs [7].

2.2.4 Preparation of inocula

The standardized inoculi of the bacterial isolates were swabbed onto the surface of nutrient agar in separate petri dishes. This was followed by placing the prepared discs of the complex and standard ampicillin (ligand) discs on to the surface of inoculated media. The isolate was incubated at 37℃ for 18 to 24 hours after which zones of growth inhibition of each sample as observed as demonstrated by [7].

3. RESULTS AND DISCUSSION

The Cu (II) and Co (II) ampicillin ligand were synthesized by reaction of metal salts with ampicillin as indicated in Scheme 1. The complexes were characterized by conductivity, melting point, solubility, infrared spectroscopy, TLC (Rf values). The complexes are generally soluble in methanol, ethanol, acetone and DMSO but insoluble in non-polar organic solvents as shown in Table 1. The decomposition temperatures of the Cu(II) and Co(II) complexes are 271℃ and 251℃, respectively, which are in agreement with vogel literatures value [8] and they are non-hygroscopic solids with melting points higher than the standard ampicillin as shown by the physical constants for ampicillin ligand and metal complexes in Table 2. The molar conductance values measured in DMSO solution (10^{-3} M) for these complexes are 5.46 x 10^{-6} and, 5.58 x 10^{-6} Ω^{-1} mol^{-1} cm^2, for the Cu and Co complexes respectively, suggesting their non electrolyte nature [9] as shown Table 2. Similarly Determination of stoichiometric ratio using Job's method [10] suggests a 1:2 metal to ligand stoichiometry for the complexes.

Ampicillin is a bidentate ligand that can provide two donor atoms to the central atom due to steric constraints. The coordination of Ampicillin occurs through the carbonyl oxygen group as indicated by the Infrared spectra. Assignment of the specific infrared frequencies directly involved in complex formation have been provided in Table 3.

The vibrational band shifts for v(C-N) of the nitrile group were observed upon complexation. Ampicillin's nitrile group band at 1373.60 cm^{-1} underwent very small shifts: Cu(II): 1368.67 cm^{-1}, Co(II): 1387.76 cm^{-1}. These insignificant shifts indicate that this group is not involved in metal-ligand coordination of the complexes. Similarly, the small spectral shifts observed at 687.45 cm^{-1} and 689.10 cm^{-1} corresponding to v(C-S) for the complexes and for which none appeared in ampicillin, suggests there is no possibility of coordination at this site to the Cu(II) and Co(II) ions. However, the band at 1396.79 cm^{-1} corresponding to v(C=O) in ampicillin shifted to lower wave numbers: 1268.55 cm^{-1} and 1156.61 cm^{-1} in the complexes, indicating that this group is possibly involved in metal-ligand coordination in the complexes. The antimicrobial activity carried out on the both ligand and the metal complexes yields a rather considerable activity

$$MX_2.nH_2O + 2L \xrightarrow[\text{Cool precipate}]{\text{Acetone/methanol reflux(2hours)}} MX_2L_2$$

$$CuCl_2.2H_2O + Amp. \xrightarrow[\text{Cool precipate}]{\text{Acetone/methonal reflux(2hours)}} Cu(Amp.)_2Cl_2$$

$$CoCl_2.6H_2O + Amp. \xrightarrow[\text{Cool precipate}]{\text{Acetone/methonal reflux(2hours)}} Co(Amp.)_2Cl_2.4H_2O$$

Scheme 1. Reactions between the metals and ampicilin (ligand)
Where AMP= Ampicillin which is the ligand

Table 4. Antimicrobial activity study of ampicillin and metal complexes of gram negative bacteria isolates

Ligand/Complex	*Escherichia coli* gram(-ve)			*Klebsiella pneumonia.* gram (-ve)		
Concentration (µg/cm^3)	**250 µg/cm^3**	**500 µg/cm^3**	**1000 µg/cm^3**	**250 µg/cm^3**	**500 µg/cm^3**	**1000 µg/cm^3**
Ampicillin (ligand)	18·0±0·30	29·10±0.13	38·40±0·28	15·10±0.80	31·08±0 39	40·0±0·53
$[Cu(Amp)_2Cl_2]$	-----	10·17±0·23	14·0±0·13	-----	10·11±0·12	13·16±0·40
$[Co(Amp)_2Cl_2]$	6.05±0.11	11·18±0.50	17·0±0·20	-----	8·05±0·30	11·00±0·15

Values are mean inhibition zone (mm) ±SD of four replicates

Table 5. Antimicrobial activity study of ampicillin and metal complexes of gram positive bacteria isolate

Ligand/complex	*Staphylococcus aureus* gram positive		
Concentration (µg/cm^3)	**250 µg/cm^3**	**500 µg/cm^3**	**1000 µg/cm^3**
Ampicillin (ligand)	20·14±0·13	33·33±0·40	46·10±0·20
$[Cu(Amp)_2CL_2]$	--------	11·14±0·20	15·20±0.13
$[Co(Amp)_2cl_2]$	--------	10·13±0·16	13.11±0·50

Values are mean inhibition zone (mm)±SD of four replicates

is used as control shown a remarkable activity on gram negative isolates of the *Escherichia coli* and *Klebsiella pneumonia*. At concentration of 1000 µg/cm^3, the activity of the ligand is 38·40±0.28mm and 40·0±0·53 mm in diameter of inhibition zones on *Escherichia coli* and *Klebsiella pneumonia* respectively. Likewise, at 500 µg/cm^3 the sensitivity of the ligand is 29·10±0·13 mm and 31·08±0·39 mm in diameter of inhibition on *Escherichia coli* and *Klebsiella pneumonia respectively*. Also, at 250 µg/cm^3 the minimum of inhibition zone of the isolates is 18·0±0·30 mm and 15·10±0·80 mm in diameter for the gram negative isolates of *Escherichia coli* and *Klebsiella pneumonia* respectively as shown in Table 4. There was a positive result on gram negative isolates of the metal complex of the Cu(II) and Co(II) at 1000 µg/cm^3 of the concentration which shown a remarkable sensitivity of 14·0±0·23 mm and 17·0±0·20 mm in diameter on *Escherichia coli* respectively as shown in Table 4. Likewise at the same concentration of 1000 µg/cm^3 of the *Klebsiella pneumonia* isolates the sensitivity of the two metal complex Cu (II) and Co(II) complex is 13·16±0·40 and 11·00±0·15 mm in diameter of inhibition respectively. At 500 µg/cm^3 the inhibition zones on *Escherichia coli* for the complex of Cu(II) and Co(II) are 10·17±0.23 and 11·18± 0·50 mm in diameter respectively. Also at 500 µg/cm^3 of the concentrations of Cu(II) and Co(II) complex of the inbition zones on *Klebsiella pneumonia* gram negative isolates are 10·11±0.12 and 8·05±0·30 respectively as shown in Table 4. Moreover at 250 µg/cm^3 there is no sensitivity of Cu(II) complex on *Escherichia coli* while there is remarkable sensitivity at 6·10±0·11 mm in diameter of inhibition zone on

the *Escherichia coli* of Co(II) complex ,whereas there is no sensitivity on *Klebsiella pneumonia* at 250 µg/cm^3 on both the Cu(II) and Co(II) complex as shown in Table 4. The gram positive isolates of *Staphylococcus aureus* shown in Table 5, There is remarkable sensitivity at 1000 µg/cm^3 of 15·20±0·13 mm and 13·08±0·39 mm in diameter of inhibition zones on *staphylococcus aureus* isolates on both the Cu(II) and Co (II) complex respectively. Also at 500 µg/ cm^3 the sensitivity of zone is 11·14±0.20 mm and 10·13±0·16 mm in diameter for the Cu(II) and Co(II) complex respectively. Likewise at 250 µg/cm^3 there is no sensitivity on the complexes of both the copper and cobalt complex as shown in Table 5. Lastly, the ligand which is the ampicillin serving as control for both gram negative and the gram positive microorganism has an activity high in the gram positive microorganism on *Staphylococcus aureus* than the gram negative isolates of *Escherichia coli* and *Klebsiella pneumonia.* It is evident that the overall zone of inhibition against bacterial species is highest in Cu(II) complex, followed by Co(II) complex which appears to have the least zone of inhibition. It is also evident that nearly all the metal complexes posses significant zone of inhibition acting against bacterial species.

4. CONCLUSION

The metal complexes of bis(ampicillin) copper(II)chloride and bis(ampicillin) cobalt(II)chloride have been successfully synthesized via careful incorporation of a bidentate ligand of ampicillin in suitable solvents by adding suitably dissolved inorganic salts of Cu(II) and Co(II) into solution of ligands, respectively. The structure of the product formed was characterized by conductivity, TLC, solubility, infrared and melting point. In all the complexes formed, Ampicillin coordinated through oxygen of the carbonyl group. Lastly, it is evident that the overall antimicrobial activity is high for the $[Cu(Amp)_2Cl_2]$ and $[Co(Amp)_2Cl_2]$ complexes, thus giving the two complexes potentiality as antidotes for metal–overload/ poisoning.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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