Sains Malaysiana 47(4)(2018): 749-754
*http://dx.doi.org/10.17576/jsm-2018-4704-13*

# Synthesis of Cephradine Metal Complexes and its Anti-bacterial Evaluation

(Sintesis Kompleks Logam Sefradin dan Penilaian Anti-bakteria)

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ABSTRACT

*Cephradine belongs to the first generation cephalosporin having a broad range of anti-bacterial activities. In the present work, Cephradine wasreacted with different metal salts. These metal salts were Iron, Copper, Cobalt and Nickel salts. All the complexes of Cephradine metals were synthesized at room temperature using a mechanical vibrator. The reactions yielded the coordinated complexes within 5-10 min with improved product yield. The synthesized complexes were analyzed for their antibacterial power using disc diffused assay. All the Cephradine complexes showed powerful antibacterial activity. The Co, Cu, Ni and Sn complexes showed good antibacterial activities 18.5 mm by Cu complexes against* S. typhi*, 17 mm against* B. subtillus *16.5 mm against* S. aureus*, 16 mm against* S. coccus*. Similarly Sn complexes exhibited 17 mm zone of inhibition against* S. coccus *and 15.5 mm against* B. subtillus*. Cobalt and Ni complexes also shed significant inhibition activities against bacterial pathogenic bacterial strains. The study is of particular importance and new, using mechanical vibrator for the first time. The product yield is also comparatively good with short reaction time.*

*Keywords: Cephalosporin; cephradine; complexation; penicillinase; synthesis*

ABSTRAK

*Sefradin tergolong dalam sefalosporin generasi pertama yang mempunyai pelbagai aktiviti antibakteria. Dalam kertas ini, Sefradin telah menggunakan garam logam yang berlainan. Garam logam ini adalah besi, tembaga, kobalt dan garam nikel. Semua kompleks logam Sefradin telah disintesis pada suhu bilik dengan menggunakan alat vibrator mekanikal. Reaksi menghasilkan kompleks yang diselaraskan dalam masa 5-10 minit dengan hasil produk yang lebih baik. Kompleks yang disintesis telah dianalisis untuk kuasa antibakteria mereka menggunakan ujian disebarkan cakera. Semua kompleks Sefradin menunjukkan aktiviti antibakteria yang kuat. Kompleks Co, Cu, Ni dan Sn menunjukkan aktiviti antibakteria yang baik 18.5 mm oleh kompleks Cu terhadap* S. typhi*, 17 mm terhadap* B. subtilus *16.5 mm terhadap* S. aureus*, 16 mm terhadap* S. coccus*. Begitu juga kompleks Sn mempamerkan zon perencatan 17 mm terhadap* S. coccus *dan 15.5 mm terhadap* B. subtilus*. Kobalt dan Ni kompleks juga menimbulkan aktiviti penghambatan yang ketara terhadap strain bakteria patogenik bakteria. Kajian ini amat penting dan baru serta menggunakan alat vibrator mekanikal untuk kali pertama. Hasil produk juga agak baik dengan masa reaksi yang pendek.*

*Kata kunci: Kompleks; penisilinase; sefalosporin; Sefradin; sintesis*

## Introduction

Many drugs have been used to from complexes with various metals which have shown modified biological properties. In this regard, the most studied metal is copper(II) which has been helpful in diseases like tuberculosis, gastric ulcers, rheumatoid arthritis and cancers (Brown et al. 1980; Sorenson 1976; Williams 1971). Various transition metal ions and their complexes have been found to play active role in many fundamental biological process such as fixing of oxygen and nitrogen and their transformation, transportation in biological systems, catalysis and bio-photo-catalysis and solar energy (Edziri et al. 2012). Keeping in mind the importance of metal complexes, the use of antibiotics as ligands is an attempt to examine the important biological activity of coordinated antibiotics and modes of binding with transition metals in the solid state (Anacona & Rodriguez 2004; Anacona & Serrano 2003; Anacona & Alvarez 2002; Anacona & Toledo 2001). Most of the synthesis of the metal complexes requires harsh reaction conditions with low product yields and recovery of the starting materials; however the present variation is of particular important using mechanical vibrator for the first time with high product yield, easy reaction condition (room temperature) and short reaction time. The synthesized complexes were allowed for the anti-bacterial activities. Most of the complexes were found active against most of the pathogenic bacterial strains with significant zone of inhibition.

## Materials and Methods

### MATERIALS

All the chemicals used in the current study were of analytical grade and of sigma brand. The chemicals were used in its original form. The hydrated salts of the metals were in their chlorideform. The metal salts were$FeCl_3.6H_2O$, $CoCl_2.6H_2O$, $NiCl_2.H_2O$, and $CuCl_2.2H_2O$. During the whole research, distilled water was used.

### SYNTHESIS OF CEPHRADINE METAL COMPLEXES

The formation of complexes of Cephradine with metal salts involved the reaction of the ligand (Cephradine) with the metal halide in 10 mL methanol. The product was obtained by stirring the mixture at room temperature for 5-10 min. The reaction progress was monitored by TLC and color change. After completion of reaction, the reaction mixtures were filtered, washed and desiccated at 37°C and % yield is calculated. The whole procedure is tabulated in Table 1 (Chohan et al. 2004; Nora 2011; Vogel 1975).

### PREPARATION OF CEPHRADINE-IRON COMPLEX

1 millimole of Ferric Chloride was dissolved in suitable volume of methanol. Then 2 mmole of Cephradine was added to the solution. The pH was adjusted to 8.0 using NaOH (0.5M). The product was obtained by stirring the reaction mixture for 5-10 min at room temperature. The precipitate of Iron-Cephradine complex was filtered, washed and desiccated at room temperature (Chohan et al. 2004; Nora 2011; Vogel 1975).

### PREPARATION OF CEPHRADINE-COBALT COMPLEX

Cobalt chloride 1 mmole was dissolved in suitable volume of methanol and was mixed with 2 mmole Cephradine at a pH of 8.0 which was adjusted by dropwise addition of 0.5 M NaOH. The reaction was performed at mild condition on stirring for 5-10 min till the complex is formed. The brown precipitate of Cobalt complex was filtered and washed at room temperature (Chohan et al. 2004; Nora 2011; Vogel 1975).

### PREPARATION OF CEPHRADINE-COPPER COMPLEX

Copper Chloride 1 mmole was dissolved in 40 mL MeOH and was mixed with 2 mmole Cephradine at a pH of 8.0 by dropwise addition of 0.5 M NaOH. The reaction was completed by stirring at room temperature for 5-10 min so that the complex was obtained. The precipitate obtained was washed and desiccated at room temperature (Chohan et al. 2004; Nora 2011; Vogel 1975).

### PREPARATION OF CEPHRADINE-NICKEL COMPLEX

For Cephradine Nickel complex, 40 mL of methanol was used to dissolve Nickel Chloride followed by the addition of with 2 m mole Cephradine at a pH of 8.0. The pH was fixed by dropwise addition of 0.5 M NaOH. The said mixture was then stirred for 5-10 min at room temperature for till the complex is formed. The product was yellow precipitate which was collected, washed and desiccated at room temperature (Chohan et al. 2004; Nora 2011; Vogel 1975).

### PREPARATION OF CEPHRADINE-ANTIMONY COMPLEX

Antimony Chloride 1 mmole was dissolved in 40 mL methanol. The solution was then mixed with 2 mmole of Cephradine and the pH was adjusted to 8.0 by adding NaOH (0.5M) drop wise. The reaction was carried out at mild condition using magnetic stirrer for 5-10 min. Off white precipitate was obtained which was filtered followed by washing with water, MeOH and desiccated at room temperature (Chohan et al. 2004; Nora 2011; Vogel 1975).

### PREPARATION OF CEPHRADINE-TIN COMPLEX

Tin Chloride (1 mmole) and Cephradine (2 mmole) were allowed to react in methanol (40 mL) at a pH of the 8.0 which was adjusted by the dropwise addition of 0.5 M NaOH. The product was obtained by stirring the reaction mixture for 5-10 min at room temperature. The precipitated yellow color complex was filtered off, washed with water, MeOH and desiccated at room temperature (Chohan et al. 2004; Nora 2011; Vogel 1975).

### PREPARATION OF CEPHRADINE-LEAD COMPLEX

Lead Chloride 1 mmole was added in 40 mL methanol was mixed with 2 mmole Cephradine at a pH of 8.0 by the drop wise addition of 0.5 M NaOH. The product was obtained by stirring the reaction mixture for 5-10 min under room temperature. The precipitated light yellow color complex was filtered off, washed with water, MeOH and dehydrated

TABLE 1 Synthesis of Cephradine metal complexes

| S. No. | Ligand | Metal | Ratio | MeOH | Reaction time | Product yield |
|---|---|---|---|---|---|---|
| 1 | Cephradine | ($SnCl_2.2H_2O$) | 2:1 | 40 mL | 5-10 min | 71% |
| 2 | Cephradine | ($NiCl_2.6H_2O$) | 2:1 | 40 mL | 5-10 min | 69.7% |
| 3 | Cephradine | ($PbCl_2.2H_2O$) | 2:1 | 40 mL | 5-10 min | 11% |
| 4 | Cephradine | ($CoCl_2.6H_2O$) | 2:1 | 40 mL | 5-10 min | 11.4% |
| 5 | Cephradine | ($CuCl_2.2H_2O$) | 2:1 | 40 mL | 5-10 min | 35.5% |
| 6 | Cephradine | ($SbCl_3.6H_2O$) | 2:1 | 40 mL | 5-10 min | 68% |
| 7 | Cephradine | ($FeCl_3.6H_2O$) | 2:1 | 40 mL | 5-10 min | 51.6% |

at room temperature using desiccators (Chohan et al. 2004; Nora 2011; Vogel 1975).

### ANTIBACTERIAL ACTIVITY

Antibacterial activity of Cephradine and its metal complexes were assessed through disc diffusion assay against five bacterial strains. All the cultures were freshened in nutrient broth media and standardized with 0.5 McFarland turbidity standards. All species were grown at nutrient agar media prepared in distil water. The standardized microbial strains were streaked on the agar plates. Discs of 6 mm diameter were placed at agar plates and then the samples (0.1, 1 and 2 mg/mL) were applied on sterilized paper discs. For complete diffusion of sample, the dishes were allowed to stand for 20 min. The petri dishes were incubated for 24 h at 37°C and after the completion of incubation period the zone of inhibition were measured in (mm) (Goren et al. 2003; Hayat et al. 2015).

### DETERMINATION OF MELTING POINT

The melting points of the synthesized metal complexes of Cephradine were determined using M.P/SPM10 equipment through open capillary method.

## Results and Discussion

Based on the results, the formula [M (cephra)Cl] has been assigned to the complexes. The results showed the endothermic decompositions in the range of 85-110°C due to the loss of water of hydration. It has been found that the complexes were stable with no coordination water and solvent molecules. The results also showed the complexes of Silver (I), Iron (II), Cadmium (II), Mercury (II) and Chromium (III) were not obtained in good stoichiometric value under the same conditions.

## IR Spectra

The Cephradine metal complexes were analyzed through IR spectroscopy. The important points of IR spectra have been shown in Table 2. The carbonyl of lactam (C=O) was present at 1750 $cm^{-1}$ in Cephradine spectra while it was also found there in Cephradine complexes. The amide C=O band was present at 1650 and 1650-1660 $cm^{-1}$ in spectra of Cephradine and its complexes, respectively. Thus the coordination of ligands occurred via oxygen of lactam carbonyl rather than the carbonyl of amide where the change was not momentous. Though the spectra of copper (II) complexes suggest that the in coordination the amide carbonyl is also involved, the change in absorption frequency from lower to higher band of the carbonyl of amide moiety in the copper(II) complex is due to great inflexibility when the group is coordinated.

The carboxylate asymmetrical stretching frequency i.e. 1600 $cm^{-1}$ was shifted to 1620-1690 $cm^{-1}$ suggesting that the group is involved in complexation with metal (II). The remaining carboxylate bands i.e. υs y m (COO), w(COO) and p(COO), formerly at 1400, 780, 604 and 525 $cm^{-1}$ correspondingly have changed their positions due to participation in complexation. In addition, the change in position of the anti-symmetric and symmetric broadening vibrations depends on the nature of the carboxylate ligand whether it act as monodentate or bidentate ligand. The IR spectra of the complexes give a separation value of >200 $cm^{-1}$ signifying mono dentate bonding of the carboxylate group.

Interestingly the M-N stretching vibrations (from $NH_2$ group) at 530-690 $cm^{-1}$ which was absent in ligand suggests the coordination of the ligand as tridentate monoanionic chelating agent. The–$NH_2$ group coordinated to the metal ion is not the evidence of these bands, but in solid complexes it is possible to coordinate the N atom of the amide group to the metal. Anyhow, due to stearic strain the coordination of N atom is prevented. The new band observed in complexes but not in free ligand in 360-380 $cm^{-1}$ are tentatively assigned to v(M-O) vibrations.

## UV Spectra

Two absorption maxima i.e. 280 and 430 nm were obtained for Cephradine and its metal complexes, respectively. The values have been assigned to ~! ~* and n~* transition within the organic ligand. The two absorption bands i.e. 300 and 366 nm were obtained for Cobalt complex possibly due to spin-orbit forbidden transitions. The two absorption bands (270 and 426 nm) were also obtained

TABLE 2. IR spectral data of Cephradine metal complexes

| Compound | v(CO) Lactum | v(CO) Amide | v(CO) Asymmetric | v(CO) Symmetric | v(M-N) | NH |
|---|---|---|---|---|---|---|
| [(Hcephra)] | 1750 | 1650 | 1600 | 1400 | | |
| [Co(cephra)Cl] ~ $2H_2O$ | | 1622 | 1620 | 1384 | 615 | 3435 |
| [Fe(cephra)$Cl_3$] ~ $6H_2O$ | | 1630 | 1632 | 1383 | 672 | 3415 |
| [Sn(cephra) $Cl_2$] ~ $2H_2O$ | | 1657 | 1657 | 1438 | 576 | 3357 |
| [Ni(cephra)Cl] | 1759 | 1689 | 1595 | 1394 | 659 | 3440 |
| [Cu(cephra)Cl] ~ $2H_2O$ | 1758 | 1626 | 1626 | 1383 | 617 | 3435 |
| [Sb(cephra)$Cl_3$] | | | | 1395 | 660 | 3434 |
| [Pb(cephra)$Cl_2$] | 1766 | | 1651 | 1355 | 657 | 3274 |

for Copper (II) assigning the spin-forbidden and 4 A 2! 4T1 (P) transitions, respectively, in tetrahedral structure. The presence of six-coordinate structure could not be ruled out. One absorptions band at 280 nm was obtained for the nickel (II) complex which is attributable to a d-d electronic transition.

## $^1$HNMR Studies

Three doublets peaks were obtained for CO-CH and N-CH of beta-lactam ring and NH, which appeared at 4.95, 5.48 and 9.03 ppm, respectively. Another peak obtained between 3.18 and 3.45 ppm having a coupling constant 17.2 Hz was corresponds for S-$CH_2$ of dihydrothiazine ring. The coupling of $NH_2$ with adjacent $CH_2$ was not prominent and a single broad peak was obtained at 3.84 ppm due to the presence of protons of $NH_2$.A multiplet peak was observed in the range of 5.60-5.67 ppm due to the presence of 1, 4-dihydrobenzene protons. The down field shifting of frequency of amino protons in spectra of metal complexes suggested that these protons are involved in coordination with metals.

## Antibacterial Activity of Cephradine Metal Complexes

The synthesized metal complexes of Cephradine were assessed *in vitro* for their biocidal power. The results are given in Tables 3 and 4. All synthesized complexes showed good antibacterial power. The Cephradine showed less activity as compare to its metal complexes. The complexes of Pb, Ni and Cu showed higher activity than the other complexes. The enhanced activity shown by Pb and Cu complexes may be due to the high toxic level of both lead and copper (Hayat et al. 2015; Joseyphus & Nair 2008). Other complexes also showed considerable amount of anti-bacterial activities which are of high importance. As literature shows that, the metal bonded to ligands increases its biological activities (Fasina et al 2012). Cephradine is itself an antibiotic agent of first generation of cephalosporin

TABLE 3 Antibacterial activity of Cephradine and its complexes with Co, Cu and Ni

| Samples | Conc. (mg/mL) | Zones of inhibition ( mm) against different bacterial species | | | | |
|---|---|---|---|---|---|---|
| | | *S. typhi* | *E. coli* | *S. aureus* | *B. subtillus* | *S. coccus* |
| Cephradine | 0.5 | 7 | 9 | 7.5 | 8 | 8.5 |
| | 1 | 8 | 9.5 | 10 | 11 | 9 |
| | 2 | 11 | 11 | 11.5 | 13 | 11.5 |
| Co complex | 0.5 | 9 | 11 | 9 | 8 | 10 |
| | 1 | 11.5 | 12.5 | 11.5 | 10 | 12.5 |
| | 2 | 13 | 14 | 13 | 12 | 14.5 |
| Cu complex | 0.5 | 13 | 11 | 12.5 | 11 | 11.5 |
| | 1 | 15 | 13.5 | 14 | 14 | 13.5 |
| | 2 | 18.5 | 15 | 16.5 | 17 | 16 |
| Ni complex | 0.5 | 9 | 9.5 | 9.5 | 10 | 9.5 |
| | 1 | 11.5 | 11 | 11.5 | 13 | 11 |
| | 2 | 14 | 13 | 14.5 | 15 | 14.5 |

TABLE 4. Antibacterial activity of Cephradine metal complexes with Fe, Sn, Sb and Pb

| Samples | Conc. (mg/mL) | Zones of inhibition ( mm) against different bacterial species | | | | |
|---|---|---|---|---|---|---|
| | | *S. typhi* | *E. coli* | *S. aureus* | *B. subtillus* | *S. coccus* |
| Fe complex | 0.5 | 8 | 8.5 | 10 | 8.5 | 11 |
| | 1 | 11 | 10 | 10 | 11 | 12.5 |
| | 2 | 13 | 12.5 | 11 | 14.5 | 14 |
| Sn complex | 0.5 | 10 | 8 | 9.5 | 10.5 | 12 |
| | 1 | 11.5 | 11 | 10 | 13 | 14.5 |
| | 2 | 12 | 13 | 13 | 15.5 | 17 |
| Sb complex | 0.5 | 10 | R | 9 | 11 | 8.5 |
| | 1 | 12.5 | 8 | 10.5 | 13.5 | 10 |
| | 2 | 14 | 10.5 | 12 | 15 | 12.5 |
| Pb complex | 0.5 | 11 | 9 | 8.5 | 10.5 | 11 |
| | 1 | 13 | 11 | 11 | 12 | 13 |
| | 2 | 15.5 | 12.5 | 13 | 13.5 | 15.5 |

TABLE 5 Melting points and colors of the synthesized metal complexes

| S. No | Complexes | Melting points (°C) | Color of complexes |
|---|---|---|---|
| 1 | [Co(cephra)Cl] ~ $2H_2O$ | 258 | brown |
| 2 | [Fe(cephra)$Cl_3$] ~ $6H_2O$ | 196 | Grey |
| 3 | [Sn(cephra) $Cl_2$] ~ $2H_2O$ | 300 | yellow |
| 4 | [Ni(cephra)Cl] | 197 | yellow |
| 5 | [Cu(cephra)Cl] ~ $2H_2O$ | 167 | Reddish brown |
| 6 | [Sb(cephra)$Cl_3$] | 208 | Off white |
| 7 | [Pb(cephra)$Cl_2$] | 220 | Light yellow |

but not so much active against drug resistant bacterial strains. Current effort was made to study the formed complexes for their enhanced anti-bacterial activities. The complexes of Cephradine were found more potent than Cephradine itself. The melting point and color of the synthesized metals are given in Table 5.

## Structure of Complexes

The suggested structure which is based on $^1$HNMR and IR spectra is shown in the Figure 2. In literature, the complexation of several antibiotics of beta-lactam class with transition and $d^{10}$ metals have been reported (Anacona & Serrano 2003; Anacona & Alvarez 2002; Anacona & Toledo 2001). Due to steric hindrance, the Cephradine can donate a maximum of three lone pair to the central metal ion. The suggestions from spectroscopic analysis, that carboxylate, lactam carbonyl and $NH_2$groups of Cephradine are involved in the coordination with metal, seems likely to molecular models.

It is possible that the metal ions in the [M(cephra)Cl] complexes (where M ¼ Mn(II), Co(II), Ni(II) and Zn(II) are tetra coordinate with ligand and the chloride anion at the vertices of a tetrahedron. In case of complexation with copper(II), the carboxylate, amide carbonyl and $NH_2$is suggested for donation of lone pair.

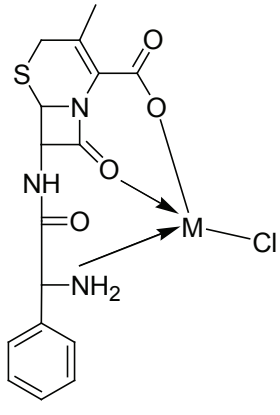


FIGURE 2. Tentative structure of the cephradine metal complexes

While in case of iron (III) complex, where in coordination sphere there are two chlorides ions. The coordination sphere is penta-coodinate with tetragonal or trigonalbipyramidal geometry; the probability of a binuclear structure cannot be ignored.

## Conclusion

Cephradine is an important drug and playing pivotal role in the health maintenance of human for the chest and related infections. Recent efforts were made to synthesize its complexes with different metal salts. The main purpose of the study was to synthesize its metal complex applying a mechanical shaker which resulted into a short reaction time with highly improved product yields. The synthesized metal complexes were then subjected to antibacterial evaluation. To the best of our knowledge it is the first report on the synthesis of the titled ligand.

## ACKNOWLEDGEMENTS

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group no. (RGP-262). No conflict of interest is noted.

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Received: 19 April 2017
Accepted: 11 November 2017