GREEN SYNTHESIS, CHARACTERIZATION & ANTICANCER ACTIVITY OF PLATINUM NANO PARTICLES

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Abstract:
The current study explains the synthesis of Platinum metal based nano particles from the extraction of the enzyme from the parts of the plant known as green route which is much economical and safe to handle. The synthesized nano particles were characterized by various techniques viz UV-Visible, IR Spectroscopy & SEM analysis. The size & shape of the nano particles was confirmed by SEM analysis.
To evaluate the potentiality of newly synthesized platinum nano particles in *in vitro* and in *in vivo* studies i.e. anti- fungal and anti-bacterial and anti cancer activities were done. Cytotoxicity of compounds was clearly established in MCR-7 (breast cancer cell line).
Key words: Green synthesis, Nano particles, UV-Visible, IR Spectroscopy, Anti cancer

Introduction:

In ISO/TS 80004, nonmaterial is defined as a "material with any external dimension in the nano scale or having internal structure or surface structure in the nano scale", the nano scale defined as the "length range approximately from 1 nm to 100 nm". This includes both nano-objects, which are discrete piece of material, and nano structured materials, which have internal or surface structure on the nano scale; a nano material may be a member of both these

categories.[1] Materials with structure at the nano scale often have unique optical, electronic, or mechanical properties.[2] Nanotechnology may be able to create many new materials and devices with a vast range of applications, such as in nano medicine, nano electronics, biomaterials energy production, and consumer products. On the other hand, nanotechnology raises many of the same issues as any new technology, including concerns about the toxicity and environmental impact of nano materials, [3]

Most transition metals can be bound to a variety of ligands, allowing for a wide variety of transition metal complexes. [4]

Transition metal complexes play a crucial role in antitumor therapy. Complexes of platinum, ruthenium as well as lanthanum and gallium have been investigated in preclinical as well as in clinical studies.

Research in anticancer agents was stimulated by the accidental discovery of cisplatin, *cis*-[$Pt^{II}(NH_3)_2Cl_2$]. However, its clinical use is restricted due to dose-dependent toxicity and resistance coupled with a narrow spectrum of activity [5, 6]. These limitations have triggered a search for platinum-based compounds that show lower toxicity, higher selectivity, and a broader spectrum of activity [7, 8]. Platinum is one of the least reactive metals. It has remarkable resistance to corrosion, even at high temperatures, and is therefore considered a noble metal. Platinum is more ductile than gold, silver or copper, thus being the most ductile of pure metals, but it is less malleable than gold. Platinum drugs, such as cisplatin, carboplatin and oxaliplatin, are the mainstay of the metal-based compounds in the treatment of cancer, but the delay in the therapeutic accomplishment of other metal-based compounds hampered the progress of research in this field.

Palladium is a soft silver-white metal that resembles platinum. It is the least dense and has the lowest melting point of the platinum group metals. Palladium is used in

jewelry, dentistry,[9,10] watch making, blood sugar test strips, aircraft spark plugs, surgical instruments, and electrical contacts[88].Palladium is also used to make professional transverse (concert or classical) flutes[11]. The palladium (II) as non platinum metal complexes highly attracted the researchers because of its significant biological activity as well as lower side effects along with higher lipophilicity or solubility compared to cisplatin [12-15].

Microorganism like fungi, yeasts (eukaryotes) or bacteria, actinomycetes (prokaryotes) are capable of interacting with metals coming in contact with them through their cells and from nano particles. The mechanism of nano particles biosynthesis include enzymatic reduction of metal ions, sorption on the cell wall and subsequent chelating with extracellular peptides or polysaccharides, leading to their aggregation and the formation of nano particles. Proteins, polysaccharides and organic acids secreted by the fungus facilitate the formation of different crystal shapes and directed the grow within to spherical crystals.[16] The enzyme nitrate reductase secreted by the fungi helps in the bio reduction of metal ions and synthesis of nano particles.

Use of plants for the fabrication of NPs has drawn attention of workers because of its rapid, economical, co-friendly protocol and it provides a single step technique for the biosynthesis process. [17]Nano particles have been produced physically and chemically for a long time, but recent developments show the critical role of microorganisms and biological systems in production of metal nano particles.

Procedure:

Horse gram and Lobia seeds were collected from the market.200 gm of mature seeds were taken and washed them thoroughly for 2-3 times with distilled water. Sterilized the seeds with 0.1%$Hgcl_2$ and again washed them. Then seeds are kept for about 24-36 hours in distilled water at room temperature for soaking process. Seeds were germinated in 24-48 hours by hanging them in a muslin cloth in at a room temperature. Seeds are moistened with sprinkling distilled water regularly after 2-3 hours. The germinated seeds were freeze dried in a refrigerator and then are generated to 60 mesh size by grinding with the help of acetone as a solvent. This homogenate was kept in sunlight for 2-3 days to completely remove the moisture content present in it. That dry homogenate was further coarsely grounded using motor and pestle at 1.2 degree Celsius to convert in powder form. This powder is suspended in buffer solution (PO_4 buffer [10m, pH8], trisHCl [10mm, pH8] and de ionized water). The extract was used for protein testing using bartered method. The mixture obtained (buffer and powder) was sprinkled with 2M NaCl and kept in incubator for 24 hours at room temperature. This mixture is further filtered and centrifuged at 10000 rpm for 8-10 minutes below 4 degree Celsius (In high speed refrigerator centrifugation) and the supernatant was collected. This supernatant will contain enzymes, were collected from supernatant is mixed with metal complex. After 2-3 days Pt (II) metal complex converted into nano particles.

U V Visible spectroscopy:

The UV-Visible spectra of these dyes were recorded using instrument model: UV 1800, made by shimadju, Japan and supplied by vignan instruments pvt. Ltd.

Ultraviolet–visible spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared [NIR]) ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved.

In this region of the electromagnetic spectrum, atoms and molecules undergo electro nic transitions. Absorption spectroscopy is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from

the excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

The nano complex form from green routes was characterized under UV spectroscopy for the study of their behavioral pattern. The following graphs of UV- visible spectra are given for the platinum and palladium metal complex.

Platinum complexes

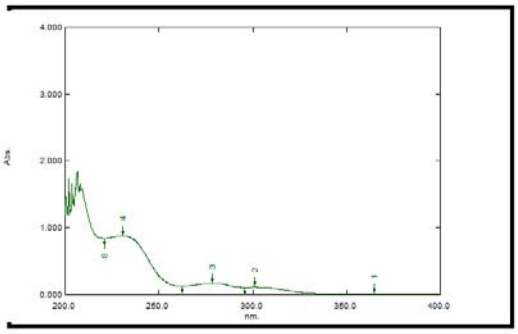
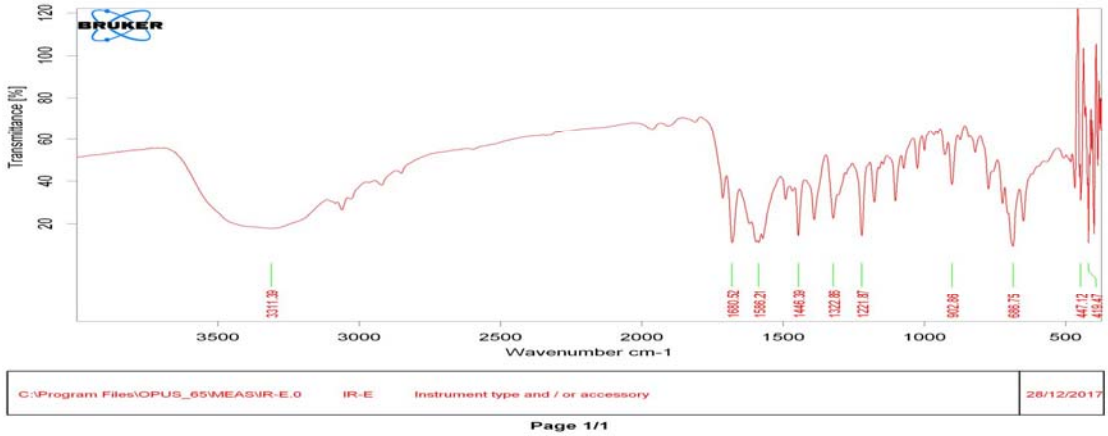


FIG. shows the UV-Visible ***(UV Double Beam Spectrophotometer, Shimadzu, Japan)*** absorption spectra of different sensitizers used in this study. A band due to the >C=N chromophore in the spectrum of the compound at 365 nm shifts to a higher wavelength. Such a shift in n-π * transition band is probably due to the donation of a lone pair of electrons by the nitrogen. Further, two bands at 260 nm and 305 nm are due to π-π * transitions, these are assigned to the benzenoid ring and (>C=N) band of the azomethine group respectively. The K band π-π * showed a red shift due to the increase in conjugation and the B-band undergoes a hypsochromic shift. The maximum wavelength is 230nm.

FTIR (FOURIER TRANSFORM INFRARED SPECTROSCOPY)

FTIR is a technique which is used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time. Fourier transforms infrared spectroscopy (FTIR) spectroscopic study of the sample was performed by ***Shimadzu, Japan*** **model,** using KBr as a reference. 0.5 g dried sample was mixed with KBr (sample/KBr ratio was 1/100) and were pressed into the transparent thin pellet.

A FTIR spectrum of Pt and Pd complexes was obtained in the range on 4000 cm-1 to 400 cm-1. The following images of graphs show the FTIR spectroscopy results of the platinum and palladium metal complex.



The FTIR spectrum of prepared dried Nano particles indicates the presences of some functional groups of hydrocarbon and oxygen. In the spectrum 3311 cm^{-1} absorption due to =CH stretching and Ar-H stretching as well as it shows the presence of primary NH_2 group two twin peaks are observed in the compounds. 1600 cm^{-1} shows the presence of C=O stretch in compounds. Band at 2450 cm^{-1} due to presence of –C≡ N stretching. Medium intensity bands around 1200-1560 cm^{-1} are also observed for the presence of aromatic rings. A band at 668cm^{-1} are observed shows substittion to the compounds.

Anti-Cancer Activity

The potential of metal-based anticancer agents has only been fully realized and explored since the landmark discovery of the biological activity of cisplatin (cis-diammine dichloro platinum (II) or cis-DDP). To date, this prototypical anticancer drug remains one of the most effective chemotherapeutic agents in clinical use. It is particularly active against testicular cancer and, if tumors are discovered early, an impressive cure rate of nearly 100% is achieved. The clinical use of cisplatin against this and other malignancies is, however, severely limited by dose-limiting side-effects such as neuro-, hepato- and nephrotoxicity.

The anticancer activity of the synthesized palladium (II) complexes was evaluated by the "MTT cell proliferation assay" [79]. The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability.

Materials:

DMEM (Dulbecco's modified Eagles medium), MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide], trypsin, EDTA Phosphate Buffered Saline (PBS) and were purchased from Sigma Chemicals Co. (St. Louis, MO) and Fetal Bovine Serum (FBS) were purchased from Gibco. 25 cm^2 and 75 cm^2 flask and 96 well plated purchased from Eppendorf India.

Maintenance of Cell Line:

The MCF-7 breast adenocarcinoma cancer cell line were purchased from NCCS, Pune and the cells were maintained in MEM supplemented with 10 % FBS and the antibiotics penicillin/streptomycin (0.5 mL^{-1}), in atmosphere of 5% CO_2 /95% air at 37 ^{0}C.

Preparation of Test Compound:

For MTT assay, Each Test compounds were weighed separately and dissolved in DMSO. With media make up the final concentration to 1 mg/ ml and the cells were treated with series of concentrations from 10 to 100 µg/ ml.

MCF-7 cell viability by MTT Assay:

Principle:

MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The assay depends both on the number of cells present and on the assumption that dead cells or their products do not reduce tetrazolium. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, dark purple coloured formazan crystals. The cells are then solubilized with DMSO and the released, solubilized formazan reagent is measured spectrophotometrically at 570 nm.

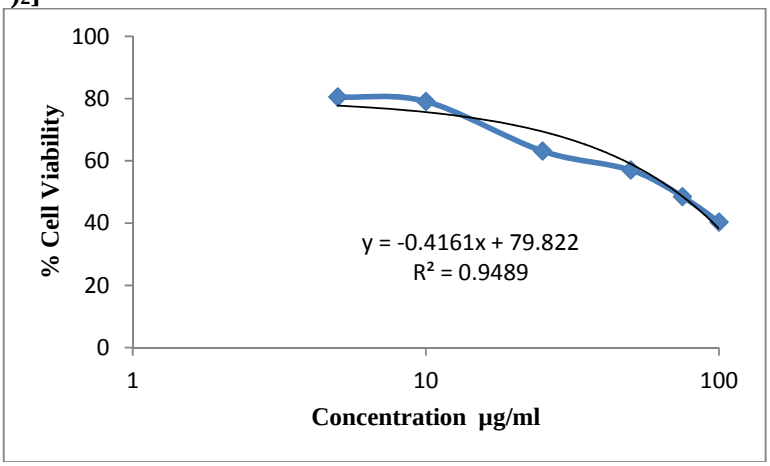
Procedure:

Cell viability was evaluated by the MTT Assay with three independent experiments with six concentrations of compounds in triplicates. MCF- 7 cells were trypsinized and perform the tryphan blue assay to know viable cells in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0 X 10^3 cells / well in 100 µL media in 96 well plate culture medium and incubated overnight at 37 ^{0} C. After incubation, take off the old media and add fresh media 100 µL with different concentrations of test compound in represntive wells in 96 plates. After 48 hrs., Discard the drug solution and add the fresh media with MTT solution (0.5 mg / $mL^{-1)}$ was added to each well and plates were incubated at 37 ^{0} C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a micro plate reader. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50 % values is

generated from the dose-response curves for each cell line using with origin software.

$$\% \text{ Inhibition} = \frac{100\ (\text{Control} - \text{Treatment})}{\text{Control}}$$

Complex 1: [Pt (L^{13})$_2$]



Graph 4.19: Cytotoxic effect of the Pt Complex on MCF 7 cell line

Table 4.1: Cytotoxic properties of Pt Complex on MCF 7 cell line

Concentration (µg/ml)	Absorbance at 570nm			Average	Aver age-Blank	%Viability	IC_{50} (µg/ml)
100	0.961	0.963	0.965	0.963	0.9484	40.351	71.68
75	1.153	1.156	1.157	1.155	1.1404	48.519	
50	1.354	1.356	1.358	1.356	1.3414	57.071	
25	1.498	1.499	1.501	1.499	1.4844	63.155	
10	1.872	1.874	1.875	1.873	1.8584	79.067	
5	1.905	1.907	1.909	1.907	1.8924	80.514	
Untreated	2.365	2.365	2.366	2.365	2.3504	100	
Blank	0.003	0.038	0.003	0.014	0		
Cisplatin							3.106

Conclusion:

The platinum nano particles were synthesized by green synthesis and they are showing good anti fungal, anti bacterial and anti cancer activities.

During the course of our screening programme for antifungal and anti bacterial activities, Pt complexes exhibited significant bioactivity. The complexes were further tested against MCF-7 cell line as no information is available on the

antimicrobial and cytotoxicity of the reported bioactive compounds. Platinum (II) complexes have been proved potent against cytotoxicity as percent inhibition is 71.68, revealed by the graphs and the table.

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