E-ISSN: 2320-1215
P-ISSN: 2322-0112

Research and Reviews: Journal of Pharmacy and Pharmaceutical Sciences

Synthesis, Characterization, and Spectroscopic Studies for New Cu(II), Co(II), Zn(II), Fe(III) And Zr (II) Complexes of Oxytetracycline Antibiotic, *In vitro* Antimicrobial Assessment Studies

Ragab R Amin[1]*, Mohammed Abo-Elkassem[2] and Alaa Anwar[3]

[1]Basic Science Department, Faculty of Engineering, Nahda University, Egypt
[2]Department of Chemistry, Faculty of Science, Beni Sueff University, Egypt
[3]Faculty of Pharmacy, Nahda University, Egypt

Research Article

Received: 16-01-2017
Revised: 30-01-2017
Accepted: 05-02-2017
***For correspondence**: Ragab R Amin, Head of Basic Science Department, Faculty of Engineering, Nahda University, Egypt, E-mail: Ragab.amin@nub.edu.eg

Keywords: Spectroscopic studies, Oxytetracycline, Antibacterial evaluation, Negative bacteria

ABSTRACT

The 1:1 and 2:1 M ratio metal complexes of new Cu(II), Co(II), Fe(III), Zn(II) and Zr(II) complexes of Oxytetracycline hydrochloride have been synthesized. Attempts have been made to ascertain their probable structures based on elemental analysis, spectra (IR, Electronic) and NMR studies. The ligand form a complex in a molar ratio 1:1 having an empirical formula ($MLSO_4.nH_2O$) and 2:1 molar ratio having an empirical formula ($M_2LSO_4.nH_2O$) where M=Cu(II), Co(II), Fe(III), Zn(II) and Zr(II), L=Oxytetracycline and n=2-6. The infrared spectral data and NMR studies were support the postulate that oxytetracycline reacts with metal ions as an ionic bidentate ligand through its carboxylate oxygen and the amide carbonyl oxygen. The antibacterial evaluation of the Oxytetracycline drug and their complexes were also performed against some gram positive and negative bacteria as well as fungi.

E-ISSN: 2320-1215
P-ISSN: 2322-0112

INTRODUCTION

Metals and metal complexes have played a key role in the development of modern chemotherapy [1,2]. For example, complexation of non-steroidal anti-inflammatory drugs to copper overcomes some of the gastric side effects of these drugs [3–7]. A number of drugs and potential pharmaceutical agents also contain metal-binding or metal-recognition sites, which can bind or interact with metal ions and potentially influence their bioactivities and might also cause damages on their target biomolecules. Numerous examples of these metallodrugs and metallopharmaceuticals and their actions can be found in the literature, for instance: (a) several anti-inflammatory drugs, such as aspirin and its metabolite salicylglycine [8–11], suprofen [12], and paracetamol [13] are known to bind metal ions and affect their antioxidant and anti-inflammatory activities; (b) the potent histamine-H_2-receptor antagonist cimetidine [14] can form complexes with Cu^{2+} and Fe^{3+}, and the histidine blocker antiulcer drug famotidine can also form stable complex with Cu^{2+} [15,16] (c) the anthelmintic and fungi static agent thiabendazole, which is used for the treatment of several parasitic diseases, forms a Co^{2+} complex of 1:2 metal to drug ratio [17] and (d) the Ru^{2+} complex of the anti-malaria agent chloroquine exhibits an activity two to five times higher than the parent drug against drug resistant strains of *Plasmodium falciparum* [18]. However, it is known that some drugs act via chelation or by inhibiting metalloenzymes but most of the drugs act as potential ligands, a lot of studies are being carried out to ascertain how metal binding influences the activities of the drugs [19]. Metal complexes are gaining increasing importance in the design of drugs on coordination with a metal. The therapeutic importance of oxytetracycline drug was behind the development of numerous methods for its determination.

The different method techniques adapted to the analysis of oxytetracycline drug have been reported [20-33]. Literature survey fell to reveal any previous work or literature regarding the complexation of oxytetracycline drug with some transition metals [34,35]. An attempt of synthesizing, characterization, and biological screening of oxytetracycline drug ligand and their metal complexes of Calcium and Magnesium have been successfully achieved. The characteristic structure of the molecule is apparent in the existence of the carboxylic and carbonyl electron-rich ligand. However, literature survey has revealed that no attempt has been made to study the complexes of some alkali earth metal ions with the above-mentioned drug ligand compounds. It is a thought of interest to study the synthesis and characterization and biological screening of metal complexes of new Cu(II), Co(II), Fe(III), Zn(II) and Zr(II) complexes of oxytetracycline drug. Drug compounds are biologically active, these compounds have become of interest to be studied biologically, and compared their activities against four species of bacteria (*Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli*, *Pseudomonas aeruginosa*), and two fungal species (*Aspergillus flavus* and *Candida albicans*).

E-ISSN: 2320-1215
P-ISSN: 2322-0112

MATERIALS AND METHODS

The chelating agent oxytetracycline complexes was prepared by mixing ethanolic solution of oxytetracycline drug (1 gm) and Metal salts (0.5 gm) in the same solvent. the reaction mixture was boiled under reflux in water bath for 3 hours then left overnight until precipitated.

Yields 70%. Identification of New complexes was carried out by elemental analysis and IR Spectral.

The complexes were prepared by adding EtOH solutions of equimolar amounts of oxytetracycline and metal (II) salts. The mixture was heated on a water bath for 3 hrs. And allowed to evaporate slowly prior to filtration. The precipitates were washed with EtOH and left overnight until dried yield 70% to 80%.

RESULTS AND DISCUSSION

Transition elements were found to form stable complexes with many ligands containing heteroatoms. there is preference for amines, halogens, CN^-, tertiary phosphrines and sulfides.

Nichel (II) is one of the transition elements was found to form complex of square shape with the general formula MLX2 where L is neutral ligand and X Non-Negative Ion [9].

Nickel(II) was found to form stable complexes with many drugs Acetylsalicylic acid [10], fluoxetine [11], vitamin C [12], and ephedrine [13]. Theoretically and from what was mentioned above Oxytetracycline could form chelate, through its nitrogen and oxygen atoms, with Nickel(II). Addition of $NiSo_4$ to oxytetracycline produced black complex that is soluble in Dimethyl sulfoxide (DMSO).

Solution Studies

Effect of pH

The influence pH was studied over the range (1-10) on the absorbance of complex at 354.3 nm, the results were evaluated as shown in **Figure 1.** The shape of the absorption spectrum, the position of the absorption maximum and the apparent molar absorptivity of oxytetracycline-Nickel (II) complex do vary with pH, where the maximum absorbance obtained at pH [4], but a neutral and basic media the oxytetracycline-Nickel(II) complex was precipitate.

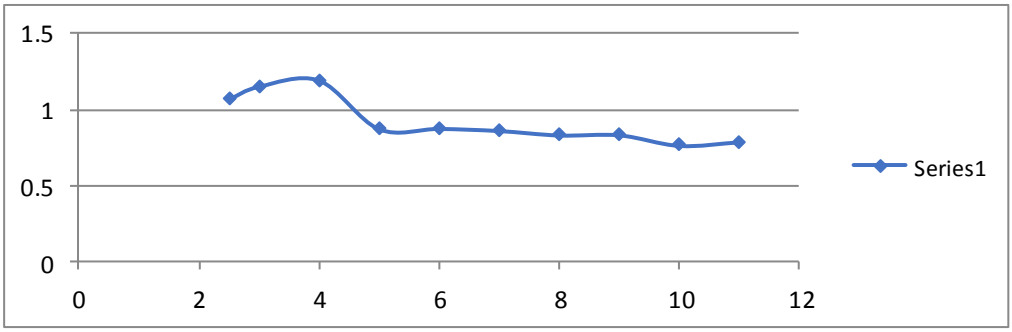


Figure 1. Effect of pH on the absorbance of Oxytetracycline-Nickel(II) complex.

E-ISSN: 2320-1215
P-ISSN: 2322-0112

Effect of Reaction Time

The stability of the absorbance with the time was studied from 1-60 min. **Figure 2** shows the relation between absorbance and the time, where the maximum absorbance was reached at the 6 and 7 min. after the addition of $NiSO_4$ to oxytetracycline, the absorbance after this optimal time decreased at 8 min. decreased and then the absorbance was almost stable till 60 min.

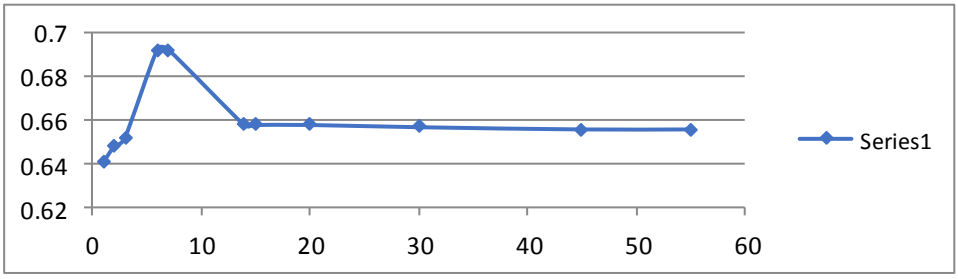


Figure 2. Effect of time on the absorbance of oxytetracycline-Ni(II) complex**.**

Effect of Temperature

The effect of temperature was studied in the range of (5°C to 60°C) on the produce of complex of oxytetracycline with Ni (II) as shown in **Figure 3,** the absorbance was reduced as the temperature was increased.

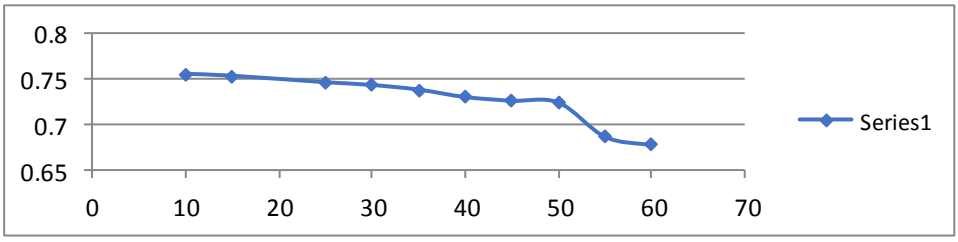


Figure 3. Effect of temperature on the absorbance of oxytetracycline-Ni(II) complex.

Effect of Volume of Ni (II) Salt

The volume of Ni(II) has a great effect on the formation of oxytetracycline-Ni(II) complex. **Figure 4** shows that as the volume of Ni(II) increase the absorbance slightly decrease.

E-ISSN: 2320-1215
P-ISSN: 2322-0112

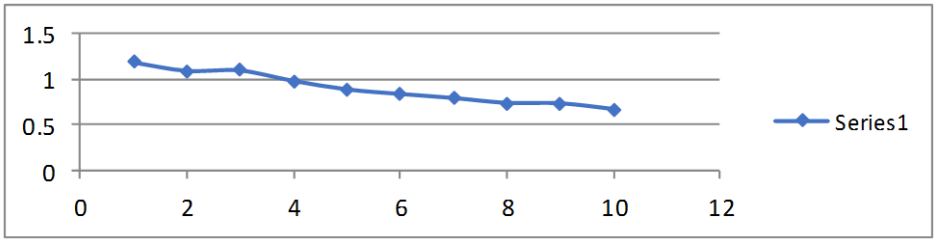
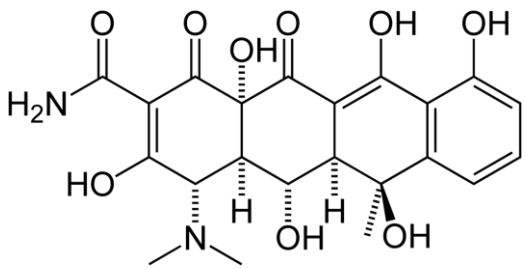


Figure 4. Effect of volume of 1 × 10^{-4} M $NiSO_4$ on the absorbance.

Solid Studies

Elemental analysis

The Elemental analysis is applied for oxytetracycline complexes to suggest the structure of oxytetracycline complexes, carbon and hydrogen percentage shown in **Table 1.** The proposal structure for oxytetracycline complexes shown in **Schemes 1-5.**



Scheme 1. Oxytetracycline antibiotic structure.

Table 1. Elemental analysis of oxytetracycline and their metal complexes.

Oxy.Complexes formula M.Wt(g/mol)	Color	Elemental analysis %			
		Calculated		Found	
		C%	H%	C %	H %
{Ni(L)SO_4}. H_2O (689.856)	Black crystalline powder	38.31	4.24	38.26	5.29
Ni_2(L)$(SO_4)_2$ (808.580)	Black crystalline powder	32.68	3.12	32.92	5.29
Cu(L)SO_4.5H_2O{ (694.646)	Black	38.04	4.4	37.32	3.95
{Cu_2(L)$(SO_4)_2$}.6 H_2O	Black	28.47	4.09	28.22	4.67

E-ISSN: 2320-1215
P-ISSN: 2322-0112

(928.226)					
Zr(L)(Cl)}. H_2O{ (642.322)	Brown near to be Orange	41.14	4.2	41.62	3.86
Fe(L) (H_2O) (570.954)	Black	46.28	4.77	46.02	4.89
{Fe_2(L)(Cl_3)} (2$H_2$0) (750.68)	Black	35.2	3.9	34.52	4.27

IR spectral studies

The infrared absorption bands were one of the important tools of the analyses used for determining the mode of chelation. Oxytetracycline Hcl behaves as an ionic mono-dentate molecule and was coordinated to the metals through its amide carbonyl oxygen. Therefore, in these complexes one metal ion was coordinated to one molecule of the Oxytetracycline. In comparison with the published spectra [14-16] of the free Oxytetracycline spectra, bands found are:

- The band at 3000 cm^{-1} to 3700 cm^{-1} in all complexes was broad. This was due to the presence of the hydroxyl group. The ligand band at 3080 cm^{-1} assigned to the secondary amide NH_2 [17] was either absent or very weak in all complexes, indicating the interference of the OH and NH_2 bands in this region (3000 cm^{-1} to 3700 cm^{-1}).
- The resolved bands at 3020 cm^{-1} and 3000 cm^{-1} in the free Ligand and copper complex due to the aromatic CH.
- The next diagnostic band in the free ligand was that of the amide carbonyl group which appears at 1583.27 cm^{-1}. This band C=O is slightly positively shifted in the spectra of the complexes due to the coordination between amide and carbonyl group (tautomeric), indicating the involvement of the C=O of the amide in the chelating process [18].
- The appearance of an acetate bands at 676 cm^{-1} to 640 cm^{-1} and 601 cm^{-1} to 525 cm^{-1} can be attributed to the carbonyl, These bands support the chelation through the O atoms [19].

Table 2. Significant IR spectral bands (cm^{-1}) of the ligand of oxytetracycline HCl and their metal complexes.

Assignments	The compounds						
	L(Ligand)	Cu (1:1)	Cu (2:1)	Ni (1:1)	Ni (2:1)	Fe (1:1)	Fe (2:1)
u(OH)	3382.5	3418.2	3424.9	3381.5	3410.4	3411.4	3418.2
u(NH_2)	3080	3100	3100	3250	3250	3220	3220
CH-aliph	2926.4	2362.3	2337.3	2362.3		2928.3	
u (C=O)	1583.2	1559.1	1580	1600	1560	1623.7	1580
C=N	1622.8	1621.8	1621.8	1636.3	1639.2	1580	1624.7

E-ISSN: 2320-1215
P-ISSN: 2322-0112

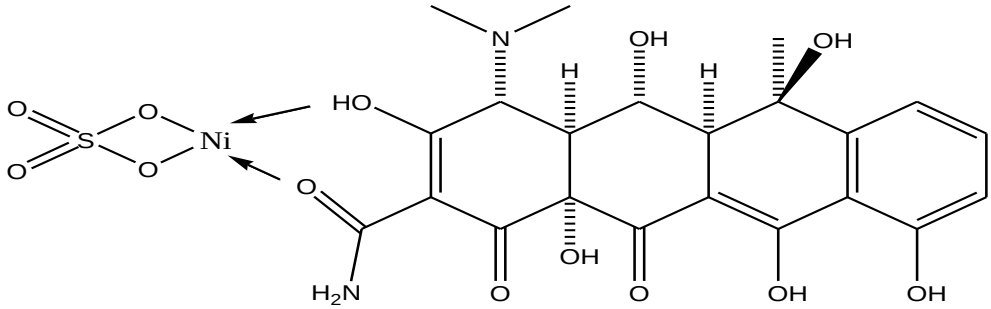
Biological Screening

The oxytetracycline Hcl ligand and their metal complexes were tested for their antimicrobial activity against some species of bacteria (*S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *N. gonorrhoeae* and *S. faecalis*) using modified Kirby-Bauer disc diffusion method (20) Briefly, 100 ml of the test bacteria/Fungi were grown in 10 ml of fresh media until they reached account of approximately 108 cells/ml for bacteria or 105 cells/ml for fungi [21]. 100 ml of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained.

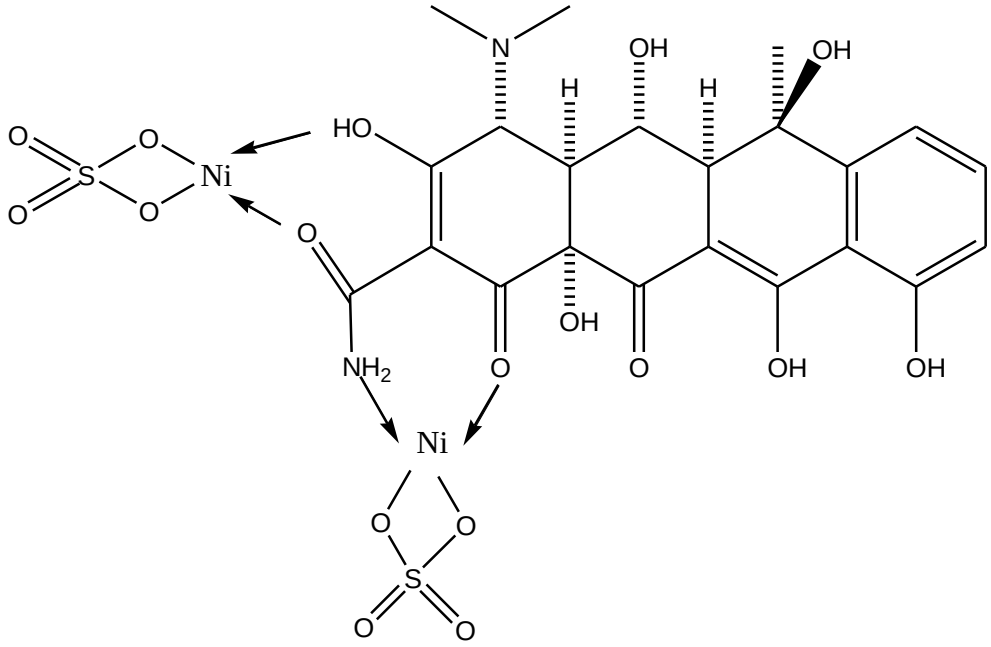
- Isolated colonies of each organism that might be playing pathogenic role should be selected from primary agar plates and tested for susceptibility by disc diffusion method.
- Of the many media, available, NCCLS recommends Mueller- Hinton agar due to: it results in good batch-to-batch reproducibility.
- Disc diffusion method for filamentous fungi tested by using approved standard method (M38-A) developed by the (NCCLS, 2002) for evaluating the susceptibilities of filamentous fungi to antifungal agents.
- Disc diffusion method for yeasts developed by using approved standard method (M44-P) by the (NCCLS, 2003).
- Plates inoculated with filamentous fungi as Aspergillus flavus at 25°C for 48 hours Gram(+) bacteria as *Staphylococcus aureus*, *Bacillus subtilis*: Gram(-) bacteria as *Escherichia coli*, *Pseudomonas aeruginosa* they were incubated at 35°C to 37°C for 24-48 hours and yeast as *Candida albicans* incubated at 30°C for 24-48 hours and then the diameters of the inhibition zones were measured in millimeters.
- Standard discs of Ampicillin (Antibacterial agent), Amphotericin B (Antifungal agent) served as positive controls for antimicrobial activity but filter discs impregnated with 10 µl of solvent (distilled water, chloroform, DMSO) were used as negative control.
- The agar used is Muller-Hinton agar that is rigorously tested for composition and pH. Further the depth of the agar in the plate is a factor to be considered in the disc diffusion method. this method is well documented and standard zones of inhibition have been determined for susceptible and resistant values.
- Blank paper disks (Schleicher & Schuell, Spain) with a diameter of 8 mm were impregnated 10 µl of tested concentration of the stock solutions.
- When a filter paper disc impregnated with a tested chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a “Zone of inhibition “or “Clear Zone”.

E-ISSN: 2320-1215
P-ISSN: 2322-0112

- For the disc diffusion, the zone diameters were measured with slipping calipers of the National Committee for Clinical Laboratory Standards (NCCLS, 1993).
- Agar-based methods such as E-test and disk diffusion can be good alternatives because they are simpler and faster than broth-based methods (Schemes 2-5) [24].

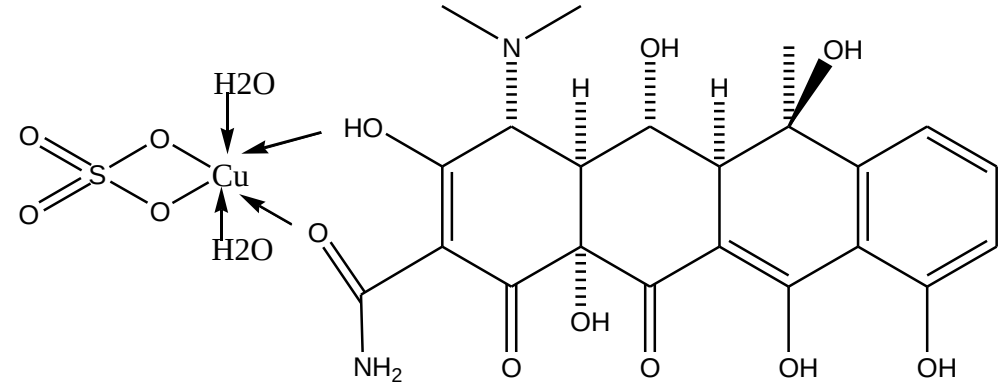


Scheme 2. The Proposal structure of oxytetracycline-Nickel(II) (1:1) complex.

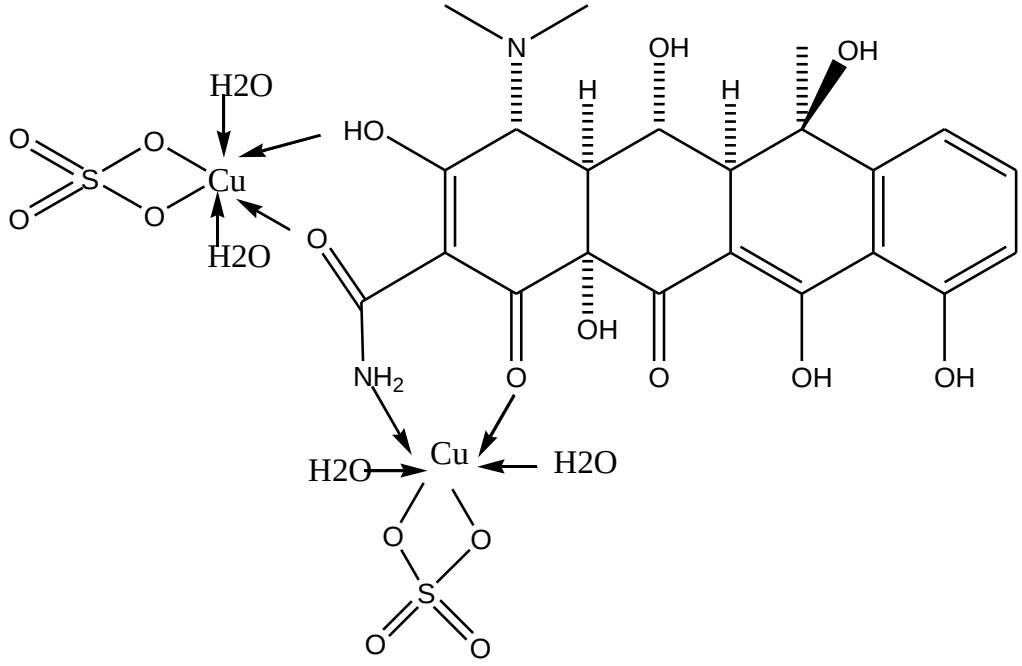


Scheme 3. The proposal structure of oxytetracycline-Nickel(II) (2:1) complex.

E-ISSN: 2320-1215
P-ISSN: 2322-0112



Scheme 4. The proposal structure of oxytetracycline-Cupper(II) (1:1) complex.



Scheme 5. The proposal structure of oxytetracycline-Cupper(II) (2:1) complex.

REFERENCES

1. Gielen M, Tiekink ERT (Eds). Metallotherapeutic drugs and metal-based diagnostic agents, the use of metals in medicine, Wiley, Chichester, 2005.
2. Bharti SK. Der Pharm Lett. 2009;1:2:39-45.
3. Weder JE, et al. Copper complexes of non-steroidal anti-inflammatory drugs: An opportunity yet to be realized. Coord Chem Rev. 2002;232(1-2):95-126.
4. Ware DC, et al. Synthesis, structures and hypoxia-selective cytotoxicity of cobalt(III) complexes containing tridentate amine and nitrogen mustard ligands. J Chem Soc Dalton Trans. 2000;925-932.

5. Nakai M, et al. Synthesis and insulin-mimetic activities of metal complexes with 3-Hydroxypyridine-2-carboxylic Acid. J Inorg Biochem. 2005;99:1275-1282.
6. Chaviara T. *In vivo* anticancer, anti-inflammatory, and toxicity studies of mixed ligand Cu(II) complexes of dien and its Schiff dibases with heterocyclic aldehydes and 2 amino 2 thiazoline. Crystal structure of [Cu(dien)(Br)(2a2tzn)](Br)(H(2)O). J Inorg Biochem. 2005;99(11):2102.
7. Sadler PJ. Metal complexes in medicine: Design and mechanism of action. Pure Appl Chem. 1998;70:863.
8. Baslas RK, et al. Studies on the metal-complex of acetyl salicylic acid (aspirin) Experientia. 1979;35:455.
9. Gonzalez BE, et al. Complex formation between transition metal ions and salicylglycine, a metabolite of aspirin. Polyhedron. 1994;13:1495.
10. Nolan KB and Soudi AA. Synthesis and characterisation of copper(II), zinc(II) and cobalt(II) complexes of salicyl glycine, a metabolite of aspirin. Inorg Chim Acta. 1995;230:209-210.
11. Muller JG and Burrows CJ. Metallodrug complexes that mediate DNA and lipid damage via sulfite autoxidation: Copper (II) Famotidine and Iron (III) Bis(Salicylglycine). Inorg Chim Acta. 1998;275:314-319.
12. Underhill AE, et al. Metal complexes of anti-inflammatory drugs. Part VIII: Suprofen complex. Inorg Biochem. 1993;52:139-44.
13. Lawal A and Obaleye JA. Synthesis, characterization and antibacterial activity of aspirin and paracetamol-metal complex. Biochemistry. 2007;19(1):9-15.
14. Kirkova M, et al. Effects of cimetidine and its metal complexes on nitroblue tetrazolium and ferricytochrome c reduction by superoxide radicals. Gen Pharmacol. 1999;33:271-276.
15. Duda AM. Histamine H2 antagonists: Powerful ligands for copper(II). Reinterpretation of the famotidine–copper(II) system. J Chem Soc. Dalton Trans. 1995;2909-2913.
16. Kubiak M, et al. Crystal structure of a copper(II)–famotidine complex and solution studies of the Cu^{2+}–famotidine–histidine ternary system. Chem Soc. Dalton Trans 1996;1905-1908.
17. Umadevi B. Metal-drug interactions-synthesis and crystal-structure of dichloro dithiabendazolecobalt (Ii) monohydrate. Eggleston, Inorg Chim Acta. 1995;234:149.
18. Sanchez-del Grado RA, et al. Toward a novel metal-based chemotherapy against tropical diseases. 2. synthesis and antimalarial activity in vitro and i*n vivo* of new Ruthenium-and Rhodium-Chloroquine Complexes. J Med Chem 1996;39:1095-1099.
19. Behrens NB, et al. Metal complexes of the antibiotics nalidixic acid. Inorg Chim Acta. 1986;125:21-264.
20. Thongnopnua P, et al. High-performance liquid chromatographic determination of enalapril in human plasma by enzyme kinetic analytical method. J Pharm Biomed Anal 2005;37:763-769.
21. Tajerzadeh H and Hamidi M. A simple HPLC method for quantitation of Enalaprilat. Pharma Biomed Anal. 2001;24:675-680.

E-ISSN: 2320-1215
P-ISSN: 2322-0112

22. Anzenbacherova E. Determination of enzyme (angiotensin convertase) inhibitors based on enzymatic reaction followed by HPLC. J Pharm Biomed Anal. 2001;24:1151-1156.
23. Qin XZ, et al. Determination and rotamer separation of enalapril maleate by capillary electrophoresis. Chromatogr A 626. 1992;251-258.
24. Gu Q. Simultaneous determination of enalapril and Enalaprilat in human plasma by liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2004;813(1-2):337-342.
25. Razak OA, et al. Spectrophotometric and polarographic determination of enalapril and Lisinopril using 2, 4-dinitrofluorobenzene. J Pharm Biomed Anal. 2003;31:701-711.
26. Ayad MM. Spectrophotometric and AAS determination of Ramipril and enalapril through ternary complex formation. J Pharm Biomed Anal. 2002;28:311-321.
27. Aboul-Enein HY. Electrochemical Sensors in Bioanalysis. Anal Lett. 1997;30:1999-2008.
28. Chandwani OD, et al. Simaltaneous spectrophotometric estimation of enalapril maleate and hydrochlorothiazide. Indian Drugs. 1996;33:401-402.
29. Ayad MM, et al. Spectrophotometric methods for determination of enalapril and timolol in bulk and in drug formulations. Anal Bioanal Chem. 2003;375:556-560.
30. Separation Methods, Budhia raj.
31. Vogel G and G. Svehla. Vogels qualtitative inorganic analysis, (3rdedn). 111-112.
32. Manoranjani M and Kamala Karuna K. UV-visible spectroscopic estimation and validation of enalapril maleate in bulk and pharmaceutical dosage forms. Int J Res Pharm Biomed Sci 2011;2:1651-6.
33. Magda M. Spectrophotometric Estimation of Lisinopril and Cefixime in Bulk and Dosage Forms. J Pharm Biomed Anal. 2002;28 (2)311–321.
34. Keshava Murthy KS, et al. Sodium Enalapril Complex and the use There of To Make Sodium Enalapril. Brantford Chemicals Inc, Patent No: 5637730, 1997.
35. Balman JA, et al. Inorg Chim Acta. 1988;81-90.