Synthesis and Antibacterial Activity of Cobalt (III) and Nickel (II) Complexes

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Abstract

Due to the ability of metal ion complexes to form octahedral and square planar molecular geometry they have very good potential to achieve unique type of antibacterial compounds. In order to evaluate the role of homoleptic monodentate ligand on its antibacterial properties, novel transition 3d metal complexes of nickel (II), cobalt (III) were synthesized as hexaamminecobalt (III) chloride and hexaamminenickel (II) chloride complexes by using cobalt chloride hexahydrate and nickel chloride hexahydrate for the purpose of investigating its antimicrobial activities. The antimicrobial potentials of these complexes were tested against gram positive human pathogenic bacteria *Staphylococcus aureus* by using paper disc diffusion method on the agar cup method. The biological activity studies indicated that cobalt (III) complex exhibited activity better than the nickel (II) complex. The complexes with high concentration on the nutrient agar showed very good anti-bacterial activity compare to low concertation complexes on the nutrient agar, in spite of, antibacterial activity on nutrient agar showed better result than blood agar.

Keywords: Antimicrobial activity, hexamminecobalt(III)chloride, hexamminenickel(II)chloride, Gram-positive, *Staphylococcus aureus.*

1. Introduction

Nowadays, Anti-bacterial and anti-fungal infections are very frequent around the world (Sanahanbi N, 2013). As the years have passed, antibiotic compounds have been used to designate contagion resulting from various environments(Mellado, 2014). These influential agents inhibit with the maturation and breeding of organisms like bacteria, fungi, parasites, virus, etc.(Sridhar.G, 2017) . Presently used anti-microbial agents are not efficacious by reason of the resistance progressed by the pathogenic bacteria and hence, it is an ongoing endeavor to synthesize new anti-microbial agents (Sanahanbi N, 2013). Likewise suppling resistance toward some fungal and bacterial strains, a many compounds are available for the cure of fungal and bacterial disease(Sridhar.G, 2017).

Although a number of drugs are available in the pharmacies, in spite of craving for attaining new anti-microbial medications with better pharmaceuticals profile and lesser perniciousness have become main targets in the field of medicinal chemistry because of rapid development of microbial resistance towards the existing molecules (Divya K, 2012) (Abhishek T, 2011). Microbial drug resistance is a dangerous issue, particularly as increasing numbers of strains are becoming resistant to multiple antimicrobial agents, with some bacteria now being resistant to all available antibiotics (Fatmah A. S. Alasmary 2015). In developing countries, synthetic drugs are not sufficient nevertheless they could be extravagant for the medication of diseases, for that reason, it is important to seek new infection-fighting strategies to control microbial disease (Ikokoh, 2015)

In view of literature the existence of metal ions substantially heighten the biological activity of organic molecules(S. L. M. a. L. S. V. Sanja O. Podunavac-Kuzmanović, 2004) . Metals have been used in the medication of infections of humans since antiquity (Eddie L. Chang 2010). Because of strong inter-atomic bonding that the transition elements possess, it made them are very important in the industrial and cause to their having high melting points and good mechanical properties (Abbas, 2000)Transition metal complexes have shown a matchless antimicrobial properties. They have been utilized as antimalarial, anti-inflammatory, antitumor, antibiotic, antimicrobial agents etc. (Ikokoh, 2015)

Coordination compounds are of great importance in biological system (Govindaraj V, 2018), and have persevere an important and popular area of research due to their easy synthesis, changeability, and different range of applications (Faridul Islam, 2015) and exhibition varied characteristic properties which depend on the metal ion to which they are bound. On the fundament of nature of the metal in addition to the type of ligand, these metal complexes have wide applications in different fields of human curiosity (R. Johari, 2009), (Uma, 2010). The oxidation state of metals is responsible to the structure–activity relationships of the metal complexes, the type and number of donor atoms, and relative disposition within the ligand (M. Rajasekary, 2010) notwithstanding many different compounds have been synthesized, and few of them are used at the time in clinical practice (Amarjit S. Naura, 2009).

Drugs which manufacture from metals are a research area of becoming larger interest for inorganic, pharmaceutical and medicinal chemistry and have emphasized much attention as a path to new drug development (Walaa H. Mahmoud, 2014). Several studies have revealed that metal complexes possess interesting toxicological and pharmacological properties. However, a substantial problem is that when they exposure to the proteins they lose their activity, which are deactivated once again when embedded in the proteins (Zahid H. Chohan, 2008). Many researchers have studied antimicrobial and toxicological activity of mixed ligand complexes of transition metals (Akalpita S. Bodkhe, 2012). The role of mixed ligand complexes in biological process has been well recognized (Mohan, 1992), (Boddu Veeraswami, 2015).

Many of metal complexes shown good biological activity against pathogenic microorganisms (R.M., 2013). Many more examples of metal-containing drugs have been reported in the literature such as Gold, rhenium, ruthenium, cobalt, platinum and nickel have all been used in medicine (Ivano Bertini, 2007).More recently, cobalt (II) and nickel (II) based polydentate ligand complexes have been found to possess both antiviral and antibacterial activities (Sridhar.G, 2017), (Eddie L. Chang 2010), (Govindaraj V, 2018), (Faridul Islam, 2015), (Walaa H. Mahmoud, 2014), (R.M., 2013), (D. M. C. a. G. S. Ć. Sanja O. Podunavac-Kuzmanović, 2004), (Hicks, 2017) . Continuing our investigations on the interaction of metal ions with antibiotics, we report here the synthesis Co(III) and Ni(II) complexes formed with mono-dentate ligands and their antibacterial properties against pathogenic bacterial strains of gram positive *Staphylococcus aureus* bacteria.

2. Experimental

2.1. Material and Methods

Cobalt Chloride (II) hexahydrate, nickel chloride hexahydrate, ammonium chloride, ammonia (%25), hydrogen peroxide (%50), concentrated hydrochloric acid (%37), ethanol, iodine, safranin, blood agar, nutrient agar and decolorizing charcoal, were purchased from commercial sources and uses as received. The bacterial subcultures were obtained from the Microbiology Laboratory, at Darbandikhan technical institute.

2.2. Synthesis of hexaamminecobalt(III)chloride

5.0g of $CoCl_2 \cdot 6H_2O$ and 3.3g of NH_4Cl to 30 mL of distilled water were added in a 250-mL Erlenmeyer flask. In the hood 1.0 g activated charcoal and 45mL conc. aqueous ammonia were added. Cooled the brown slurry in an ice bath to 0°C, then 4.0 mL (% 50) H_2O_2 was added dropwise. The temperature fixed and must not been rise above 10°C. The red-brown solution formed then is heated to 60°C, and kept this temperature for 30min. the mixture was cooled to 0°C; the product was precipitated from the solution. The product and the charcoal were collected by filtration. To separate the resulting product from activated charcoal recrystallization was necessary. The solid product was placed in a 250-mL Erlenmeyer flask, and added 40 mL hot water and 1.0 mL conc. HCl. The mixture was heated to 70°C, and filtered while still hot. Placed the filtrate in an ice bath, and added 1.0 mL cold conc. HCl. The orange precipitate was collected by filtration, then washed with 25 mL ice-cold ethanol, and allowed to air-dry at room temperature. The bright orange precipitate formed (4.320 g) with % 75.8 yield, and melting point couldn’t determine because it was decomposed.

2.3. Synthesis of hexaamminenickel(II)chloride

6g of nickel chloride hexahydrate to 5 ml of distilled water were added in a 100 ml beaker, added slowly 12 ml of concentrated aqueous ammonia (% 25), to a rapidly stirred solution of nickel chloride. The color of the solution was changed from pale green to intense violet. The solution allowed standing at room temperature for 5 minutes. Cooled in an ice-bath, without disturbance, for about 15 minutes. The violet crystals of $[Ni(NH_3)_6]Cl_2$ have formed. The solution filtrated, and washed the crystals with concentrated aqueous ammonia solution (5 ml). The crystals dried at room temperature. A Blue-violet crystals formed (4.195g) with %70.7 yield, and 182ºC melting point.

2.4. Antimicrobial sensitivity test

The antibacterial activities of synthesized complexes were screened in vitro using the disc diffusion method. The chosen strain, *Staphylococcus* bacteria were obtained from the Microbiology Laboratory, at Darbandikhan technical institute. Test solutions of complexes were prepared in distilled water. The bacteria were cultured for 24 h at 37ºC in an incubator. Blood and nutrient agar was used for preparing basal media for the bioassay of the organisms, nutrient agar and blood agar was poured onto petri-dish and allowed to solidify. 0.1 mL of inoculums of the test organism was spread uniformly on the surface of the agar medium in a petri plate by using a spreader. The sterilized filter paper discs of 5 mm diameter were dipped into the different concentration of solution of the complexes (10mg in 10 mL distilled water, and 20mg in 10 mL distilled water). The discs were left in an incubator for 48 h at 37ºC, and then applied on the bacteria grown agar plates, each agar plates were divided into four parts. The plates were incubated at 37°C for 24 hours. The width of the growth inhibition zone around the disc was measured in millimeters (mm) after 24 h incubation.

3. Result and Discussion:

3.1. Synthesis of hexaamminecobalt(III)chloride.

Hexamminecobalt(III) chloride (Cohex) (Figure 1), a fully coordinated complex of Co(III) ion surrounded by six ammonia ligands, as antibacterial compound have been synthesized. Because of this complex is full octahedral coordination, the Co (III) ion interacts with its environment via outer-sphere coordination and through its triply positive charge. The biological activities of Co(III) complexe formed with ammine ligands has been investigated.

Synthesizing a 6-coordinate cobalt (III) compound from $CoCl_2.6H_2O$ is difficult because of Co^{2+} ion is more stable than Co^{3+} for simple salts. There are only a few salts of cobalt(III), such as CoF_3, that are known. However, cobalt(III) can be stabilized when it form octahedral coordinated compounds**.** The most important thing is the oxidation of cobalt(II) to cobalt(III). Which was carry through the addition of hydrogen peroxide(equation 1).

The simple Co^{3+} ion is unstable in water, but it was stabilized against reduction to Co^{2+} by coordination to monodentate ligands (ammine). Cohex forms a very stable and inert complex with its six ammonia ligands. The purpose of sing the activated charcoal to serve as a catalyst for the reaction that forms the bonds between NH_3 and Co. Conveniently, it also catalyzes the transformation of Co^{2+} into Co^{3+} by the H_2O_2

$$2CoCl_2 + 10NH_3 + 2NH_4Cl + H_2O_2 \rightarrow 2[Co(NH_3)_6]Cl_3 + 2H_2O \quad \text{(Equation 1)}$$

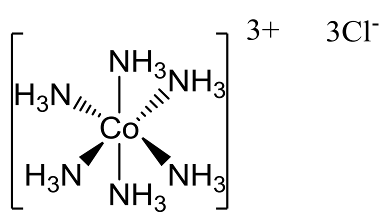

Figure 1: Hexamminecobalt (III) chloride (Cohex)

3.2. Synthesis of hexaamminenickel(II)chloride

Nickel ion Ni^{2+}form octahedral complexes with H_2O in aqueous solution which is surrounded by six water molecules. This complex is called the hexaquonickel(II) ion. By addition of ammonia to this solution substitution reaction was occurred in which the water molecules were replaced by ammonia molecules (equation 2) because the ammonia forms a stronger bond than water and the solution color was changed to blue-violet. The addition of ammonia to nickel chloride hexahydrate solution was dropwise which allow the replacement occur properly, and each replacement is accompanied by the release of heat

$$[Ni(H_2O)_6]Cl_2(aq) + 6\ NH_3(aq) \rightarrow [Ni(NH_3)_6]Cl_2(aq) + 6\ H_2O\ (l)\ \text{(equation 2)}$$

Nevertheless nickel(II) complexes acquire a wide variety of coordination geometries and change from octahedral to square planar, but may also include five-coordinate and tetrahedral coordination modes. The most common geometry is the six-coordinate octahedral orientation. Aqua and ammine nickel(II) complexes always adopt this geometry. So hexaamminenickel(II)chloride possess octahedral orientation (Figure 2).

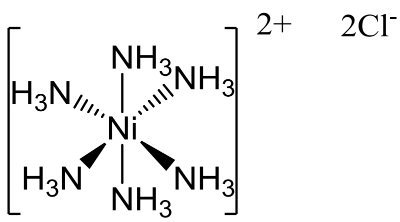

Figure 2: Hexamminenickel (II) chloride

3.3. Biological Activities:

It has been investigated that both of the metal complexes with monodentate ligand showing biological activity. The antibacterial activities of the complexes have been studied against the pathogenic bacteria *(Staphylococcus aureus)*. The bacteria samples were taken from the patients with suspected tonsillitis were swabbed on blood and nutrient agar for beta-hemolytic activity it was showed that all of them were beta hemolytic that shown in (Table 1), because they were appear lightened and transparent. Moreover, all bacteria samples were confirmed as gram positive that shown in (Table 1), because during the gram staining the bacteria with thick peptidoglycan layer retain the primary stain, and crystal violet, during alcohol decolorizing and are referred to gram positive.

Table 1: *In-vitro* screening of anti-bacterial activity of the synthesized compounds by Agar diffusion method.

Sample	concentration	Agar plate	Case suspected	β- hemolytic	Gram strain	Inhibitation zone (mm)

$Co(NH_3)_6^{+3}$	10mg/10mL	Nutrient agar	tonsillitis	+ ve	+ ve	15-17
$Co(NH_3)_6^{+3}$	10mg/10mL	blood agar	tonsillitis	+ ve	+ ve	12-13
$Co(NH_3)_6^{+3}$	20mg/10mL	Nutrient agar	tonsillitis	+ ve	+ ve	15-25
$Co(NH_3)_6^{+3}$	20mg/10mL	blood agar	tonsillitis	+ ve	+ ve	10-20
$Ni(NH_3)_6^{+2}$	10mg/10mL	Nutrient agar	tonsillitis	+ ve	+ ve	7-12
$Ni(NH_3)_6^{+2}$	10mg/10mL	blood agar	tonsillitis	+ ve	+ ve	6-10
$Ni(NH_3)_6^{+2}$	20mg/10mL	Nutrient agar	tonsillitis	+ ve	+ ve	10-18
$Ni(NH_3)_6^{+2}$	20mg/10mL	blood agar	tonsillitis	+ ve	+ ve	7-14

The paper disc diffusion method has been used to study the antibacterial activity against *staphylococcus aureus*. The solution was prepared by dissolving complexes in distilled water because they were dissolved in distilled water properly. The activity was assessed by measuring the diameter of the zone of inhibition in mm after 24 hours incubation. The antibacterial activity data of synthesized complexes is shown in (Table 1). It has been observed that the bacteria colonies were grown, those complexes with different solutions used for current investigation showed that the disc that dipped in cobalt complex solution with concentration (20mg in 10mL distilled water) on the nutrient agar gave the good result against growing bacteria which its inhibitation zone diameter was about (15-25 mm), while the disc which dipped in same solution but on the blood agar showed inhibitation zone diameter about (10-20 mm). While the same complex with different concentration (10mg in 10mL distilled water) on nutrient agar gave the good result against growing bacteria which its inhibitation zone diameter was about (15-17 mm), while the disc which dipped in same solution but on the blood agar showed inhibitation zone diameter about (12-13 mm). That is because of the concentrations, the complex with high concentration gave higher resistance against bacteria.

On the other hand, the disc that dipped in nickel complex solution with concentration (20mg in 10mL distilled water) on the nutrient agar gave resistance against certain bacteria with inhibitation zone diameter which was about (10-18 mm), while the disc which dipped in same solution but on the blood agar showed inhabitation zone diameter about (7- 14 mm). While the same complex with different concentration (10mg in 10mL distilled water) on nutrient agar gave the good result against growing bacteria which its inhibitation zone diameter is about (7-12 mm), while the disc which dipped in same solution but on the blood agar showed inhibitation zone diameter about (6-10 mm). That is because of the concentrations, the complex with high concentration gave higher resistance against bacteria. The data shows that the antibacterial activity of the complex with monodentate ligands is significantly enhanced biological activities as the concentration increase as shown in (Figure 3).

Figure 3. Agar diffusion methods: (A) disk-diffusion method of Cobalt complex in concentration (20mg/10mL distilled water) on nutrient agar (B) disk-diffusion method of Cobalt complex in concentration (20mg/10mL distilled water) on blood agar against *Staphylococcus aureus* bacteria.

4. Conclusion

The octahedral and square planar geometrical structures of the metal ion complexes have a good ability to be a good antibiotic. All the compounds showed good activity against the anti-bacterial organisms tested *Staphylococcus aureus* bacteria. The compounds with high concentration on the nutrient agar showed very good anti-bacterial activity. All the tested compounds exhibited moderate to good anti-bacterial activity against gram positive bacteria.

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A

B

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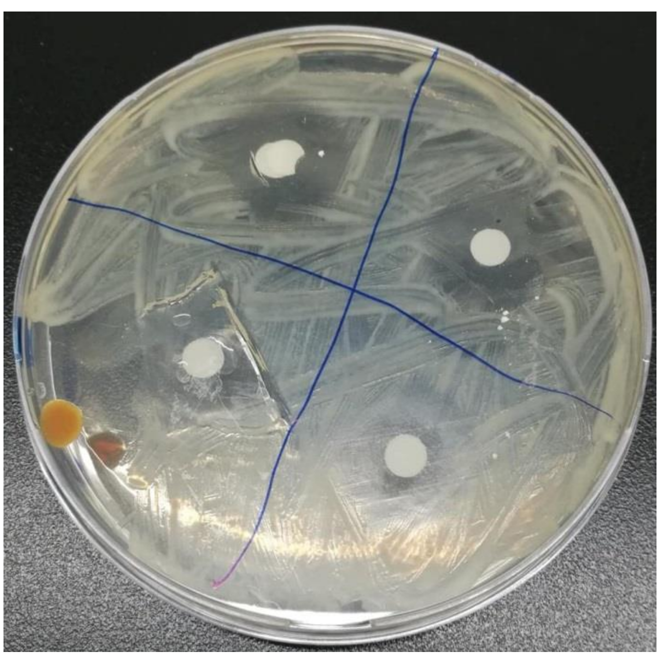

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