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Spectral Characterization and Antibacterial Activities of Benzyloxybenzaldehydethiosemicarbazone, 3,4- Dihydroxybenzaldehydeisonicotinoylhydrazone and their Transitional Metal Complexes

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Abstract: The synthesis and spectral characterization of benzyloxy-benzaldehydethiosemicarbazone (BBTSC) and the study of antibacterial activity of ligands BBTSC, 3,4-dihydroxybenzaldehydeisoni-cotinoylhydrazone (3,4-DHBINH) and their transition metal complexes was studied. The composition of the metal complexes was also evaluated by using Job's method, molar-ratio method and Asmus' method. The antibacterial activities of BBTSC, 3,4-DHBINH and their complexes *i.e.,* Cu(II)-BBTSC, Pd(II)-BBTSC, Cr(VI)- 3,4-DHBINH, Ti(IV)- 3,4-DHBINH and Pd(II)-3,4-DHBINH were studied against two gram positive bacteria and two gram negative bacteria. Antibacterial activities were evaluated by agar cup well method with the help of Luria Bertoni plates.

Keywords: BBTSC, 3,4-DHBINH, Antibacterial activity,Transitional metal complexes

Introduction

Thiosemicarbazones form a class of mixed hard-soft oxygen/nitrogen-sulphur chelating ligands that show a variety of coordination modes in metal complexes. The thiosemicarbazones can act as a monodentate ligand that binds to the metal ion through the sulphur atom[1] or as a bidentate ligand that coordinates to the metal ion through the sulphur atom and one of the nitrogen atoms of the hydrazine moiety to form four or a five membered chelate rings[2,3].

The coordination capacity of thiosemicarbazones can be increased due to the presence of aldehydes or ketones containing additional functional group(s) in position(s) suitable for chelation[4]. Besides their interesting coordination chemistry, thiosemicarbazones have attracted considerable interest because of their potentially beneficial biological activities such as antiviral[5], antitumour[6], antimalarial[7], antifungal[8] and antibacterial[9]. The biological activity of thiosemicarbazones and metal-thiosemicarbazones has been receiving considerable attention recently[10-12].

A good number of reports are available on the biological activity of transition metal complexes of thiosemicarbazones[13-15]. The mechanism of biological activity of thiosemicarbazones is due to their ability to inhibit the biosynthesis of DNA, possibly by blocking the enzyme ribonucleotide diphosphate reductase; binding to the nitrogen bases of DNA, hindering or blocking base replication; creation of lesions in DNA stands by oxidative rupture[16]. In some cases the activity is associated with a metal atom. Several palladium(II) and platinum(II) thiosemicarbazone complexes having potential antitumor activity have been recently reported[17-20].

Collins *et al*[21] have reported the correlation between structure and antibacterial activity in a series of 2-acetylpyridinethiosemicarbazone. In many cases, by coordination to different transition metal ions that can be found in biological systems, it is possible to obtain complexes that are more efficient drugs than the corresponding free ligands[22-24]. Some important thiosemicarbazones with antibacterial activity reported earlier are discussed here under.

Kovala-Demertzi *et al*[25] have reported the anti-tumor activity of 2-acetylpyridine-4*N*-ethylthiosemicarbazone and its platinum(II) and palladium(II) complexes. Elena Bermejo *et al*[26] have reported the biological activities of complexes of zinc(II), cadmium(II), mercury(II), palladium(II) and platinum(II) with 2-acetylpyridine- 4-methylthiosemi-carbazone and its metal complexes assayed *in vitro* for antifungal activity against the pathogens *Aspergillus niger* and *Paecilomyces variotii*. Among the above mentioned transition metal complexes only zinc(II) complex shows some activity at a concentration of 600 μg mL^{-1}. The minimum inhibition zone for zinc(II) complex is found as 9.5 mm.

Dimitra Kovala-Demertzi *et al*[27] have reported the antibacterial and antifungal activities of nickel(II) and palladium(II) complexes of 2-acetyl-pyridinethiosemi-carbazone. Antibacterial activities are evaluated against gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Escherichia coli.* Antifungal activity is evaluated against *candida albicans.*

Sulekh Chandre *et al*[28] have reported the antimicrobial activities of copper(II) and nickel(II) complexes with benzil bisthiosemicarbazone against the gram positive bacteria (*Bacillus macerans)* and gram negative bacteria (*Pseudomonas striata).* The authors reported that the metal chelates exhibit more inhibitory effects than the parent ligand.

Antibactrial activity of hydrazones and their complexes

Interest in the study of hydrazones has been growing because of their antimicrobial, antituberculosis and antitumor activities[29-30]. Hydrazones play an important role in inorganic chemistry, as they easily form stable complexes with most of the transition metal ions. The development of the field of bioinorganic chemistry has increased the interest in hydrazone complexes, since it has been recognized that many of these complexes may serve as models for biological important species[31]. Coordination compounds derived from aroylhydrazones have been reported to act as enzyme inhibitors and are useful due to their pharmacological applications[32-35].

Hydrazones possessing an azometine –NHN=CH-proton constitute an important class of compounds for new drug development. Therefore, many researchers have synthesized these compounds as target structures and evaluated their biological activities.

Hydrazones and their metal complexes are biologically very active compounds. For example Ragavendran *et al*[36] have reported anticonvulsant activity of 4-aminobutyri-cacidhydrazone, Abdel-Aal *et al*[37] have reported antiviral activity of *N*-arylaminoacetyl-hydrazones against *Herper simplex virus-1* and *Hepatitis-A virus(HAV),* Walcourt *et al*[38] have reported antimalarial activity of 2-hydroxy-1-naphthaldehyde -isonicotinoyl-hydrazone and Savini *et al*[39] have reported antitumor activity of 3- and 5-methylthio-phene-2-carboxaldehyde α-(*N*)-heterocyclichydrazone derivatives. Recently critical reviews have been published by various authors on antibacterial activity of hydrazones[40-42].

A thorough literary survey has revealed that a good number of thiosemicarbazone and hydrazone complexes are evaluated for antibacterial activity against various bacteria. But potentiality of these complexes is not fully utilized. Hence, the newly synthesized copper(II) and palladium(II) complexes of benzyloxybenzaldehydethiosemicarbazone (BBTSC) and vanadium(V), chromium(VI), titanium(IV) and palladium(II) complexes of 3,4-dihydroxy-benzaldehydeisoni-cotinoylhydrazone(3,4-DHBINH) are tried to evaluate antibacterial activity.

Experimental

Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli and *Pseudomonas aeruginosa* were grown at 37 ^{o}C and maintained on LB plates (Luria-Bertani broth, with 2.0% agar) for 18-20 h.

Assay of antibacterial activity by Agar cup well method

Preparation of overnight bacterial culture

The LB broth (Hi media) medium was inoculated with selected bacterial culture under sterile conditions and incubated at 37 ^{o}C for 18-20 h. This developed inoculum was used for further assay.

Preparation of LB plates

The LB agar medium was prepared and poured in petri plates and allowed to solidification.

Inoculation of bacterial culture on LB medium by spread plate method

The overnight bacterial culture having 0.1; O.D (spectrophotometric value at 600 nm) of 0.1 mL was spread inoculated on LB agar plate and left undisturbed for 3 min.

Preparation of agar cup wells and addition of metal solution

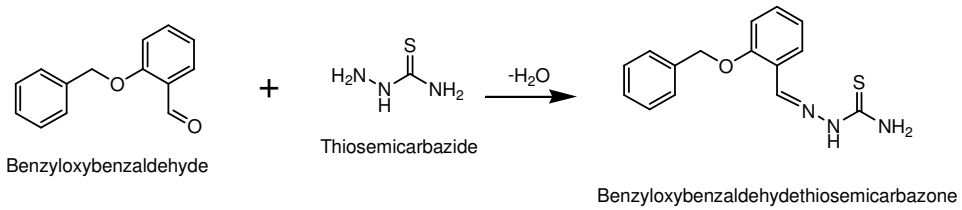
Using a sterile cork borer, wells were created on LB agar plate and the chelated metal stock solution (Conc 2.0 mg mL^{-1}) 30 micro L was added and kept for incubation at 37 ^{o}C for 20-24 h.

Synthesis of benzyloxybenzaldehydethiosemicarbazone (BBTSC)

Benzyloxybenzaldehydethiosemicarbazone was synthesized by refluxing a methanolic solution containing 2.0 g of benzyloxybenzaldehyde (M.Wt. 212 g) and 1.0 g of thiosemicarbazide (M.Wt. 91.13 g) for about 1 h 30 min. in a round bottom flask. The light yellow colored product obtained was separated by filtration and dried. The product was recrystalized from methanol. The purity of the reagent was checked by ascertaining the melting point and elemental analysis.

Elemental analysis

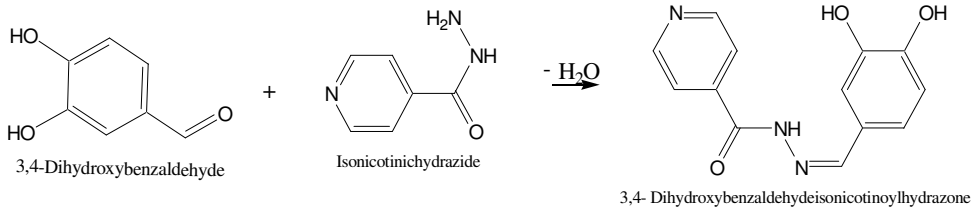
The calculated data for $C_{15}H_{15}N_3SO$, (C, 63.15; H, 5.26; N, 14.73; S, 11.22%) is in good agreement with the experimentally found data (C, 61.27; H, 5.37; N, 14.05; S, 9.90%) to the above proposed molecular formula for BBTSC. The synthesis of BBTSC is presented in Scheme 1.



Scheme 1. Synthesis of BBTSC

Preparation of 3,4-dihydroxybenzaldehydeisonicotinoylhydrazone[43]

Equimolar quantities of 3,4-dihydroxybenzaldehyde (dissolved in alcohol) and isonicotinichydrazide (dissolved in water) were taken in a 500 mL round bottom flask and refluxed for 2-3 h. The contents were cooled and then filtered. The product (Scheme 2) obtained was washed with aqueous methanol and finally recrystallized from rectified spirit. The purity of the reagent was checked by TLC and melting point analysis (m.p. 491-492 K).



Scheme 2. Formation of 3, 4-DHBINH

Results and Discussion

Characterization of BBTSC

The synthesized reagent BBTSC was characterized by melting point determination, elemental analysis, IR (Figure 1), ^{1}H NMR (Figure 2), ^{13}C NMR (Figure 3) and Mass spectra (Figure 4).

Characterization of 3,4-DHBINH

It is evident from I.R. spectrum (Figure 5) that, C=N group is present in the compound because the peak appears at 1600 cm^{-1}. C=O(Stretching) amide peak appears at 1656.9 cm^{-1}. -OH group peak appears at 3484.6 cm^{-1}, -NH peak appears at 3245 cm^{-1}. From the above data it is confirmed the formation of 3,4-dihydroxybenzaldehydeisonicotinoylhydrazone. A 0.1 mol L^{-1} stock solution was prepared by dissolving 2.57 g of 3,4–DHBINH in 40% aqueous dimethyl formamide(DMF).

Antibacterial activity

In the present investigation, we studied the antibacterial activity of ligands BBTSC and 3,4-DHBINH and their complexes *i.e.,* Cu(II)-BBTSC, Pd(II)-BBTSC, V(V)-3,4-DHBINH,

Cr(VI)-3,4-DHBINH, Ti(IV)-3,4-DHBINH and Pd(II)-3,4-DHBINH against two gram positive bacteria (*Klebsiella pneumoniae* and *Staphylococcus aureus)*and two gram negative bacteria *(Escherichia coli* and *Pseudomonas aeruginosa).* The results are reported Table 1 and Table 2.

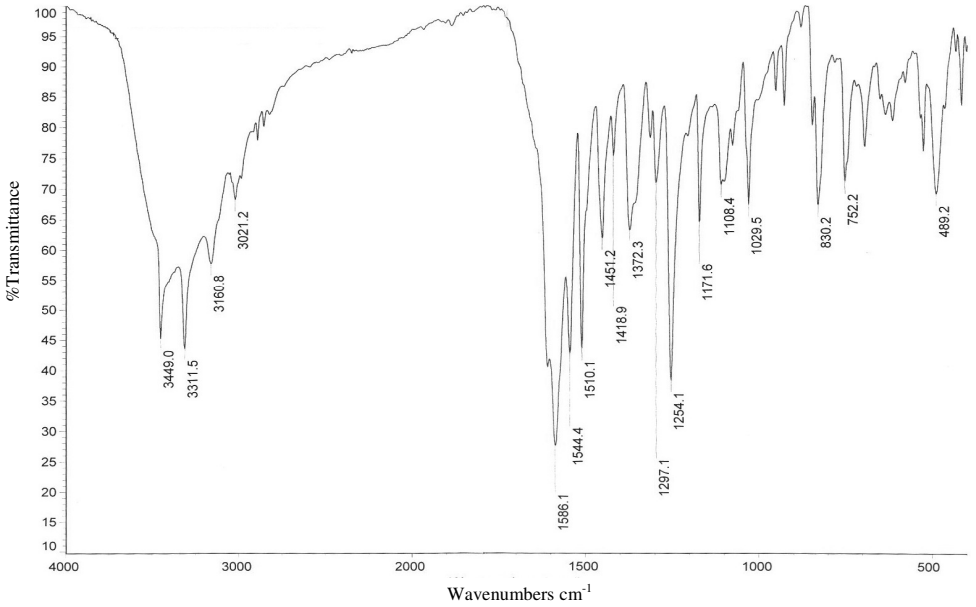


Figure 1. Infrared spectrum of BBTSC

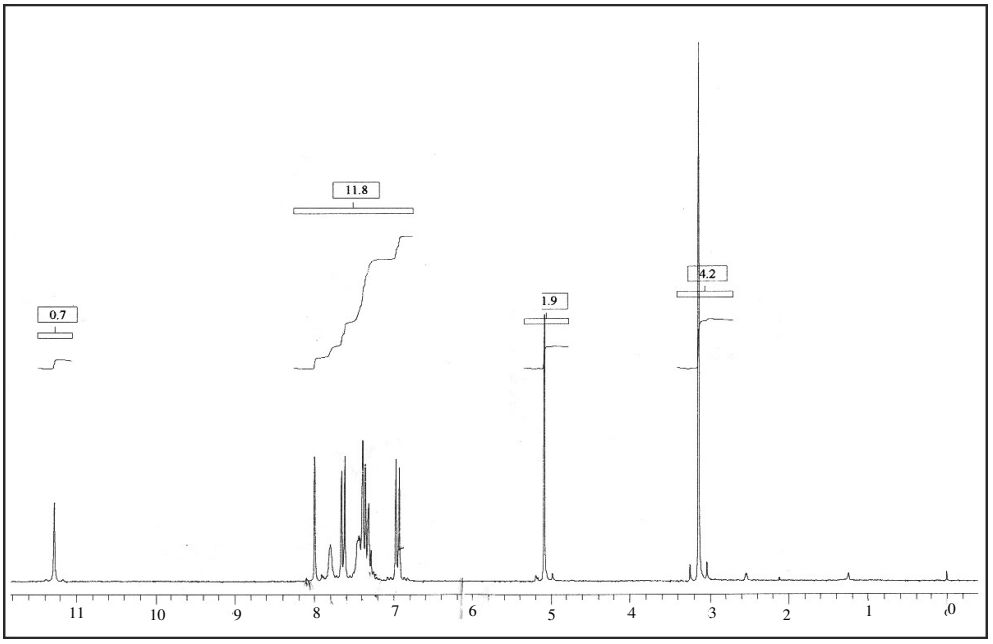


Figure 2. ^{1}H NMR spectrum of BBTSC

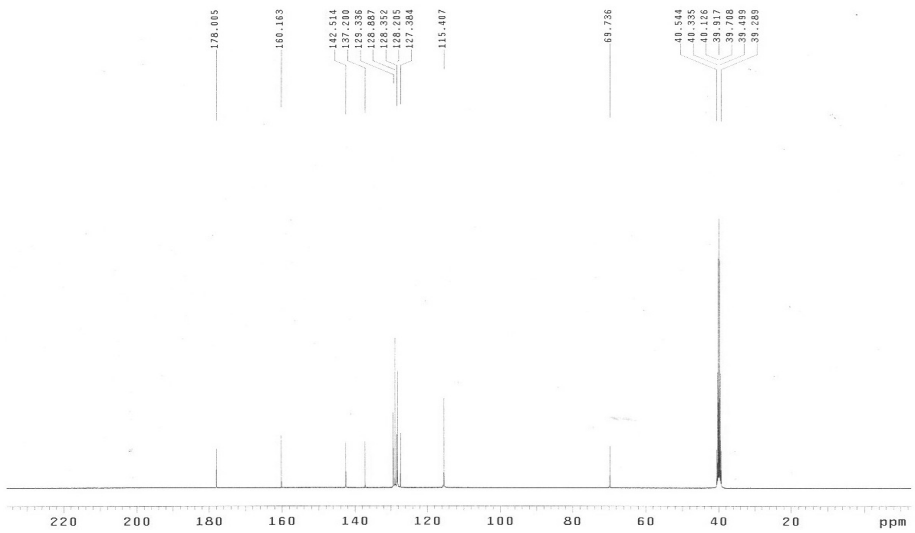


Figure 3. ^{13}C NMR spectrum of BBTSC

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================================================================================
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Injection Date  :  3/26/2007                         Injection vol    :   3  uL
SAMPLE NAME        JBL004-EJ0-A11045-SM              Acq Method       :   D:\MSDATA\260307MS1->
================================================================================
    Method info  :   Analytical Conditions:
                     Mobile Phase(A) :  90%H2O+10%ACN+0.02%FA
                     Mobile Phase(B) :  10%H2O+90%ACN+0.02%FA
                     Flow rate : 0.2 mL/ min
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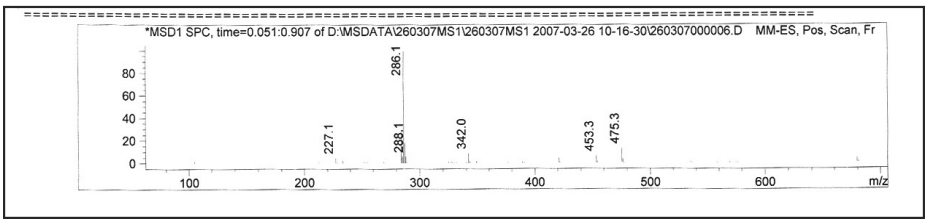

Figure 4. Mass spectrum of BBTSC

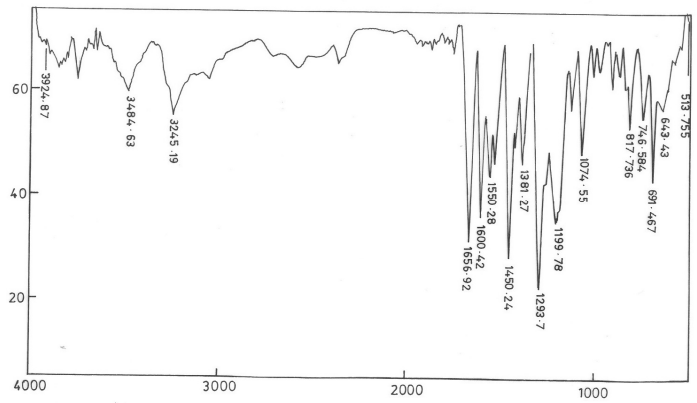

Figure 5. IR SPECTRUM of 3, 4-dihydroxybenzaldehydeisonicotinoylhydrazone

From the data in Tables 1 and 2, it is clear that the free ligands BBTSC and 3,4-DHBINH is inactive against the tested bacteria. BBTSC, on complexation with Pd(II) and 3,4-DHBINH with Ti(IV) and Pd(II) there is a notable antibacterial activity. From the results, it is conclusive that the Pd(II) complex with 3,4-DHBINH is effective against *S. aureus* and *E.coli* than the Ti(IV) complex of 3,4-DHBINH. At the same time the Ti(IV) complex of 3,4-DHBINH is effective against *K. pneumoniae* and *P. aeruginosa* than the Pd(II) complex of 3,4-DHBINH. In case of Pd(II)-BBTSC complex it is effective about *S. aureus* than the remaining tested bacterial stains. The minimum inhibition concentration (MIC) of these complexes found to be 1000 µg mL^{-1}. From our observations, we conclude that the antibacterial activities are strongly dependant on the central metal ions.

Table 1. Antibacterial activity of BBTSC and its complexes

S. No.	Compound	Concentration, µg mL^{-1}	Minimum inhibition zone, mm[a]			
			K.pneumoniae	*S.aureus*	*E.coli*	*P.aeruginosa*
1	BBTSC	1000	-	-	-	-
		2000	-	-	-	-
2	Cu(II)-BBTSC	1000	-	-	-	-
		2000	-	-	-	-
3	Pd(II)-BBTSC	1000	7.5	10.0	5.0	8.0
		2000	10.0	12.5	7.5	10.0

[a]*The results are average of two replicates*

Table 2. Antibacterial activity of 3,4-DHBINH and its complexes

S. No.	Compound	Concentration, µg mL^{-1}	Minimum inhibition zone, mm[a]			
			K.pneumoniae	*S.aureus*	*E.coli*	*P.aeruginosa*
1	3,4-DHBINH	1000	-	-	-	-
		2000	-	-	-	-
2	V(V)-3,4-DHBINH	1000	-	-	-	-
		2000	-	-	-	-
3	Cr(VI)-3,4-DHBINH	1000	-	-	-	-
		2000	-	-	-	-
4	Ti(IV)-3,4-DHBINH	1000	10.0	8.0	7.0	10.5
		2000	15.0	9.5	8.5	13.5
5	Pd(II)-3,4-DHBINH	1000	7.5	9.0	10.0	7.5
		2000	10.5	11.5	12.0	9.5

[a]*The results are average of two replicates*

Absorption spectra of ligands and their metal complexes

Formation of complexes by ligands with the transition metal ions was also proved by the absorption spectra of the complexes and ligands. It is further confirmed by composition studies.

Absorption spectra of BBTSC and Pd(II)-BBTSC

The absorption spectrum of the Pd(II)-BBTSC complex was recorded against reagent as blank and the absorption spectrum of BBTSC was recorded against solvent blank. The obtained

spectra have revealed that the Pd(II)-BBTSC complex and the reagent (BBTSC) have maximum absorbances at 365 nm and 340 nm, respectively. The reagent has minimum absorbance at the maximum absorbance of the complex and the reagent does not interfere with the determination of palladium(II)[44].

Absorption spectra of 3,4-DHBINH, Ti(IV)- 3,4-DHBINH and Pd(II)- 3,4-DHBINH

The absorption spectrum of Ti(IV)-3, 4-DHBINH complex was recorded against the reagent blank. Similarly the absorption spectrum of the reagent (3,4-DHBINH) was recorded against the solvent blank. From the absorption spectra it is cleared that the complex and reagent have shown maximum absorptions at 370 nm and 340 nm, respectively. The reagent has minimum absorbance at the maximum absorbance of the complex. The reagent absorbance at the maximum absorbance of metal complex was further suppressed using suitable concentration of reagent as blank and further absorbance measurements were made at 370 nm. The absorption spectra in the 300-600 nm range for 3,4-DHBINH and Pd(II)-3,4-DHBINH complex[45] were recorded. The formation of the 380 nm band in the spectra must be connected with the chelate formation between Pd(II)- 3,4-DHBINH.

Composition of the metal complexes

Composition of Pd(II)-BBTSC complex

Job's method of continuous variation, molar-ratio method and Asmus' methods were employed to elucidate the composition of the complexes.

Job's method of continuous variation

Equimolar solutions (0.5×10^{-4} M) of palladium(II) and BBTSC were prepared. The metal and reagent solutions were mixed in different proportions, keeping the total volume of the mixture constant at 2.0 mL. In each case, 3.0 mL of sodium acetate-acetic acid buffer (pH 5.0) was added to the mixture and the aqueous phase was shaken thoroughly with 10.0 mL of cyclohexanol. The organic phase was collected into a 25.0 mL standard flask and made up to the mark with cyclohexanol. The absorbances of all the organic phases were recorded at 365 nm against their reagent blanks. A plot was drawn between absorbance and V_M / V_L+V_M (where V_L and V_M are the volumes of the reagent and the metal, respectively). From the graph, it was observed that one mole of palladium(II) reacts with one mole of ligand and the composition of metal to ligand complex as 1:1.

Molar ratio method

In molar ratio method, different aliquots containing 1.0 mL of 0.5×10^{-4} M palladium(II), 3.0 mL of sodium acetate-acetic acid buffer (pH 5.0) and varying concentrations (0.125×10^{-4}-2.5×10^{-4} M) of BBTSC were used to determine the metal to ligand ratio. The absorbances of the extracted organic phases were recorded at 365 nm against their respective reagent blanks. A plot was drawn between the absorbance and the concentration of the reagent. From the obtained curve it was confirmed that one mole of palladium(II) complexes with one mole of BBTSC. The composition of the metal to ligand was further confirmed by Asmus' method also.

Composition of the Ti(IV)-3,4-DHBINH complex

Job's method of continuous variation

Equimolar solutions (1.0×10^{-4} M) of titanium(IV) and 3,4-DHBINH were prepared. The metal and reagent solutions were mixed in different proportions, keeping the total volume of metal and ligand constant at 1.2 mL. In each case, 4.0 mL of sodium acetate-acetic acid buffer (pH 3.5) was

added to the mixture and the total volume of the solution was made up to 10.0 mL with double distilled water. The absorbances of all the solutions were recorded at 370 nm against their reagent blanks. A plot was drawn between absorbance and V_M / V_L+V_M (where V_L and V_M are the volumes of the reagent and the metal, respectively). From the graph, it was observed that one mole of titanaium(IV) reacts with two moles of ligand shows the composition of metal to ligand complex as 1:2.

Molar ratio method

In molar ratio method, different aliquots containing 1.0 mL of 1.0×10^{-4}M titanium(IV), 4.0 mL of sodium acetate-acetic acid buffer (pH 3.5) and varying concentrations (0.25×10^{-4}-3.0×10^{-4} M) of 3,4-DHBINH were used to determine the metal to ligand ratio. The absorbances of the solutions were recorded at 370 nm against their respective reagent blanks. A plot was drawn between the absorbance and the concentration of the reagent. From the obtained curve it was confirmed that one mole of titanium(IV) complexes with two moles of 3,4-DHBINH.

Composition of the Pd(II)-3,4-DHBINH complex

Job's method of continuous variation

Equimolar solutions (1.0×10^{-4} M) of palladium(II) and 3,4–DHBINH was prepared. The metal and reagent solutions were mixed in different proportions, keeping the total volume of metal and ligand constant at 1.0 mL. In each case, 4.0 mL of sodium acetate-acetic acid buffer (pH 3.0) was added to the mixture and the total volume of the solution was made up to 10.0 mL with double distilled water. The absorbance of all the solutions was recorded at 380 nm against their reagent blanks. A plot was drawn between absorbance and V_M / V_L+V_M (where V_L and V_M are the volumes of the reagent and the metal, respectively). From the graph, it was observed that one mole of palladium(II) reacts with one mole of ligand shows the composition of metal to ligand complex as 1:1.

Molar ratio method

In molar ratio method, different aliquots containing 1.0 mL of 1.0×10^{-4} M palladium(II), 4.0 mL of sodium acetate-acetic acid buffer (pH 3.0) and varying concentrations (0.25×10^{-4}-2.75×10^{-4} M) of 3,4-DHBINH were used to determine the metal to ligand ratio. The absorbances of the solutions were recorded at 380 nm against their respective reagent blanks. A plot was drawn between the absorbance and the concentration of the reagent. From the obtained curve it was confirmed that one mole of palladium(II) complexes with one mole of 3,4-DHBINH.

Conclusion

We confirmed the formation of BBTSC and 3,4-DHBINH with the help of various spectral techniques. The composition of the metal complexes was also evaluated by various methods. From the anti bacterial activity results, we concluded that the ligands BBTSC and 3,4-DHBINH were inactive against the tested bacteria strains. Out of six complexes the three complexes *i.e.,* Pd(II)-BBTSC, Ti(IV)-3,4-DHBINH and Pd(II)-3,4-DHBINH are effective against the two gram positive bacteria (*Klebsiella pneumoniae* and *Staphylococcus aureus*) and two gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The minimum inhibition zone of the complexes is increased with the increase in concentration. From our results we conclude that the antibacterial activities of the transitional metal complexes are strongly dependant of the central metal ions.

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