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Metal Chelates of Cellulose-antibiotics and Their Antimicrobial Activities.

Adedibu C. Tella, Mercy O. Obaleye and Emmanuel O. Akolade

Department of Chemistry, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria

Abstract: Five Chelates cellulose antibiotics [Cu(Clox)(Cell)Cl_2], [Zn(Tetra)(Cell)Cl_2] [Zn(Clox)(Cell)Cl_2], [Mn(Clox)(Cell)Cl_2] and [Mn(Tetra)(Cell)Cl_2] were synthesized. The chelates were characterized by solubility, melting point, conductivity, infrared and ultraviolet-visible spectroscopies analysis. The octahedral geometry is assigned for all the five chelates. Both Cloxacillin and Tetracycline act as bidentate ligands. The metal ions coordinate with the Cloxacillin through the Oxygen of the carbonyl and oxygen in the lactam ring and also through both oxygens of the hydroxyl group in the cellulose with two chloride ions to complete octahedral geometry. For tetracycline, the metal ions coordinate through both oxygens of the hydroxyl groups and oxygens of hydroxyl in the cellulose with two chloride ions to complete octahedral geometry. The antimicrobial activities of the chelates were investigated against six microorganisms. The chelates were found to be active against these organisms.

Key words: Chelate · Coupling, antibacterial activity · Spectroscopy

INTRODUCTION

Cellulose is a natural polymer, polysaccharide produced by linking additional sugar in exactly the same, the chain varies greatly from a few hundred-sugar unit in wood pulp[1,2]. Investigation of coupling of a number of antibiotics to cellulose under a series of coupling conditions showed that it is possible to produce insoluble derivatives of antibiotics that possess antibacterial activity [3-5]. It was found that the antibiotics became physically bound to cellulose-antibiotics complexes but covalent binding of the antibiotics via their amino group to the cellulose extended the range of antibacterial activity [3]. It has been shown that chelation of titanium to cellulose yields complexes that can be used as an insoluble matrix for immobilization of enzymes [6,7]. Coupling of this insoluble matrix with antibiotics produces antimicrobial surfaces with wide application. This immobilized antibiotics product could serves as selective protector against microbial attack of paper and legal documents, of canvas and chromatographic media based on cellulose materials and of cellulose-based packing of cooling towers [8]. Literature survey revealed that few works were reported on transition metals coupling of antibiotics into cellulose especially the first and second row metals. The present study is aimed at synthesis, characterization and antimicrobial activities of some metal chelates of cellulose- antibiotics. To the best of our knowledge, this is the first work on Co(II), Zn(II) and Mn(II) chelates of cellulose antibiotics.

MATERIALS AND METHODS

The materials used in this research work are Cloxacillin and Tetracycline which were all obtained from Rajrab Pharmaceutical Company Ilorin, Kwara state while the cellulose were also obtained Biomedical Pharmaceutical Company, Ilorin, Kwara state.

Melting point was performed in the Department of Chemistry, University of Ilorin , Nigeria using Griffin melting point apparatus.

Investigation of antimicrobial activities of the complexes were carried out by screening against the tested organisms: *Escherchia Coli, Staphylococcus aureus and Candida albican, Aspergillus niger, Aspergillus flavus and Pseudomonas aureginosa.* These organisms were obtained from Microbiology Department, University of Ilorin Teaching Hospital, Ilorin, Nigeria.

Synthesis of Cellulose Antibiotics- Metal Chel Ates.
Preparation of Metal Chloride Solution: 12.5 w/v solution in Hcl of each of the metal chloride ($CuCl_2.2H_2O$,

Corresponding Author: Adedibu C. Tella, Department of Chemistry, University of Ilorin. P.M.B. 1515, Ilorin, Nigeria.
E-mail: ac_tella@yahoo.co.uk.

$MnCl_2.4H_2O$, $ZnCl_2$) were prepared. Each of the metals chloride was obtained as 3.125 g/dm^3 in 25 cm^3 solution of hydrochloric acid. It was allowed to filter immediately before used in order to remove any impurities or precipitated oxide or hydroxide which might be present.

Activation of Cellulose by Chelation: 5.0 g of Cellulose was mixed together with the 50 ml solution of each of metal chlorides for 5 minutes at a room temperature which resulted into a green precipitate and the damp material was maintained at a temperature of 45°C for 24 hours in an oven. The resulting dried material was ground into a powder form and kept in an ambient temperature.

Coupling of Antibiotic to Metal Cellulose: 0.75 g of the prepared cellulose metal chelate was washed twice with 0.02 M sodium phosphate buffer(pH 5.1, 30ml). The solution of sodium phosphate (buffer) was prepared by reacting 2.4 g of sodium phosphate in 1000 ml of distilled H_2O and the pH of the 5.1.

Mixture of the prepared metal Cellulose was stirred together with a solution of antibiotics used. It was then allowed to cool in cold water for about 1 hour at a temperature of 4°C. The precipitate obtained was washed with 0.1 M, sodium phosphate buffer (pH 5.1, 30 ml) and 0.5 M, 30 ml of sodium chloride. The complexes formed were dried in a dessicator at room temperature.

Preparation of Microorganism Culture: Microorganism tested in the study were provided from the culture collection of Microbiology Laboratory of Department of Microbiology, University of Ilorin, Nigeria. In the study , *Escherchia. coli, Pseudomonas aureginosa, Staphylococcus aureus, Candida albicans, Aspergillus mger, Aspergillus flavus* were used as the test organisms in antibacterial study.

The bacteria and yeast shown were inoculated into nutrient broth and incubated for 24 hours and 48 hours.

In the disc diffusion method, the sterile Muller Histon agar(Oxoid) for bacterium and sabourad agar for yeast were separately inoculated with the test organism. The compound dissolved in DMSO as 50 μg/disc were placed in well(6mm diameter) and cut in the agar media and the plates were incubated at 32°C for bacteria (18-24 hours) and at 25°C for yeast(72 hours). The resulting inhibition zones in the plates were measured after 48 hours. The control samples were only absorbed in DMSO.

RESULTS AND DISCUSSION

The chelates were synthesized by coupling antibiotics to cellulose chelated with metals in molar ratio of 1:1:1. The chelates were characterized by Melting point, Infrared, Ultraviolet-visible Spectroscopies and Conductivity measurements. The physical properties of the various chelates are shown in table 1.

The chelates are non-hygroscopic solids with different melting point ranging from 200-280°C. All the chelates have higher melting point than their respective parent antibiotics.

The molar conductance values measured in DMSO solution (10^{-3}M) for these chelates are 10-20 $\Omega^{-1}cm^2$ mol^{-1}. The results indicate that the chelates are non-electrolytes[9].

It can be observed in Table 2 that chelates are almost insoluble in distilled water but some are soluble in acetone, ethanol and methanol.

The infrared spectra of the cellulose, antibiotics and the chelates are shown in able 3. In the IR spectra of cloxacillin, the band at 1790 cm^{-1} was assigned to ν (C=O) lactam band [10]. The position of this band appeared at frequency ca 1650-1725cm^{-1} for Cloxacillin- cellulose

Table 1: Melting Point of Cellulose Antibiotics-Metal Chelates.

Ligands/Complexes	Colour	Melting point(°C)	Molar conductivity Ω^{-1} Cm^2 mol^{-1}
Cellulose	Cream	195-198	-
Cloxacillin	White	200-202	-
[Cu(Clox)(Cell)Cl_2]	Green	273-275	10
[Zn(Clox)(Cell)Cl_2]	Off white	205-207	15
[Mn(Clox)(Cell)Cl_2]	Off white	242-245	20
Tetracycline	Yellow	170-173	-
[Zn(Tetra)(Cell)Cl_2]	Light yellow	294-296	16
[Mn(Tetra)(Cell)Cl_2]	Light yellow	262-265	10

Table 2: Solubility Test of Cellulose Antibiotics-Metal Chelates

	Distilled H_2O		Acetone		Ethanol		Methanol		Chloroform	
Complexes	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot
[Cu(Clox)(Cell)Cl_2]	NS	NS	SS	SS	NS	SS	NS	SS	SS	SS
[Zn(Clox)(Cell)Cl_2]	NS	SS	NS	SS	SS	NS	SS	NS	NS	SS
[Mn(Clox)(Cell)Cl_2]	NS	NS	SS	NS	NS	SS	SS	SS	SS	SS
[Mn(Tetra)(Cell)Cl_2]	NS	NS	SS	NS	SS	SS	SS	SS	NS	NS
[Zn(Tetra)(Cell)Cl_2]	NS	NS	SS	SS	SS	SS	SS	SS	SS	SS

NS= Not soluble , SS= soluble

Table 3: Infra- Red Spectra of Cellulose Antibiotics-Metal Chelates.

Ligands/Complexes	ν(O-H)/(N-H)	ν(C-H)	ν(C=O)	ν(C-N)
Cellulose	3449	2935	-	-
Cloxacillin	3361	2973	-	-
[Cu(Clox)(Cell)Cl_2]	3405	2931	1724	1228
[Zn(Clox)(Cell)Cl_2]	3407	2933	1710	1243
[Mn(Clox)(Cell)Cl_2]	3424	2933	1655	1245
Tetracycline	3447	2940	1617	1228
[Zn(Tetra)(Cell)Cl_2]	3404	2931	1609	1231
[Mn(Tetra)(Cell)Cl_2]	3419	2931	1609	1230

Table 4: Uv-Visible Spectra Of Cellulose Antibiotics-Metal Chelates.

Ligands/Complexes	Wavelength(nm)	Energies (cm^{-1})	Assignment
Cellulose	210 , 220	47619 , 45455	π - π *, n- π *
Cloxacillin	220 , 240	45455 , 41667	π- π*, n- π*
[Cu(Clox)(Cell)Cl_2]	510 , 680	19608 , 14706	d-d transition
[Zn(Clox)(Cell)Cl_2]	240 , 270	41667 , 37037	π- π*, n- π*
[Mn(Clox)(Cell)Cl_2]	210 , 220	47619 , 45455	π- π*, n- π*
Tetracycline	265 , 300	37736 , 33333	π - π *, n- π *
[Zn(Tetra)(Cell)Cl_2]	240, 300	41667 , 33333	π - π *, n- π *
[Mn(Tetra)(Cell)Cl_2]	205 , 230	48781, 43478	π- π*, n- π*

metal chelates. The band at 3361 cm^{-1} due to ν(O-H) also shifted to higher frequency (3400-3439 cm^{-1}). All these suggest that, coordination of the cloxacillin occurs through the oxygen atom of the lactam carbonyl group[11]. The chelation of the cellulose with metal occurs via the oxygen of the hydroxyl group. The band ν(O-H) at 3449 cm^{-1} is shifted to lower frequency in the chelates. Cloxacillin acts as bidentate ligand. The chelates consists of metal ion which coordinates with 1 molecule of cloxacillin through the oxygen of hydroxyl group and carbonyl oxygen in the lactam ring of the cloxacillin and 1 molecule of cellulose through each oxygen of the hydroxyl groups with two chloride ions to complete octahedral structure. The mode of coordination is in agreement with the previous work of Kennedy and Humphreys [8].

In the infrared spectra of tetracycline, the band at 3447 cm^{-1} assigned to ν(O-H) and 1617 cm^{-1} ascribed to ν(C=O) both shifted to lower frequencies of 3400-3420 cm^{-1} and 1609 cm^{-1} respectively. These observations confirm coordination of the metal ion Mn(II), Zn(II) through the oxygen of the hydroxyl group and oxygen of the carbonyl group in the tetracycline[12].

The chelation of the cellulose with Mn(II) and Zn(II) was evidenced by the shifting of the band 3449 cm^{-1} ascribed to ν(O-H) to lower frequency in the chelates. This suggests that the chelate consists of metal ions which coordinate with 1 molecule of tetracycline through the oxygen of the carbonyl, oxygen of the hydroxyl group and each oxygen in the hydroxyl groups with two chloride ions to complete octahedral geometry [2,12].

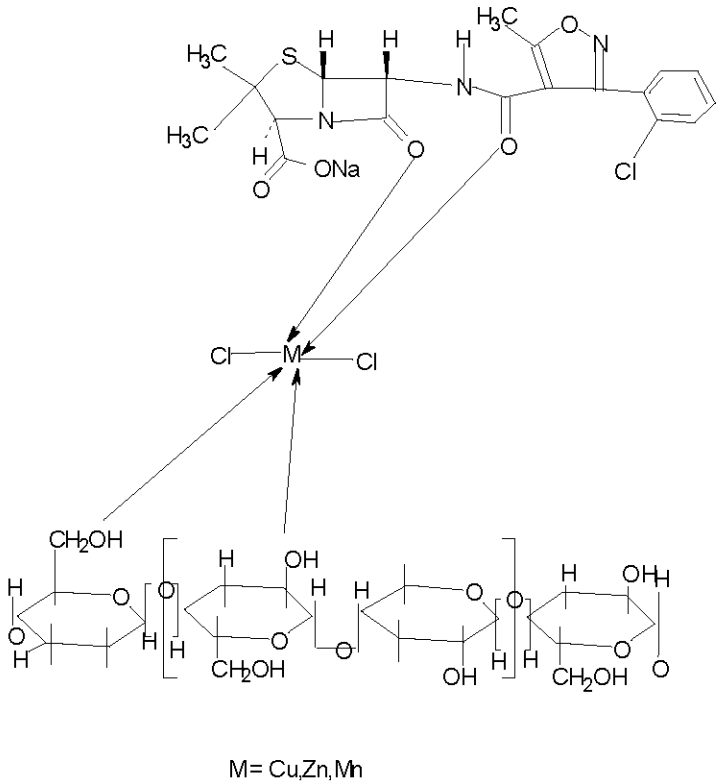

Fig. 1: Proposed structure of metal cellulose cloxacillin chelates.

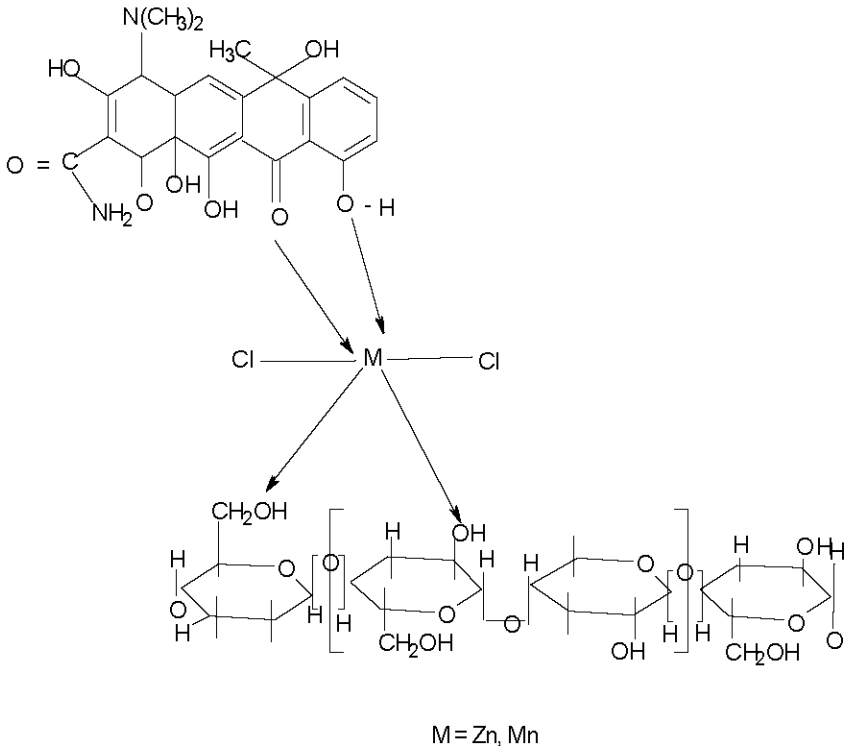

Fig. 2: Proposed structure of metal cellulose Tetracycline chelates.

Table 5: Antimicrobial Activity Data For The Cellulose Antibiotics Metal Complexes.

Inhibition zone(mm)				[Zn(Tetra)	[Zn(Clox)	[Mn(Clox)	[Mn(Tetra)	[Cu(Clox)
Bacteria and yeasts	Cellulose	Cloxacillin	Tetracycline	$(Cell)Cl_2$]	$(Cell)Cl_2$]	$(Cell)Cl_2$]	$(Cell)Cl_2$]	$(Cell)Cl_2$]
Escherichia Coli	-	3	4	6	6	4	6	3
Pseudomonas aureginosa	-	4	2	4	2	5	2	4
Staphylococcus aureus	-	4	3	5	3	6	3	4
Candida albicans	1	1	-	6	3	4	3	3
Aspergillus niger	1	1	-	2	2	5	2	5
Aspergillus flavus	1	2	1	3	3	4	3	4

The UV-Visible spectra of the ligands, cellulose and their chelates in DMSO solution are presented in Table 4. A band at 230-300nm is assigned to $\pi - \pi^*$, $n - \pi^*$ transitions within the organic ligands[13]. Co(II), Cu(II) chelates presents bands between 510-680 nm ascribed to d-d transitions. Mn(II) and Zn(II) chelates did not exhibit d-d transition. [13]. All these bands are in agreement with octahedral geometry proposed for the chelates [14]. The proposed structures are shown in figures 1 and 2.

The cellulose-antibiotics metal complexes were found to be highly active against *Escherichia Coli, Pseudomonsa aureginosa, Staphylococcus aureus, Candida albicans* and *Aspergillus flavus* than the parent Cloxacillin, tetracycline and cellulose as shown in Table 5. All the cellulose-antibiotics chelates showed antibacterial activity against strains of both Grams +ve and Gram –ve such as *Escherichia Coli, Pseudomonas aureginosa* and *Staphylococcus aureus* and also showed antifungal activity against *Candida albican, Aspergillus niger, Aspergillus flavus* [15].

In conclusion, active immobilized antibiotics based on cellulose-metal chelates are reported. The chelates were characterized by melting point , electronic, IR and AAS spectroscopies. It can be seen that it is possible to form active immobilized antibiotics by a sample chelation with metal salts. All the chelates are found to have octahedral geometry. They are active against all the six organisms. The immobilized antibiotics product might be of greater applicability as food packaging material, antibacterial surface(water storage tanks, industrial membranes and chromatographic columns). They can also be useful to protect paper and legal documents.

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