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MECHANOCHEMICAL SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL SCREENING OF METAL (II) COMPLEXES DERIVED FROM AMOXICILLIN

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ABSTRACT
Mechanochemical synthesis of Co(II), Fe(II), and Mn(II) amoxicillin complexes by simple grinding of metal (II) chlorides with amoxicillin. The mechanochemical products were characterized by solubility, melting points, conductivity values, magnetic moments and Infrared spectral studies. The IR spectral data of amoxicillin complexes the ligand acted as bidentate which coordinated to the metal ion through υ(COO) and υ(C=O). The Job's method analyses suggested 1:2 metals to ligand ratio and molar conductivity values is in the range of 5.58-11.67 $\Omega^{-1}cm^{-1}mol^{1}$ indicating that the complexes behave as non-electrolytes nature in DMSO. The complexes are all soluble in DMSO, while two are soluble in ethanol, DMF and methanol, one is not. The complexes decompose at a range of 217-278^{o}C Indicating higher stability than their respective ligands. The magnetic moment values have proved that all the synthesized complexes are paramagnetic. The ligands and complexes were screened against three bacterial strains; Escherichia coli, Salmonella typhi and Staphylococcus aureus for antimicrobial activity. The results show that both the ligand and the complexes are active against the test bacteria; most complexes are active in all test concentration while others are only active at high concentration against bacteria.
Keywords: Mechanochemistry, Synthesis, Antibiotic, Transition Metals

INTRODUCTION

'Mechanochemistry' refers to reactions, normally of solids, induced by the input of mechanical energy, such as by grinding in ball mills(James *et al.,*2012). It is becoming more intensely studied partly because it can promote reactions between solids quickly and quantitatively, with either no added solvent or only nominal amounts. Historically it has been a sideline approach to chemical synthesis, and solution-based methods have been adopted by default. However, mechanochemistry could in future become a more mainstream technique for two reasons.

Firstly, it is increasingly clear that is effective, and even advantageous, in ever-widening types of synthesis. Secondly, our current dependence on solvents appears increasingly unsustainable since it is wasteful of fossil-derived materials (e.g. 85% of chemicals used in the pharmaceutical industry are solvents and even if recycled typical recovery rates are only 50–80%), environmentally problematic, hazardous and energy-demanding with regard to solvent production, purification and recycling (James*et al.,*2012).

Solvents can be present in the solid starting materials, such as in hydrated metal salts or in molecular solvates. There may even be (smaller) amounts of moisture in non-formally hydrated materials or in the atmosphere which aid the reaction (James *et al.,* 2012). Under some experimental conditions, it is advantageous to add a few drops of solvent to the solids leading to so-called liquid-assisted grinding (LAG). In some cases, chemical reactions can also be initiated between a solid reactant and a liquid: in that case, the process is called kneading (Dubois *et al.,*2014).

This research work is aimed at synthesizing and characterizing metal (II) complexes of amoxicillin via machanochemical means. The objectives of this research include; Finding an alternative synthetic route for the synthesis of Co(II), Mn(II)and Fe(II) complexes of amoxicillin and characterizing them by Solubility, Melting/decomposition temperature, Conductive measurement, Magnetic susceptibility measurement and Infrared spectroscopy.

Italso includes determining the antimicrobial activity of metal (II) complexes of amoxicillin in order to establish how metal-drug binding influence the activity of these drugs.

MATERIALS AND METHODS

All the reagents were of analytical grade and used without any further purification. The active pharmaceutical ingredient, amoxicillin was obtained from Sigma Aldrich. Metal salts used include metal (II) chlorides of Co, Fe,and Mn.

All weighing was done using weighing balance model B154 METTLER TOLEDO. Molar conductance measurement was done using Jenwey conductivity meter model 4010 in DMSO. Jenwey 6305 UV-Visible Spectrophotometer was used for UV-absorbance measurement. Decomposition/ melting temperature were recorded using Stuart melting point apparatus SMP 10. Magnetic susceptibility measurement of the complexes was recorded using Magnetic Susceptibility balance of Sherwood Scientific Cambridge UK. The bacterium isolates;*Escherichia Coli, Staphylococcus Aureus* and*Salmonella typhi*were obtained and identified at Department of Microbiology, Bayero University Kano. Metal to Ligand ratio was determined using Job's method of continuous variation and Infrared spectra of ligands and complexes was recorded using Fourier Transform Infrared Spectrophotometer of Agilent Technologies.

Synthesis of the Complexes

The methods described by Kurawa and Yammama (2014) was adopted and modified for the synthesis of all the complexes. Each metal salts of (1mmol, 0.238g of $CoCl_2.6H_2O$,0.3654g of $FeCl_2.4H_2O$ and0.198g of $MnCl_2.4H_2O$) were mixed with 0.7308g (2mmol) of amoxicillin in 1:2 mole ratios. The mixture was ground in an agate mortar for 10-15minutes with 0.1ml drop of methanol to obtain a fine powder as product. The complexes were then dried in vacuum for 24hrs at 110^oC.

$Mcl_2.nH_2O$ + 2Amox $\xrightarrow[LAG(CH_3OH)]{\text{Grinding}}$ $[Co(amox)_2Cl_2]$

(M=Fe, Mn & Co)

Melting points / DecompositionTemperature

Melting point of the ligand as well as decomposition temperature of the complexes were determined by introducing a pinch of each sample into a capillary tube and then inserted into Stuart melting point apparatus (SMP 10), the temperature at which the ligand melts and the complexes decomposed were recorded. Similar to the report of Waziri *et al*., (2017)

Solubility Test

The solubility's of the complexes were determined in different solvents ranging from polar to non-polar such as distilled water, methanol, ethanol, ethylacetate, chloroform, hexane,CCL_4, dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO), in which 0.1g of each sample was tested in 10ml of each solvent. Kurawa and Yammama (2014)

Molar Conductance Measurement of the Ligand and their Complexes

Molar conductance of the ligand and the complexes were carried out in dimethyl sulfoxide (DMSO) by dissolving 0.001g of each sample in 10ml of the solvent in a test tube, the electrode was inserted and the reading taken. Results obtained was compared with the one reported by (Mustapha *et al*., 2014)

Magnetic Susceptibility Measurement

The prepared metal complexes were introduced into the balance's capillary tube up to a given mark and the reading recorded using the magnetic susceptibility balance. The formula below was used to calculate the magnetic susceptibility (Xg). That is; $Xg = C \times L(R-R_0)/10^9M$

Where; C=1, Constant of proportionality

L = sample length in cm

R = reading obtained of the sample placed in tube

R_0 = reading obtained of the pre-weight empty sample tube

$M = W_2-W_1$, mass of sample in the tube in (g)

The effective magnetic moment can then be calculated thus;

Xm = Xg x molar mass (molar magnetic moment)

Effective magnetic moment (μ_{eff}) $=2.828(Xm \times T)^{1/2}$

T=Absolute temperature

Determination of Metal to Ligand ratio

The number of ligand coordinated to the metal ion was determined using Job's method of continuous variation. 3.0mmol of Dimethylsulfoxide (DMSO) solution of the ligands and the metal chlorides were prepared. The following ligand to metal salt ratio (in ml) 00:16, 01:15, 03:13, 05:11, 07:09, 09:07, 11:05, 13:03 were taken from the ligand solution and each of the metal chloride solution respectively.

A total volume of 16ml was maintained (in that order) throughout the process and the mole fraction of the ligand was calculated in each mixture. The solution of the metal salts were scanned (as blank) to find the wavelength of maximum absorbance (λ_{max}) for that particular metal ion (Skoog*et al*., 2007). The machine was fixed at λ_{max} (in each case) before taken the absorbance values.

The absorbance values were extrapolated against mole fraction of the ligand and the number of coordinated ligand (coordination number) was determined using the relation below:

$$\overline{n} = x_i / (1-x_i)$$

Where $\overline{n}$ = number of coordinated ligand and X_i=mole fraction at maximum absorbance

Antibacterial Activity Test

According to method describe by (Yusha'u, & Salisu, 2011). 0.02g of ligand and metal (II) complexes each were dissolved in 1ml of Dimethyl sulfoxide (DMSO) to give stock solutions of three different concentrations (5µg, 10µg and 20µg) which has been prepared by half serial doubling dilution method. They were placed on the surface of the culture media and incubated at room temperature for 48hrs. Then in vitro antibacterial activity against *Escherichia coli,Staphylococcus aureus* and *Salmonella typhi* were carried out by agar-well diffusion method. Standard was used to compare with the diameter of zone of inhibition produced by ligand and complexes.

RESULT AND DISCUSSION

The interaction between amoxicillin and the metal salts yielded coloured complexes of blue, brown and yellow cream for Co(II), Fe(II) and Mn(II) complexes respectively,which is similar with the result reported by Al-Mudhafar, (2009). Amoxicillin melted at a temperature of 189^{o}C where as its interaction with metal (II) ions resulted complexes with decomposition temperatures of 278^{o}C, 217^{o}Cand 245^{o}C for Co(II), Fe(II)and Mn(II) complexes respectively. This higher values than that of free Amoxicillin provide evidence of coordination of the ligand to the respective metal ions and also revealed the more stable nature of the complexes as shown in Table 1.These values are in agreement with similar metal (II) complexes reported by Waziri *et al.,* (2017).

Table1: The Physical Properties of Amoxicillin and its Metal (II) Complexes

Compounds	Colour	Melting point (^{o}C)	Decomposition temperature (^{o}C)
Amoxicillin	White	189	
Amox-Co(II)	Blue	-	278
Amox -Fe(II)	Brown	-	217
Amox -Mn(II)	Yellow cream	-	245

Amoxicillin is soluble in methanol, ethanol, ethyl acetate, DMF and DMSO whereas its complexes are all insoluble in hexane and CCL_4, all soluble in DMSO, soluble in some organic solvents and slightly soluble in other organic solvents (table 2). This is because, polar compounds are expected to be soluble in polar solvent, while non-polar compounds are expected to be soluble in non-polar solvent (like dissolves like),which is in agreement with results reported by Kurawa and Yammama (2014).

Table 2: Solubility of Amoxicillin and its Metal (II) Complexes

Ligand/ Complexes	Methanol	Ethanol	Chloroform	Ethyl Acetate	Hexane	CCL_4	DMF	DMSO
Amoxicillin	S	S	IS	S	IS	IS	S	S
Amox-Co(II)	IS	S	S	IS	IS	SS	S	S
Amox - Fe(II)	S	IS	IS	IS	IS	IS	IS	S
Amox - Mn(II)	S	S	S	IS	IS	IS	S	S

Key: S-soluble, SS-slightly soluble, IS-insoluble

The suggestion of the likely geometry of complexes can also be provided by their magnetic moment in which magnetic susceptibility studies shows that complexes of Co(II), Fe(II) and Mn(II) were paramagnetic with the values range from 3.8-5.3BM (Table 3), in which all the values lies within the range that correspond to spin-only value magnetic moment for high spin octahedral geometry around Co(II), Fe(II) and Mn(II) ions respectively, which is similar with the one reported by (Adekunle 2013;Al-Noor *et al*., 2013; Anacona & Rodriguel, 2004; Cotton & Wilkinson, 1972 , p. 906).

The μ_{eff} values which are higher or lower than spin-only values for the respective metal (II) ion concerned may be due to spin-orbit coupling.

Table 3: Effective Magnetic Moment and Molar Conductance of Metal (II) Amoxicillin Complexes

Complex	Magnetic Susceptibility(Xg) (g^{-1})	Xm (mol^{-1})	Effective magnetic moment (BM)	Molar Conductivity ($\Omega^{-1}cm^2mol^{-1}$)
Amox-Co(II)	$7.8x10^{-6}$	$6.7x10^{-3}$	3.8	11.67
Amox -Fe(II)	$8.4x10^{-6}$	$7.2x10^{-3}$	4.0	8.42
Amox -Mn(II)	$1.7x10^{-5}$	$1.3x10^{-2}$	5.3	5.58

The molar conductance of metal (II) amoxicillin complexes measured at room temperature in $1x10^{-3}$M in DMSO, the results obtained range from 5.58-11.67$\Omega^{-1}cm^2mol^{-1}$ respectively, which indicate few ionsor absence of ions. The results indicate non-electrolytes of all the complexes prepared due to the lower values. The results which is presented in Table 3 are in line with the report of (Adekunle 2013; El-waheed *et al*2008; Kurawa & Yammama 2014; Mustapha *et al*., 2014; Refat *et al*., 2014;Shah & Sharma 2013)

The IR spectra data of amoxicillin and its metal(II) complexes are presented in table 4. The bands at 1689.54cm^{-1} and 1774.50cm^{-1} in the spectrum of free amoxicillin are assigned to υ(C=O) of amide and carboxylic acid respectively, as these shifted to different frequencies, 1599.51cm^{-1} and 1778.83cm^{-1}, 1584.78cm^{-1} and 1778.92cm^{-1}, 1737.91cm^{-1} and 1778.93cm^{-1}, for Amox-Co(II), Amox-Fe(II)and Amox-Mn(II) respectively, which suggested the coordination of carboxylic oxygen after deprotonating (Waziri *et al*., 2017)

Table 4: The IR Spectra Data of Amoxicillin and its Metal (II) complexes

Compounds	υ(C=O)cm^{-1} of amide	υ(C=O)cm^{-1} of carboxylic acid	υ(N-H)cm^{-1}	M-O
Amoxicillin	1689.54	1774.50	3454.83	-
Amox-Co(II)	1599.51	1778.83	3409.70	575.26
Amox-Fe(II)	1584.78	1778.92	3361.89	579.09
Amox-Mn(II)	1737.91	1778.93	3324.66	553.00

The bands at the range 575.26cm^{-1}, 579.09$cm^{-1}$and 553.00cm^{-1}, in the spectra of amoxicillin complexes which could not traced to the free ligand spectrum is tentatively assigned to M-O stretching frequencies for Co(II), Fe(II)and Mn(II). Similar amoxicillin complexes have been reported by other researchers (Imran *et al,* 2006; Waziri, *et al,* 2017; Reiss *et al*, 2015; Al-Mudhafar, 2009) using solution based synthesis.
The octahedral geometry assumed agrees with IR spectrum and similar to the structures reported by Imran *et al,* (2006); Waziri, *et al,* (2017); Reiss *et al*, (2015) and Al-Mudhafar, (2009), supporting coordination of Amoxicillin to respective metal ions as shown in Table 4.
Estimation of the Metal to Ligand ratio was achieved by using Job's method of continuous variation, the results showing mole fraction of the Ligand and absorbance for the respective metal ions (Co^{2+}, Fe^{2+} and Mn^{2+}) are presented in Table 5 as reported in the literature by (Skoog *et al*., 2007).

Table 5: Mole Fraction of the Ligand (Amoxicillin) and Absorbance of Co^{2+}, Fe^{2+} and Mn^{2+} with the Ligand

Mole fraction X(total volume=9ml	Co:L_2' λmax=620nm	Fe:L_2' λmax=580nm	Mn:L_2' λmax=635nm
0.063	0.052	0.099	0.065
0.188	0.071	0.100	0.066
0.313	0.069	0.177	0.069
0.438	0.070	0.212	0.077
0.563	0.078	0.253	0.078
0.688	0.073	0.224	0.070
0.813	0.063	0.223	0.050
0.938	0.052	0.127	0.040
1.000	0.050	0.095	0.030

The plot of absorbance against mole fraction in each case gives a curve with maximum absorbance corresponding to the ligand mole fractions which were used in calculating the number of coordinated ligand, which suggest 1:2 Metal-Ligand ratio in all the prepared complexes as reported by Anacona and Rodriguez(2004)

Table 6: Anti-bacterial Activity Test of Amoxicillin and its Metal (II) complexes

		Inhibition Zones		
Compounds	Concentration (µg/agar-well)	*S. aureus* (mm)	*E.coli* (mm)	*S. typhi* (mm)
Amoxicillin	5	24	18	20
	10	28	28	33
	20	30	35	42
Amox-Co(II)	5	23	19	18
	10	28	27	20
	20	38	33	31
Amox-Fe(II)	5	-	-	17
	10	-	10	24
	20	-	15	37
Amox-Mn(II)	5	29	20	18
	10	37	25	21
	20	40	34	29
Standard (Gentamycin)	20	48	37	33

Key: S*. aureus = Staphylococcus aureus* *E. coli = E scherichia coli* *S. typhi=Salmonella typhi*

Antimicrobial screening results shows that the amoxicillin complexes are active against all bacteria isolates in all concentration except Amox-Fe(II) which is inactive against *Staphylococcus aureus* and *Escherichia coli* at 5µ/agar-well. Amox-Mn(II) has highest inhibition zone against *Staphylococcus aureus* at all concentrations with Amox-Fe(II) been least, as reported by Waziri *et al.,* (2017).

On the basis of the analytical data obtained viz: melting point, conductivity measurement, magnetic susceptibility (effective magnetic moment), Job's method and FTIR spectroscopic studies, the structures proposed for the metal (II) complexes of amoxicillin would be;

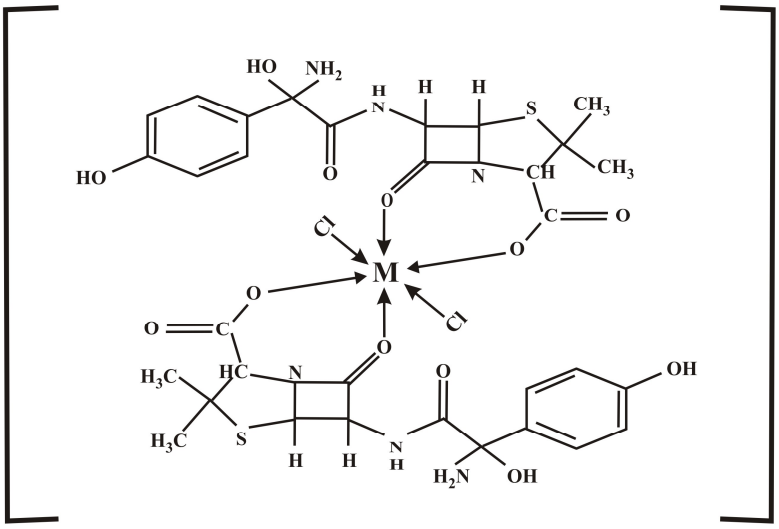

Fig 1: Proposed structure of Amoxicillin Complexes (M= Co^{+2}, $Fe^{2+}$and Mn^{+})

CONCLUSION

This research work has demonstrated the use of mechanochemical synthesis approach for the reliable synthesis of coordination metal (II) complexes of active pharmaceutical ingredient (API). It was discovered that, the solvent free solid-solid state can be used to obtain the same

product as that obtained from conventional For all complexes, the obtained IR data, molar conductivity and effective magnetic moment values suggested the geometry and coordination of molecules of Amoxicillin to their respective metal ions. Amoxicillin coordinates u(COO) and u(C=O) given rise to octahedral geometry.

Contribution of Authors

All the reported authors, Jibril S, performed the experimental work, Kurawa M. A supervised the method.

research works. Sani S, assist in the interpretation of results while Shehu S. M contributed in the literature search. All the authors agreed with the final manuscript.

Conflict of Interest

All authors accepted and declare that, no conflict of interest.

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