PART IV

CHAPTER II

BIOLOGICAL EVALUATION
In this chapter, the biological activity of thiourea, N-substituted thioureas, N-Benzoyl N'-substituted thioureas, mixed ligands involving heterocyclic bases as secondary ligands and their corresponding nickel(II) bianionic complexes on test organisms is discussed.

A) EVALUATION OF ANTIBACTERIAL ACTIVITY

The antibacterial activity of the ligands and their complexes was assayed against one gram positive microorganism *Staphylococcus Aureus* and one gram negative microorganism *Escherichia Coli*, which are known to cause diseases in human beings, hence highly pathogenic in nature.

Preparation of Subcultures

The cultures, *Staphylococcus Aureus* and *Escherichia Coli* were inoculated in nutrient broth (20 ml) and were incubated over night at 37°C, one day prior to test.

Nutrient broth was prepared by dissolving peptone (1 gm, 1%), beef extract (0.5 gm, 0.5%) and sodium chloride (0.5 gm, 0.5%) in distilled water (100 ml). The pH of this solution was adjusted to 7.2 by using sodium hydroxide solution (4%) and was autoclaved for 20 minutes under 15 lbs pressure.
Preparation of nutrient agar

Nutrient agar which served as the basal medium was prepared by dissolving bacteriological peptone (10 gm, 1%), yeast extract (0.5 gm, 0.25%), beef extract (0.5 gm, 0.25%) and agar (20 gm, 2%) in distilled water (1000 ml). The pH of this solution was adjusted to 7.2 and sterilized in an autoclave, for 20 minutes at 15 lbs pressure.

Glucose Solution (3 ml, 10%) was added to the sterilized basal medium to hasten the bacterial growth and then it was poured (~30 ml) in sterile petri dishes. After the solidification of the medium, holes of 10 mm diameter were carefully bored with the help of a sterile cork borer.

Preparation of drug solution

The drug solutions were prepared by dissolving ligands and complexes (5 mg each) in pure dimethyl formamide (5 ml) to get the concentration of 1000 μg/ml. This solution (0.1 ml) was used for testing antimicrobial activity. In the same way the compounds were also tested at a concentration of 500 μg/ml.

Method of testing

A saline solution (0.4 cc) of twenty four hours old culture is spread uniformly over the basal medium in
petri dishes. To the cups prepared in the basal medium as mentioned above, drug solution (0.1 ml) was added using a sterile pipette. The petri dishes were kept in a cold room to facilitate the diffusion of the drug solution for about two hours. Then the dishes were incubated at 37°C for 24 hours. The extent of inhibition was obtained by measuring the diameter or width of the zone (mm). Aqueous phenol (5%) was employed as the standard substance for comparison of the inhibition values.

B) EVALUATION OF ANTIFUNGAL ACTIVITY

Antifungal activity of representative ligands and complexes was tested against the pathogenic fungi Aspergillus niger. The fungal growth inhibitory action of the compound was measured by the turbidity method using Klett-Sommerson colorimeter.

Preparation of Subculture

One day before the tests, the above mentioned fungi was inoculated in Sabouroud's broth and incubated at 37°C for 48 hours.

Sabouroud's broth was prepared by dissolving bacteriological peptone (1 gm, 1%), dextrose (4 gm, 4%)
streptomycin (0.01 gm, 0.01 %) and a pinch of rose bengal in distilled water (~100 ml) and autoclaved for 20 minutes at 15 lbs pressure.

**Preparation of drug solution**

This was prepared by dissolving the ligands and complexes (5mg each) to be tested in purified dimethyl formamide (5 ml) to get a concentration of 1000 μg/ml. Similarly the compounds were also tested at the concentration of 500 μg/ml.

**Method of testing**

Sabouroud's medium (5 ml) was placed in each of the sterile matching test tubes and then treated with 48 hours old culture (0.1 ml) separately. After shaking thoroughly, the drug solution (0.1 ml) was added. Then each of the test tubes was plugged with cotton and aeration was facilitated by constant shaking for 48 hours on a shaker. The extent of inhibition was determined by measuring the decrease in turbidity in terms of percent transmission at 420 nm.

Salicylic acid (5 %) was used as the standard and dimethylformamide as controlling solvent.