



## Studies on Isomeric Complexes of Nickel with Biguanide molecule

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**Abstract:** The antibacterial activities<sup>1-3</sup> of biguanide and substituted biguanide complexes have been studied for a long time but no work is done regarding the antifungal activities<sup>4-9</sup> of biguanides and substituted biguanide complexes. In this work, isomeric  $\alpha$ ,  $\beta$ ,  $\gamma$  nickelphenylbiguanide salts have been isolated and characterized from the studies of their colour, magnetic susceptibilities, infrared vibrations and electronic absorption spectra. The metal complexes of phenylbiguanide ligands were dissolved in DMSO and was tested with dilution test method. The inoculation of fungus was done in PDA & SDA medium at 25°C and checked daily for a week. The fungus used is *Aspergillus Niger*. The MIC was then calculated. It was found that the complexes were effective in controlling 100% of the fungal growth if the concentration was raised to 800  $\mu\text{g/ml}$  and 1000  $\mu\text{g/ml}$ . The solutions was used in the ratio 1:10 and the concentration of the solution was 400 $\mu\text{g/mg}$ , 200  $\mu\text{g/ml}$  and 100  $\mu\text{g/ml}$ .

**Keywords:** Phenylbiguanide, Magnetic susceptibilities, Absorption spectra and *Aspergillus Niger*

### I. Introduction

In this research we have tried to drag the attention of people towards the antifungal properties and spectroscopical aspects of metallic complexes of phenyl biguanide. Biguanide can refer to a molecule or to a class of drugs based upon this molecule. Biguanide can function as oral anti-hyperglycemic drugs used for diabetes mellitus or prediabetes treatment. They are also used as antimalarial drugs<sup>10-14</sup>. Biguanides have a replaceable hydrogen atom in a potentially imino group and also has a donor nitrogen (amino) atom for chelation, thus behaving as bidentate ligand. In formula V suggested by Ray<sup>15</sup>, it has been shown that in the inner metallic complexes formed by biguanide, it is the hydrogen atom of one of the imino groups (=NH) of biguanide, which is replaced by the metal atom to form a primary valence bond.

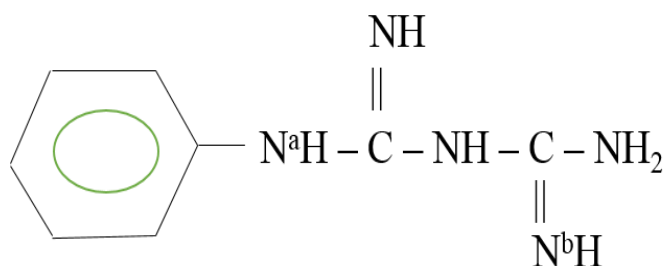
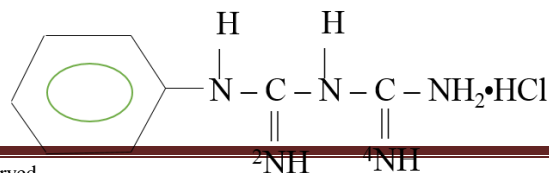


Fig 1. For Phenylbiguanide we can represent the bonding N by labelling it as a and b.

### II. Experimental

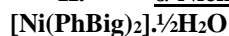
#### I. Phenybiguanide hydrochloride (Melting Point 244 – 247°C) [C<sub>8</sub>H<sub>11</sub>N<sub>5</sub>.HCl]

Phenylbiguanide hydrochloride was prepared by refluxing alcoholic solution of both aniline hydrochloride C<sub>6</sub>H<sub>5</sub>NH<sub>2</sub>.HCl and dicyandiamide C<sub>2</sub>H<sub>4</sub>N<sub>4</sub> in round bottom flask on water bath. This process continues for about 2 hours<sup>16</sup>. The crystals get deposited on cooling the mixture obtained. The crystals were filtered and recrystallized with hot water.



## Fig 2. Phenylbiguanide hydrochloride

### II. $\alpha$ -NickelPhenylbiguanide and its hydrate



To cold ( $8^{\circ}$ - $10^{\circ}$ ) concentrated solution of nickelphenylbiguanidium chloride  $[\text{Ni}(\text{PhBigH}^+)_2]\text{Cl}_2 \cdot 2\frac{1}{2}\text{H}_2\text{O}$ , a cold concentrated solution of caustic soda was rapidly added in excess. The red ppt. of  $\alpha$ -Nickel phenylbiguanide  $[\text{Ni}(\text{PhBigH}^+)_2] \cdot \frac{1}{2}\text{H}_2\text{O}$  was immediately obtained which was washed with ice cold water, dried first on a porous plate and then over caustic potash (carbon dioxide free atmosphere).

Found: N=33.09%, Ni=13.88%  
 $[\text{Ni}(\text{PhBigH})_2] \cdot \frac{1}{2}\text{H}_2\text{O}$  Requires: N=33.09%, Ni=13.99%

When heated to  $110^{\circ}\text{C}$  for 2 hours it loses 2% of its weight without any change in colour.

Calc.  $\text{H}_2\text{O}=2.14\%$

Found for anhydrous product: Ni=14.11%, Calc. Ni =14.22%

The substance is soluble in  $\text{CH}_3\text{OH}$  (methanol),  $\text{C}_2\text{H}_5\text{OH}$  (ethanol) and also in acetone-  $\text{CH}_3\text{COCH}_3$  forming an orange red solution, but rapidly separates out in form of a yellow powder ( $\beta$  variety). The same change is also observed to occur in moist state. It decomposed at  $255^{\circ}\text{C}$  without melting.

### III. $\beta$ -NickelPhenylbiguanide

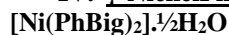


This can be prepared by adding a concentrated solution of caustic soda to a warm solution of the nickel phenylbiguanidium chloride. The precipitate so obtained was washed with water and dried over caustic soda.

Found: N=33.73%, Ni=14.67%  
 $[\text{Ni}(\text{PhBig})_2]$  requires: N=34.09%, Ni=14.22%

The substance is insoluble in alcohol, acetone or water and decomposes, without melting at about  $265^{\circ}\text{C}$ . The compound has also been described by Smolka and Friedrich (loc.cit).

### IV. $\gamma$ -NickelPhenylbiguanide and its hydrate



This was prepared by keeping the moist  $\alpha$  variety  $[\text{Ni}(\text{PhBigH}^+)_2] \cdot \frac{1}{2}\text{H}_2\text{O}$  at room temperature when after 2 to 3 days the mass changed completely into a deep yellow product. This was dried over caustic soda.

Found: N=33.10%, Ni=13.93%,  $\text{H}_2\text{O}$  (by loss at  $110^{\circ}\text{C}$ ) – 2.04%  
 $[\text{Ni}(\text{PhBigH})_2] \cdot \frac{1}{2}\text{H}_2\text{O}$  requires: N=33.36%, Ni=13.99%,  $\text{H}_2\text{O}=2.14\%$   
The anhydrous product gave: Ni=14.17% calc. 14.22%

There is no change of colour on dehydration.

The substance is insoluble in alcohol, acetone or water and decompose without melting at  $263^{\circ}\text{C}$ . This is likely to be a molecular compound or a solid solution of  $\alpha$  and  $\beta$  variety.

### V. Preparation of PDA(Potato Dextrose Agar)

Potato tubers were taken peeled off and weighed 200g. It was chopped into small pieces and transferred to a beaker containing about 100ml of distilled water and boiled for 20minutes and filtered with muslin cloth. 20g Dextrose, 15g agar and 2g peptone were added into the extract and gently heated. The filtrate so obtained was made to 1 litre. The pH of the solution was maintained at 5.6 by using 1N HCl or NaOH and kept in Erlenmeyer flask. This solution so obtained was PDA medium and autoclaved at  $121^{\circ}\text{C}$  for 20minutes before using.

### VI. Preparation of SDA (Sabourand and Dextrose Agar Medium)

It was prepared by combining the ingredients water, dextrose, agar, peptone & antibiotics separately, in many different variations. In the case of using premix, the proper amount (around 70gms) was mixed with one litre of water and heated to dissolve the agar. pH of the medium was adjusted with one molar solution of hydrochloric acid to lower pH. The pH was maintained at 5.5. The medium was then autoclaved and stored at room temperature. The medium can be used to inoculate with fungal spores and mycelium inhibition growth was counted by usual method. The observation of the study is represented in the Table A.

### III. Results and Discussion

Nickel phenylbiguanide hydrates and its salts – the nickelphenylbiguanidium chloride or Bis(phenylbiguanide) nickel(II) chloride was prepared which was then used to prepare further other complexes. The anhydrous nickel phenylbiguanidium have proved to be monomeric by ebullioscopic measurements. The  $\alpha$  – Nickel Phenylbiguanide has been converted to  $\gamma$  Nickel phenylbiguanide by keeping  $\alpha$  variety moist for 2 or 3 days when the mass changed completely into a deep yellow product and dried over caustic soda. The hydrates are of three types:  $\alpha$ ,  $\beta$  and  $\gamma$  differing in colour, melting point and solubility.

The planar configuration of the 4 coordinated inner metallic complexes of bivalent Ni is now been well established. Evidences in support of this are, derived not only from the classical methods of stereochemistry but also from a substantial mass of physical data, specially relating to X-ray measurement of crystalline salts.

The occurrence of cis-trans isomerites of the nickel derivatives of benzylmethyleglyoxime<sup>9</sup> is a definite illustration of the point. From the magnetic criterion of the bond type deduce from Pauling theory<sup>17</sup> planar configuration with dsp<sup>2</sup> hybridization a generally established for red and yellow Ni complexes like Nickeldimethylglyoxime, Nickeldicyndiamide, Potassiumnickelcyanide, Nickelbiguanide etc. which are all diamagnetic<sup>18</sup>.

#### Electronic Spectra

##### A. UV spectra

For Bis(phenyldibiguanidium)nickel(II)chloride

250nm  $\text{CT} \rightarrow {}^1\text{A}_1\text{g}^1\text{E}_\text{g}$

340nm CT

For  $\alpha$ -Nickel Phenylbiguanidine (II)

340 nm CT

230 & 240nm  $\longrightarrow$  CT +  ${}^1\text{A}_1\text{g}^1\text{E}_\text{g}$

For  $\beta$ -Nickel Phenylbiguanidine (II)

460nm  ${}^1\text{A}_1\text{g}^1\text{E}_\text{g}$

340nm CT

240nm  $\text{CT} \rightarrow {}^1\text{A}_1\text{g}^1\text{E}_\text{g}$

##### B. IR spectra

K<sub>1</sub> (Phenyldibiguanidine hydrochloride)

Band Position (in cm <sup>-1</sup> )	Assignment
3550.8	N-H (w)
3419.1	$\nu$ (NH <sub>2</sub> ) + $\nu$ (N-H)
3377.6	$\nu$ (NH <sub>2</sub> ) + $\nu$ (N-H)
3287.7	N-H(str)(H-bonded)
3165.0	C-H(str) in aromatic ring
2915.5	$\nu$ (C-H)
2718.6	N-H(str) in >N <sup>+</sup> H
2508.8	
2455.6	
2369.7	
1748.1	N-H (scissoring)
1626.4	N-H def for NH <sub>2</sub> <sup>+</sup> , C=N(str)
1544.3	N-H def for NH <sub>3</sub> <sup>+</sup> , C=N(str)
1291.4	C-N str in aromatic amines
1218.8	In plane bending bands of ring C-H bond
1068.9	C-N str
831.0	Out of plane bending of the ring C-H bond
771.3	
678.6	
576.7	N-C-N (def)
520.1	N-C-N (def)

K<sub>2</sub> Bis(Phenyldibiguanidium)nickel(II)chloride

Band Position (in cm <sup>-1</sup> )	Assignment
3595.9	N-H (wagging) and v(H <sub>2</sub> O)
3384.7	v (NH <sub>3</sub> ) & v (N-H)
3302.9	v N-H (H-bonded)
3021.6	v (C-H) in aromatic group
2401.4	N-H(str) in>N <sup>+</sup> H
1586.8	v C=C(str), C-N(str)
1519.7	C=C(str)
1293.5	In plane bending bands of ring C-H bond C-N(str) in aromatic amines
1216.2 1068.7 928.7	In plane bending bands of ring C-H bond C-N(str) in aliphatic amines
757.9	C-H def in mono substituted aromatic compounds
670.5	Out of plane C-H bending in benzene ring
503.5	v Ni-N

K<sub>3</sub> (α-Nickel phenyldibiguanidine)

Band Position (in cm <sup>-1</sup> )	Assignment
3433.0	v (NH <sub>2</sub> ) & v (N-H)
3021.8	v (C-H) in aromatic group
2401.1	N-H(str) in>N <sup>+</sup> H
1583.3	C-C (str)
1216.4 926	C-N (str) in aliphatic amines In plane bending of C-H bond
765.3	C-H def in monosubstituted
670.9	Out of plane C-H bending benzene ring
514	N-C-N (def)
470.3	v Ni-N

K<sub>4</sub> (β-Nickel phenyldibiguanidine)

Band Position (in cm <sup>-1</sup> )	Assignment
3398.7	N-H (wagging) and v(H <sub>2</sub> O)
3327.3	v (-NH <sub>2</sub> ) & v (N-H)
3223.4	v N-H (H-bonded)
3022.0	v (C-H) in aromatic group
2401.4	N-H(str) in>N <sup>+</sup> H
2367.9	N-H(str) in>N <sup>+</sup> H
1586.9	C=C and phenyl ring skeletal
1523.1	C=C (str)
1428.0	N-H (def)
1216.6 1026.7	In plane bending band in C-H bond C-N(str) in aliphatic amines

928.3	
761.9 671.2	Phenyl ring C-H out of plane bending
484.0	$\nu$ (Ni-N)

K<sub>5</sub> ( $\gamma$ -Nickel phenyldibiguanidine)

Band Position (in cm <sup>-1</sup> )	Assignment
3449.0	N-H (wagging) and $\nu$ (H <sub>2</sub> O)
3393.8	$\nu$ (N-H) & $\nu$ (NH <sub>2</sub> )
3022.0	$\nu$ (C-H) in aromatic group
2402.3	N-H(str) in >N <sup>+</sup> H
2363.3	N-H(str) in >N <sup>+</sup> H
1520.9	C=C (str) and phenyl ring
1425.6	N-H (def)
929.5	1216.6 str C-N in aromatic amines in plane bending of C-H band in phenyl
763.7 671.5	Phenyl ring C-H out of plane bending
522.0	N-C-N (def)
485.2	$\nu$ (Ni-N)

The metal complexes of phenylbiguanide were dissolved in DMSO using the concentration which was most effective from Dilution test method. The inoculation of fungus was done in PDA and SDA medium at 25°C and checked daily for a week. The fungus are *Aspergillusniger* and *Aspergillusversicolor*. Both are from ascomycetes group. The MIC was then calculated i.e. the minimum inhibition concentration of the fungus. During this process it was found that the complexes was effective in controlling 100% of the fungal growth if the concentration was raised to 800 $\mu$ g/ml to 1000 $\mu$ g/ml. The solution was used in the ratio 1:10 and the concentration of the solution was 400 $\mu$ g/ml, 200 $\mu$ g/ml and 100 $\mu$ g/ml. Refer to table I for **Micelle count inhibition of fungal growth**.

Metal complex	% Inhibition of fungal growth		
	Concentration ( $\mu$ g/ml)	PDA Medium	SDA Medium
<b>Bis(phenylbiguanidium)nickel(II)chloride</b>	400 $\mu$ g/ml	84.01%	82.09%
	200 $\mu$ g/ml	65.72%	62.37%
	100 $\mu$ g/ml	44.36%	42.27%
<b><math>\alpha</math>-Nickelphenylbiguanidine</b>	400 $\mu$ g/ml	85.3%	84.70%
	200 $\mu$ g/ml	60.51%	57.92%
	100 $\mu$ g/ml	40.17%	38.72%

**Table I Percentage inhibition of growth of fungus *Aspergillusniger* at indicated dose**

#### IV. Conclusion

From the stoichiometry, spectral studies (IR & electronic absorptions) and the physio-chemical properties studied in the nickel complex with the ligand phenylbiguanide confirms cis-trans attachment in complexes as shown below:

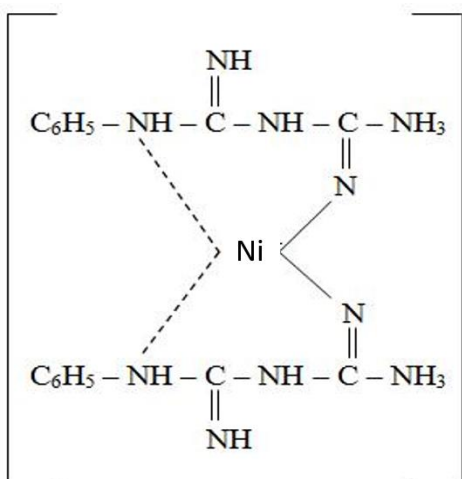


Fig 3. Cis- Bis(phenyldibiguanidine)nickel(II)

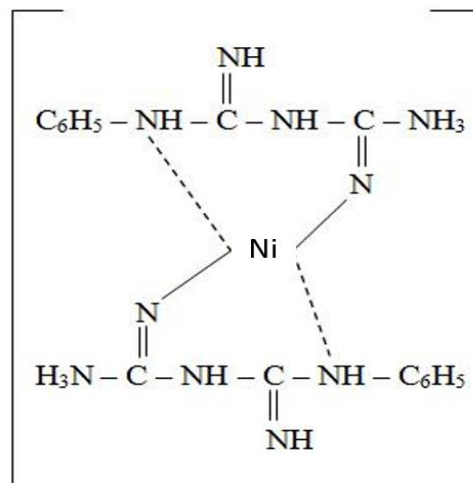


Fig 4. Trans- Bis(phenyldibiguanidine)nickel(II)

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