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Solvent-free synthesis, characterization and antimicrobial studies of calcium and potassium complexes with some cephalosporin antibiotics

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Abstract

The solid complexes of potassium and calcium were synthesized with some cephalosporin antibiotics which include: cefixime (CFI), cefuroxime (CFU) and cefradine (CFA). The complexes were synthesized by solvent-free technique and characterized by solubility, molar conductivity, melting point, UV-Vis, IR and elemental analysis. The analytical data of these complexes showed that the antibiotics coordinated to the metals through oxygen atom of the carboxylate anion, oxygen atom of nitrate ion and oxygen atom of carbonyl in both complexes due to similarity in structures of the antibiotics to give a coordination number of five with proposed molecular formula as [M(CFI)NO_3], [M(CFU)NO_3] and [M(CFA)NO_3] where M represents potassium or calcium. The molar conductance values of the complexes suggest that they are non-electrolytes, while the result of antimicrobial activity showed that the complexes have more activity than the antibiotics. The melting point, color and electronic spectra of the complexes were different from those of the antibiotics, which suggest the formation of coordination compounds.

Keywords: Antimicrobial resistance, cephalosporin, metals, potassium and calcium.

Introduction

Antimicrobial resistance or bacteria resistance to antibiotic is growing at alarming rate globally, and this has result to high rate of death annually. The resistance has become a worldwide issue which is fast spreading into most countries in which Nigeria is not an exception. Different species of microbes has their mode developing resistance and varies considerably from fungi which is known antifungal resistance, viruses has theirs as antiviral resistance and bacteria with antibiotic resistance[1]. The Resistant microorganism usually present difficulty in their treatment, and this result to seeking new platform and medication techniques so as to find alternative to the menace. But the alternative way or approach to tackle this menace is becoming expensive, while dosage modification is usually associated to toxicity or leading to toxic[2]. Research and findings had attributed the increase in drug resistance by bacteria to rampant use of the antibiotics for treatment of illness that requires little or no antibiotics for their treatment[3]. The increase in bacteria resistance to antibiotics has necessitated the ongoing effort toward seeking for another alternative or means for the treatment of bacteria related diseases. Recently, there was an effort toward proving new set antibiotics and this mandate seems to be difficult because up to now no new antibiotic that has been develop[4]. World Health Organization (WHO) has issued a statement recently which indicates that issue of bacteria resistance is on the rise globally within all the region of the world. The report further stressed that the resistance of bacteria to antibiotics can affect all ages groups and every country if adequate measures were not put in place[5].

Centers for Disease Control and Prevention (CDC), also stated that the number of people that are infected with bacteria is fast growing every year in the United States. Their report further predicts high number of people that will become infected with bacteria and will be resistance to antibiotics[6]. High rate of bacterial resistance to antibiotics is associated to the mode of antibiotic prescription and also non-adherence to dose specification[7]. Indiscriminate prescription of antibiotics has been linked to the different instances of bacteria resistance. Some of the issues relating to bacteria resistance has to do with the patient and physicians both of whom are contributing significantly by way of demanding for antibiotics by the patients and prescribing it to them without considering professional applications or explaining the need for antibiotics in treatments[8]. Antibiotic resistance usually increases due to long time use of antibiotics in the treatments. Therefore, there is need to minimize the duration when using antibiotics for treatments which will in turn reduce the rate of bacteria resistance to antibiotics[9].

Cephalosporin antibiotic are usually indicated for the treatment of infection which is due to particular bacterium that is resistant to the antibiotic needed for it treatment. Most of the initial cephalosporin are effective against Gram-positive bacteria, and other types also proved to be active against Gram-negative bacteria[10]. Although the use of broad-spectrum cephalosporin has increased, resistance cephalosporin still exists.

Metals have a long history of used as antimicrobial agents since time in memories. Some studies showed that various types of

metals has the ability to cause different effect to the cell wall of the micro-organism, and these varies based on the target location within the microbes. Several researchers have reported the effectiveness of transition metals against micro-organisms. Most of these transition metals show increased activity against micro-organisms when coordinated with ligands in complex compounds. Coordination compounds have diverse applications in many aspects of human life due to their numerous exciting properties. They play critical roles, especially during the biological processes. Several studies have demonstrated an increase in biological activity following the interaction of several compounds with metal ions[11].

In biological system, inorganic compounds are vital players with diverse role which has far out weight organic compounds. This is because the inorganic compounds are usually stable within the biological system without undergoing further transformation[12]. These complexes exhibit applications in clinical, analytical, and industrial processes.

Human body system required adequate amount of potassium, sulfur and chlorine for the maintenance of body system. These metals includes: calcium, phosphorus, potassium, magnesium, sodium and chlorides functions as electrolytes in the body[13,14]. Potassium is usually required by the body for proper metabolic functioning of the body system and enzymatic path ways[15]. Potassium is a key player in terms of heart functions, and also maintenance of body skeleton and muscle functions[16]. Potassium is also required in form ions in nerve cells for proper functioning[17]. Calcium (Ca) is one of the most prominent alkaline earth metals that have received increasing attention. Ca complexes has wide range of application in catalysis, it's usually employed in most of organic reactions as catalyst[18]. Calcium is one of the essential mineral needed for by both human and animals. It is essentially required for the maintenance of bone. Inadequate amount of calcium in the body contribute to the absorption of some metals like cadmium (Cd) and lead (Pb)[19,20]. This may result to improper action of calcium in living organism[21,22].

Some research showed that the toxicity posed by Pb and Cd when present in the body, affect the nutritional requirements of calcium[23]. The influence of monovalent and divalent cations on excitable tissues has been of interest to physiologists and pharmacologists for many years and the properties of pharmacological agents and physiological mechanisms are in some cases most readily understood in terms of ion distribution and ion movements. Ca^{2+} channels, for example, play the key role in cell physiology[24]. Even though, the process of ion passage is unclear[25]. Proposed mode coordination of calcium ions in most of its complexes are characterized in term of its ionic radii and their high polarity and electro positivity. Calcium usually form complexes with d^0 configuration due absence of electrons, and it result to the formation ionic bonding around the metal. Coordination compounds have diverse applications in many aspects of human life due to their numerous interesting properties. They play critical roles especially in biological processes. Several studies have demonstrated an increase in biological activity following the interaction of several compounds with metal ions[12]. The bonding of metals to ligands frequently results in synergistic activity. Metal complex such as cisplatin, has vast application in bioinorganic chemistry, some them demonstrate promising activity while others are undergoing clinical trial and preclinical respectively. Despite this milestone in the field of organometallic chemistry, fundamental information and research on the complexes of calcium and potassium remain obsolete. Hence, there is need for more exploration into complexes of calcium and potassium.

In continuation of our work on bacteria resistance to cephalosporin using transition metals[1,26], this paper reports the solvent-free synthesized complexes of calcium and potassium using some cephalosporin antibiotics.

Materials and methods

Chemicals and instruments: The chemicals and other reagents that are used in this work are of analytical grade. These chemicals were used as purchased without additional purification. The source of metals that were used includes: calcium nitrate tetrahyrate ($Ca(NO_3)_2$. $4H_2O$ and potassium nitrate (KNO_3). The ligands are Cefuroxime (Cfu), Cefradine (Cfa) and Cefixime (Cfi). Infra red spectra analyses of the complexes in KBr pellets were obtained in the range of 4000-400 cm^{-1} using FTIR spectrometer. The metal estimation analysis was determined using atomic absorption spectroscopy (AAS) Perkin-Elmer Spectrometer, model 3110. The ultra violet spectra of the complexes and the ligands were obtained on UV-2550 Shimadzu Spectrophotometer in the wavelength range of 200-800 nm.

Synthesis of the complexes: Cefuroxime (10mmol, 4.25g) and calcium nitrate tetrahydrate (10mmol, 2.36g) were carefully weighed and transferred into a mortar. The two reactants were then crushed (ground) for forty-five (45) minutes to obtain a homogenous powder. After which, TLC was used to determine the progress of the reaction. The homogenous mixture (powder) was removed from the mortar and stored in a desiccator. Same procedure was repeated for Cefuroxime (10mmol, 4.25g) and potassium nitrate (10mmol, 1.01g), Cefixime (10mmol, 4.53g) and calcium nitrate tetrahydrate (10mmol, 2.36g), Cefixime (10mmol, 4.53g) and potassium nitrate (10 mmol, 1.01g), Cefradine (10mmol, 3.63g) and calcium nitrate tetrahydrate (10 mmol, 2.36g) and Cefradine (10mmol, 3.63g) and potassium nitrate (10mmol, 1.01g) respectively (Scheme-1).

$$Ca(NO_3)_2 + X \xrightarrow[rtp/45min]{grinding} [Ca(X)NO_3]$$

$$KNO_3 + X \xrightarrow[rtp/45mins]{grinding} [K(X)NO_3]$$

Scheme-1: Synthesis of the complexes.

Where: X = Cefuroxime (CFU), Cefixime (CFI), Cefradine (CFA).

Antimicrobial Screening: The antimicrobial activities of the antibiotics and their metal complexes were assayed in-vitro, using disc diffusion method against the following microorganisms; Streptococcus pneumonia, Bacillus subtitle, Salmonella typhi, Klebsiella pneumonia, Escherichia coli, Methicillin-resistance Staphylococcus aureus (MRSA), Pseudomonas aeruginosa and Staphylococcus aureus. The suspension containing specie of the micro-organism was added to the freshly prepared nutrient agar medium contain in Petri dish and allowed to set. The solutions of the antibiotics were introduced with various concentrations (30, 20 and 10) mg/mL of antibiotics and their metal complexes in methanol were placed on the culture media and incubated for 24hrs at 37^0C. Activities were determined by measuring the diameter of the zone of inhibition (mm). The antibiotics and their complexes that showed a zone of inhibition of 10mm and above were further assayed for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using samples concentration of (6, 4 and 2) mg/mL in methanol using same bacterial species in peptone water[27].

Results and discussion

The complexes synthesized were found stable in air, they are soluble in polar solvents like ethanol, water, DMSO and methanol, while slightly soluble in chloroform; insoluble in ethyl acetate, ether and benzene. The percentage yield of the compounds is above 80%, while the color range from milky to white, yellow and brown. The molar conductance values are in the range of 2.1-4.8Scm^2 mol^{-1} in 1x10^{-3} molL^{-1}, which suggest that they are non-electrolytes[28]. The melting point of the complexes is different from those of the antibiotic (Table-1), which indicate the formation of a compound and this was further confirmed by TLC.

Infrared analysis: The main infrared absorption bands of the antibiotics and its complexes are shown in Table-2. It can be seen from the results that the characteristics absorption peaks of all the complexes are similar. The vibrations centered around 3019-3216cm^{-1} in the antibiotics was assigned to ν(O-H) stretching frequency, which upon complexation undergo a shift in the complexes. The band at 3503- 3535 cm^{-1} in the antibiotics was also assigned to ν(N-H$_2$) vibration of the amine group, while the band at 1233-1334cm^{-1} was assigned to ν(C=N)[29]. The sharp intensity band attributed to ν(C=O) vibration stretching was observed in the spectra of the antibiotics at 1021-1028 cm^{-1}. The relevant bands were observed in the metal complexes with lower wavelength shift as compared to the antibiotics couple reduction in their intensities (Table-2). The appearance of new bands at 667- 820cm^{-1} in the spectra of the complexes which is assignable to ν(M-O) stretching suggest the formation of the complexes.

Table-1: The physical properties of the antibiotics and their complexes.

Compounds	Molecular formula (Molar mass)	Color	Yield (g) (%)	M.pt/d ($^{\circ}$C)	Conductivity (Scm^{2}mol^{-1})
Cefixime	[$C_{16}H_{15}N_5O_7S_2$] (453.452)	White	-	218-225	-
Cefuroxime	[$C_{16}H_{16}N_4O_8S$] (424.386)	White	-	65-71	-
Cefradine	[$C_{16}H_{19}N_3O_4S$] (349.406)	White	-	95-110	-
[K(CFI)NO$_3$]	[K($C_{16}H_{15}N_6O_{10}S_2$)] (554.452)	Milky	5.28 (95.20)	180	2.1
[K(CFU)NO$_3$]	[K($C_{16}H_{16}N_5O_{11}S$)] (525.386)	White	5.12 (96.77)	122	3.2
[K(CFA)NO$_3$]	[K($C_{16}H_{19}N_4O_7S$)] (450.406)	White	4.50 (96.87)	180	2.7
[Ca(CFI)NO$_3$]	[Ca($C_{16}H_{15}N_6O_{10}S_2$)] (555.452)	White	6.59 (95.50)	130	4.5
[Ca(CFU)NO$_3$]	[Ca($C_{16}H_{16}N_5O_{11}S$)] (526.386)	Brown	6.33 (95.80)	135	4.0
[Ca(CFA)NO$_3$]	[Ca($C_{16}H_{19}N_4O_7S$)] (451.406)	Yellow	5.21 (86.70)	135	4.8

Where, CFI = Cifixime, CFU = Cefuroxime and CFA = Cefradine.

Ultra violet visible spectral analysis: Electronic absorption spectroscopy is one of the tools that is used to analyzed the nature of binding mode of the metal to the ligands through the vacant orbital of the metal. These interactions between metals to ligands is usually through empty d or f orbital in transition metals, which can result to d-d or f-f transition in the visible region and also corresponds to a particular energy level of transition. But for metals without electrons in the d orbital or without d orbital, the interrelation is usually through pie orbital in ligand and finally ligand-metal charge transfer (LMCT) or metal ligand charge transfer (MLCT), which is rare among non-transition metals. In non-transition metals, the π* orbital of the interacted ligand can couple with the π electron in s-orbital thus reducing the π- π* transition energy and resulting bathochromic shift. But if the coupling π is partially filled with electrons, it results in decreasing the transition probabilities which will lead to hypochromism and this concept is usually applicable to DNA binding study of complex compounds[30]. The electronic absorption spectra of the antibiotics and the complexes are presented in Table-3.

From the results obtained, all the antibiotics absorbed in the region of 320-348 nm which corresponds to 2865 – 3125 cm^{-1} energy level assignable to π- π*. Similarly, the complexes show absorption spectra in the region of 276 -360 nm in all the complexes and this corresponds to 2857 -4237 cm^{-1} energy region of the complexes. These transitions were assigned to MLCT due to the π- π* of the organic ligands (antibiotics).

Table-2: Infrared spectra for the antibiotics and their metal complexes.

Compounds	v(O-H)	v(N-H)	v(C=O)	v(NH_2)	v(C=N)	v(C-S)	v(C=C)	v(N-O)	v(M-O)
CFI	3216	1662	1021	3526	1334	2050	15191	-	-
CFU	3019	1558	1021	3503	1233	2050	1517	1353	-
CFA	3020	1565	1028	3535	1245	2063	1554	-	-
[K(CFI) NO_3]	3526	1666	1025	3220	1334	2112	1543	-	700
[K(CFU) NO_3]	3056	1595	1039	3473	1326	2020	1539	1326	670
[K(CFA) NO_3]	3410	1684	1022	3504	1233	2050	1558	1353	667
[Ca(CFI) NO_3]	3343	1640	1021	-	1319	-	-	-	820
[Ca(CFU) NO_3]	3451	1669	1039	-	1244	-	1546	1326	820
[Ca(CFA) NO_3]	3030	1684	1017	3421	1233	-	1558	1349	749

Table-3: UV-Visible Spectra of the Antibiotics and their metal complexes.

Compounds	Formula	Wavelength (nm)	Energies(cm^{-1})	Assignments
CFI	$C_{16}H_{15}N_5O_7S_2$	320	3125	π→π*
CFU	$C_{16}H_{16}N_4O_8S$	349	2865	π→π*
CFA	$C_{16}H_{19}N_3O_4S$	330	3030	π→π
[K(CFI) NO_3]	[K($C_{16}H_{15}N_6O_{10}S_2$)]	348	2874	LMCT
[K(CFU) NO_3]	[K($C_{16}H_{16}N_5O_{11}S$)]	340	2941	LMCT
[K(CFA) NO_3]	[K($C_{16}H_{19}N_4O_7S$)]	276 300	3623 3333	LMCT
[Ca(CFI) NO_3]	[Ca($C_{16}H_{15}N_6O_{10}S_2$)]	360	2778	LMCT
[Ca(CFU) NO_3]	[Ca($C_{16}H_{16}N_5O_{11}S$)]	360	3248	LMCT
[Ca(CFA) NO_3]	[Ca($C_{16}H_{19}N_4O_7S$)]	235 280 350	4237 3571 2857	LMCT

Microanalysis: The elemental analyses of the complexes are presented in Table-4. From the results obtained, the percentage C, H and N appears to be in good agreement with the proposed structures. This suggest that the complexes analyzed as [M(CFI) NO_3], [M (CFU)NO_3], and [M(CFA)NO_3]. Where M = K and Ca.

Antimicrobial studies: Metals have a long history of being antimicrobial agents. Some of these have now been widely studied as pharmaceutical agents and extensive investigation in the field of metal complexes have been reported[31]. The resistance of bacteria to antibiotics is growing at an alarming rate globally, and this necessitates the quest effective antimicrobial agent. In continuation of this discovery, the present study synthesized potassium and calcium complexes with cefixime, cefuroxime, and cefradine using a solvent-free method. The antimicrobial effects were observed to see whether the compounds involved in this study exhibit any activity or not. In the present study, both ligand and complexes have been evaluated against both Gram-positive and negative bacteria such as *Streptococcus pneumoniae*, *Bacillus subtilis, Salmonella typhi, Klebsiella pnuemoniae, Escherichia coli, MRSA, Pseudomonas aeruginosa and Staphylococcus aureus.* The results of the inhibition zones of the selected bacteria due to the effect of the ligand and its complexes are presented in (Tables 5 and 6).

This results in Table-5 shows that only potassium complex with cefradine and cefixime showed increased activity, while potassium complex with cefuroxime had less activity as compared with the parent drugs.

From the results obtained in Table-6, calcium complex with cefradine did not show any activity against MRSA and *pseudomonas aruginosa* at all concentrations tested. The result also indicate the complexes has activity on the other organisms as compared with cefradine against the same organism at the same concentration. Calcium complex with cefixime showed activity on *Staphylococcus aureus* at all concentrations, while cefixime was resistant by *Staphylococcus aureus* at all the concentrations. The complex of calcium with cefuroxime displays increased activity at a concentration of 20 and 30mg/ml respectively when compared with cefuroxime at the same concentration.

Structure of the complexes: The analytical data of this study revealed that the mode of binding of the antibiotics to the metal ions occurs through oxygen atom of the carboxylate anion, oxygen atom of nitrate ion and the oxygen atom of carbonyl for both complexes to give a coordination number of five (Figure-1 and 2). This is similar to our previous reports[26,1].

Conclusion

Based on the results obtained from the analysis of both compounds, five coordinated complexes were proposed. Measurements of inhibition zones of the ligand and complexes showed that the prepared complexes have enhanced antibacterial activity as compared to the antibiotics.

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Table-4: Microanalysis of the Complexes.

Compounds	Molecular formula (Molar mass)	Microanalysis: found (calculated)%			
		C	H	N	M
[K(CFI)NO_3]	[K($C_{16}H_{15}N_6O_{10}S_2$)] (554.452)	33.45 (34.62)	2.50 (2.70)	15.03 (15.15)	6.93 (7.03)
[K(CFU)NO_3]	[K($C_{16}H_{16}N_5O_{11}S$)] (525.386)	36.01 (36.54)	3.00 (3.04)	13.28 (13.32)	7.18 (7.42)
[K(CFA)NO_3]	[K($C_{16}H_{19}N_4O_7S$)] (450.406)	42.20 (42.62)	4.18 (4.21)	12.38 (12.43)	8.32 (8.65)
[Ca(CFI)NO_3]	[Ca($C_{16}H_{15}N_6O_{10}S_2$] (555.426)	34.48 (34.57)	2.56 (2.70)	15.09 (15.10)	7.15 (7.20)
[Ca(CFU)NO_3]	[Ca($C_{16}H_{16}N_5O_{11}S$)] (526.386)	36.28 (36.47)	3.00 (3.03)	13.17 (13.29)	7.25 (7.59)
[Ca(CFA)NO_3]	[Ca($C_{16}H_{19}N_4O_7S$)] (451.406)	42.49 (42.53)	4.15 (4.21)	12.00 (12.04)	8.57 (8.86)

Table-5: Antimicrobial activities of antibiotics and their potassium complexes.

Compounds	Conc. mg/mL	MRSA	S. aureus	S. pneumoniae	B. subtilis	E. coli	S. typhi	K. pneumoniae	p. aeruginosa
CFA	10	13±0.1	22±1.0	0.0±0.0	24±1.0	0.0±0.0	14±0.5	16±0.3	8.0±0.6
	20	17±0.6	25±0.6	0.0±0.0	29±1.0	9.0±0.6	19±0.6	22±0.5	11±1.0
	30	19±1.0	29±1.0	0.0±0.0	34±1.0	13±1.0	25±1.0	28±1.0	14±0.4
CFI	10	0.0±0.0	0.0±0.0	0.0±0.0	9.0±0.6	12±0.2	13±0.6	0.0±0.0	7.0±0.8
	20	0.0±0.0	0.0±0.0	8.0±0.2	12±1.0	16±1.0	18±0.4	7.0±0.5	11±0.0
	30	0.0±0.0	0.0±0.0	11±0.4	15±0.7	19±0.7	22±1.0	11±0.6	14±1.0
CFU	10	7.0±0.8	10±0.5	0.0±0.0	12±0.5	0.0±0.0	10±0.4	0.0±0.0	0.0±0.0
	20	11±0.2	14±0.6	0.0±0.0	14±0.3	0.0±0.0	13±0.6	0.0±0.0	0.0±0.0
	30	14±0.5	16±0.4	0.0±0.0	18±0.6	0.0±0.0	16±1.0	0.0±0.0	0.0±0.0
$[K(CFA)NO_3]$	10	0.0±0.0	21±0.4	0.0±0.0	7.0±0.5	14±0.5	13±0.8	0.0±0.0	0.0±0.0
	20	0.0±0.0	27±0.5	8.0±0.3	10±0.6	19±0.3	19±0.1	0.0±0.0	0.0±0.0
	30	0.0±0.0	32±0.6	11±0.4	12±0.3	24±0.6	23±0.3	0.0±0.0	0.0±0.0
$[K(CFI)NO_3]$	10	10±0.1	10±0.2	0.0±0.0	8.0±1.0	7.0±0.3	13±0.8	13±0.3	0.0±0.0
	20	15±0.6	15±0.3	0.0±0.0	11±1.1	10±0.2	19±0.5	19±0.2	0.0±0.0
	30	19±0.7	20±0.4	0.0±0.0	14±0.7	13±0.4	24±0.4	24±0.8	7.0±0.5
$[K(CFU)NO_3]$	10	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	20	0.0±0.0	7.0±0.5	0.0±0.0	7.0±0.4	0.0±0.0	0.0±0.0	7.0±0.6	0.0±0.0
	30	0.0±0.0	11±0.3	0.0±0.0	9.0±0.5	0.0±0.0	0.0±0.0	10±0.4	0.0±0.0

Table-6: Antimicrobial activities of antibiotics and their Calcium complexes**.**

Compounds	Conc. mg/mL	MRSA	S. aureus	S. pneumoniae	B. subtilis	E. coli	S. typhi	K. pneumoniae	p. aeruginosa
CFA	10	13±0.1	22±1.0	0.0±0.0	24±1.0	0.0±0.0	14±0.5	16±0.3	8.0±0.6
	20	17±0.6	25±0.6	0.0±0.0	29±1.0	9.0±0.6	19±0.6	22±0.5	11±1.0
	30	19±1.0	29±1.0	0.0±0.0	34±1.0	13±1.0	25±1.0	28±1.0	14±0.4
CFI	10	0.0±0.0	0.0±0.0	0.0±0.0	9.0±0.6	12±0.2	13±0.6	0.0±0.0	7.0±0.8
	20	0.0±0.0	0.0±0.0	8.0±0.2	12±1.0	16±1.0	18±0.4	7.0±0.5	11±0.0
	30	0.0±0.0	0.0±0.0	11±0.4	15±0.7	19±0.7	22±1.0	11±0.6	14±1.0
CFU	10	7.0±0.8	10±0.5	0.0±0.0	12±0.5	0.0±0.0	10±0.4	0.0±0.0	0.0±0.0
	20	11±0.2	14±0.6	0.0±0.0	14±0.3	0.0±0.0	13±0.6	0.0±0.0	0.0±0.0
	30	14±0.5	16±0.4	0.0±0.0	18±0.6	0.0±0.0	16±1.0	0.0±0.0	0.0±0.0
$[Ca(CFA)NO_3]$	10	0.0±0.0	8.0±0.5	13±0.4	10±0.1	11±0.5	12±0.4	10±0.3	0.0±0.0
	20	0.0±0.0	12±0.5	19±0.3	15±0.6	15±0.4	17±0.6	16±0.5	0.0±0.0
	30	0.0±0.0	17±0.6	24±0.3	19±0.8	20±0.2	22±0.4	19±1.1	0.0±0.0
$[Ca(CFI)NO_3]$	10	0.0±0.0	11±0.3	7.0±0.9	9.0±0.6	11±0.2	7.0±0.3	0.0±0.0	0.0±0.0
	20	0.0±0.0	15±0.2	11±0.5	13±0.3	14±0.1	11±0.4	0.0±0.0	0.0±0.0
	30	0.0±0.0	19±0.6	15±0.4	17±0.2	19±0.4	14±0.6	0.0±0.0	0.0±0.0
$[Ca(CFU)NO_3]$	10	0.0±0.0	13±0.4	8.0±0.1	0.0±0.0	0.0±0.0	0.0±0.0	13±0.4	10±0.2
	20	0.0±0.0	15±0.4	12±0.3	7.0±0.6	7.0±0.5	7.0±0.5	18±0.6	13±0.3
	30	0.0±0.0	22±0.4	17±0.3	10±0.8	9.0±0.2	10±0.6	22±0.5	17±0.4

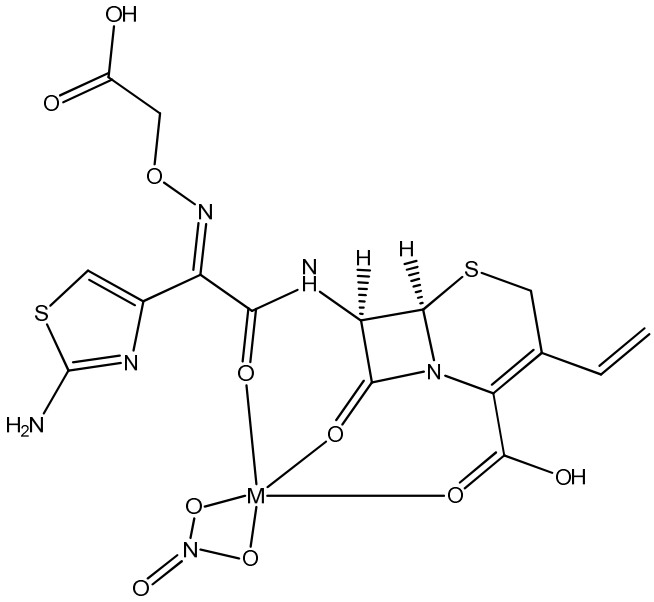

Figure-1: [M(CFI)NO_3], where M = K and Ca.

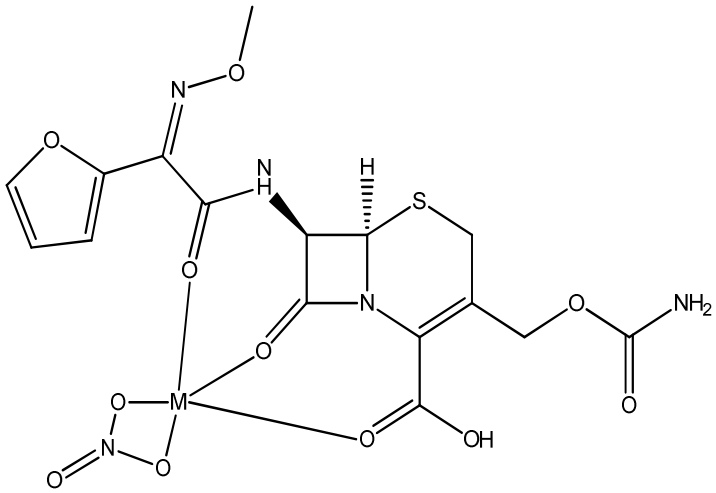

Figure-2: [M(CFU)NO_3] where M = K and Ca.

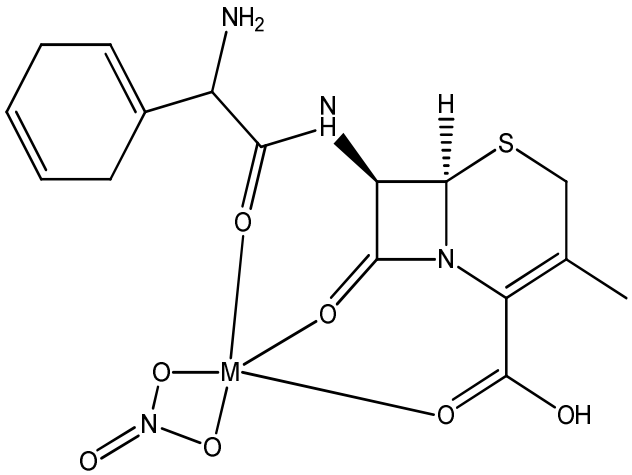

Figure-3: [M(CFA)NO_3], where M = K and Ca.

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