INVESTIGATION ON NON-CONVENTIONAL BIOLOGICAL ROUTE OF PREPARATION OF PD NANO-PARTICLES AND CYTOTOXICITY AGAINST TUMOUR CELL

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Abstract

Palladium nanoparticles have attained a special focus due to its unique physical, chemical and biological properties. Green protocol of synthesizing nanoparticles has emerged as an alternative to overcome the limitation of conventional methods. Thus to solve the objective biological approaches derived from plant sources in the form of extracts exhibits superiority over chemical or biological methods. The nanoparticles synthesis with plant based biological molecules makes it suitable .The present study describes the preparation of Palladium metal based nano particles from the green economic method. 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 predict Potentiality of newly synthesized nano particles anti- fungal and anti-bacterial and anti cancer activities were done. Cytotoxicity of compounds was clearly visible in MCF- 7 (breast cancer cell line). The area of target to the cancer cell to trigger the anti cancer reaction was calculated by the bioinformatics i.e. ADMET technique.

Key words: Green synthesis, Nano particles, SEM, Anti cancer.

Introduction

Cancer represents one of the major causes of death in humans worldwide, only overcome by cardiovascular diseases, and represents a huge burden on society (both sociologically and economically). About 20 million cancer cases are expected to occur in the next two decades, which renders the quest for new and improved antineoplastic agents (namely, based on natural compounds [1]) an urgent issue in the field of Biomedicine and Human Health. Over the past decade, efforts have been made in the way of understanding the carcinogenesis process, which is recognised to consist in a progressive disorganisation at both the cellular and tissue levels. This knowledge is essential to develop new chemotherapeutic strategies, in order to control the incidence of the most recurrent cancer types.

Nanotechnology is one of the most active area in modern science. This field is developing day by day in making an impact in humans life. A nanotechnology deals with the synthesis, strategy and manipulation of particles structure ranging from approx 1-100nm in size .All the properties (chemical, physical & biological) of the atom /molecules and their corresponding bulk takes place within the size, shape and distribution. Nanocrystalline particles have found tremendous application in the field of high sensitivity bimolecular detection and diagnostic, therapeutic and antimicrobials[2], catalysis & micro electronics. [3]

Nanoparticles 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 nanoparticles. The use of organisms in this area is rapidly developing due to their growing success and ease of formation of nanoparticles. Moreover, biosynthesis of metal nanoparticles is an environmentally friendly method (green chemistry) without use of harsh, toxic and expensive chemicals.[4-5] For instance, production of silver nanoparticles by

chemical reduction (e.g., hydrazine hydrate, sodium borohydride, DMF, and ethylene glycol) may lead to absorption of harsh chemicals on the surfaces of nanoparticles raising the toxicity issue.

Many metal-containing compounds have been utilized throughout history to treat a wide variety of disorders [6]. In medicinal chemistry — traditionally dominated by organic chemistry— metal complexes have gained favor as diagnostic tools and anticancer agents [7]. 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 [8].

Globally, the demand for platinum-based drugs has grown steadily, although several side effects ranging from nephrotoxicity to drug resistance of the tumour cells have posed real challenges to researchers.[9-12] Side effects associated with cisplatin administration, alongwith limited applicability arising from specificity shown towards certain cancer cell lines have prompted researchers to look for alternatives.

One of the alternatives that has shown considerable promise has been the development of other transition-metal based drug candidates.[13-18]The combination of transition metals with biologically active molecules has also been exploited showing promising activity due to their unique ability to bind different biological targets.[19-21] Palladium-based complexes are closely related to their platinum analogues, due to their structural similarities and significant overlap of coordination chemistry for the two metals

The palladium (II) as nonplatinum 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 [22-23]. Palladium metal is a suitable candidate for metallodrugs because it displays structural properties similar to those of platinum and also exhibits promising in vitro cytotoxicity. Numerous Pd (II) complexes with different benzylamine ligands have been synthesized and their interesting in vitro biological activities have been reported [24]. The antimicrobial activity of different palladium (II) complexes on the growth and metabolism of various groups of microorganisms has been studied and reported elsewhere. Garoufis et al. reviewed numerous scientific papers on antiviral, antibacterial, and antifungal activity of Pd (II) complexes with different types of ligands (sulfur and nitrogen donor ligands, Schiff base ligands, and different drugs as ligands) [25]. There are other interesting works which are reported recently in the literature showing different intensities of palladium complex activity on various species of bacteria and fungi [26-27].

Synthesis of Palladium nano-particles

Horse gram and Lobia seeds are collected from the super market. 200 gm of mature seeds were taken and washed thoroughly for 2-3 times with distilled water and sterilized seeds with 0.1%$HgCl_2$ and again washed them. Now keep the seeds for about 24-36 hours in distilled water at room temperature for soaking process. Seeds will germinate in 24-48 hours by hanging them in a muslin cloth in at a room temperature, moistened the seeds with sprinkling distilled water regularly after 2-3 hours regularly. The germinated seeds allowed to freeze and dried in a refrigerator and then are converted 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. This dry homogenate was further coarsely ground using motor and pestle at 1.2 degree Celsius to convert in powder form.this powder is suspended in buffer solution(po4 bufer[10m,ph8],tris Hcl[10mm,ph8]and deionized water). The extract is use for protien testing using bartard method. The mixture obtained(buffer and powder) is sprinkled with 2MNacl 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.

Enzymes collected from supernatant is mixed with metal complex and after 2-3 days metal complex will automatically convert into nano particle

Why biological route is important than any other method

Biosynthesis involves using an environment-friendly green chemistry based approach that employs unicellular and multicellular biological entities such as actinomycetes , bacteria , fungus, plants, viruses, and yeast . Synthesising nanoparticles via biological entities acting as biological factories offers a clean, nontoxic and environment-friendly method of synthesizing nanoparticles with a wide range of sizes, shapes, compositions, and physicochemical properties . Another interesting feature of many biological entities is their ability to act as templates in the synthesis, assembly and organisation of nanometre scale materials to fabricate well-defined micro and macro scale structures.

In comparison with microorganisms, the plant approach is more advantageous since it does not need any special, complex, and multi-step Materials procedures such as isolation, culture preparation, and culture maintenance. Furthermore, synthesis in plants tends to be faster than microorganisms, is more cost-effective and is relatively easy to scale up for the production of large quantities of nanoparticles .

Characterization of Palladium nano-particles UV

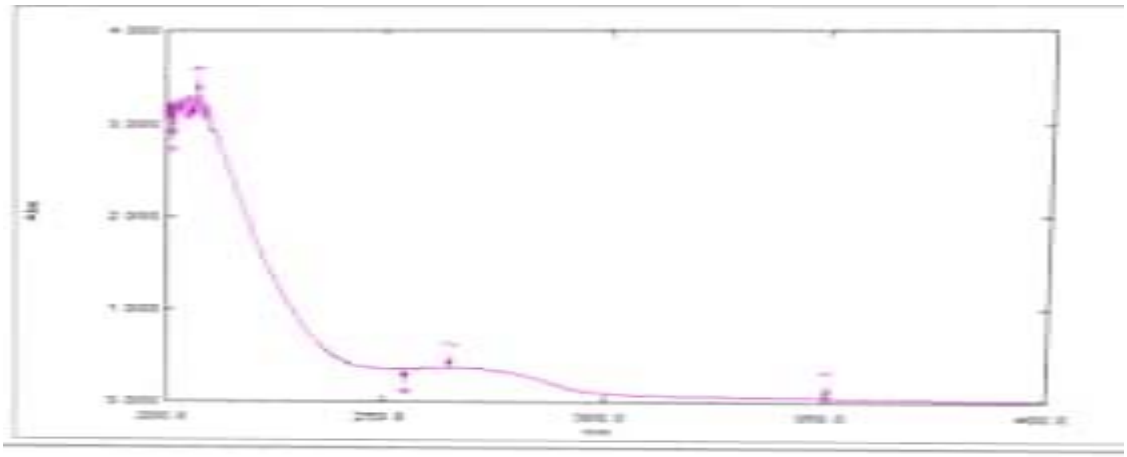
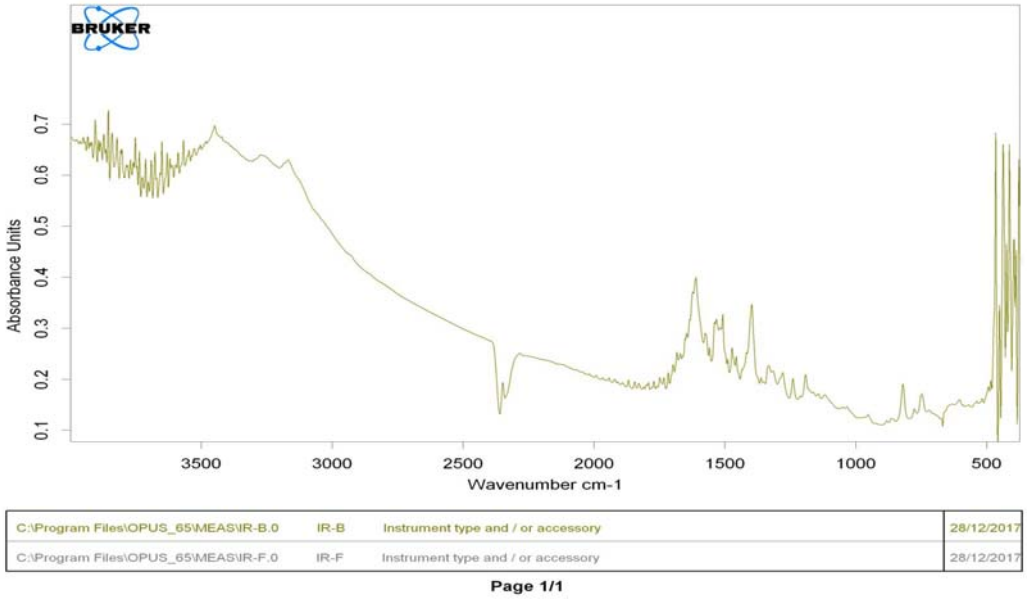


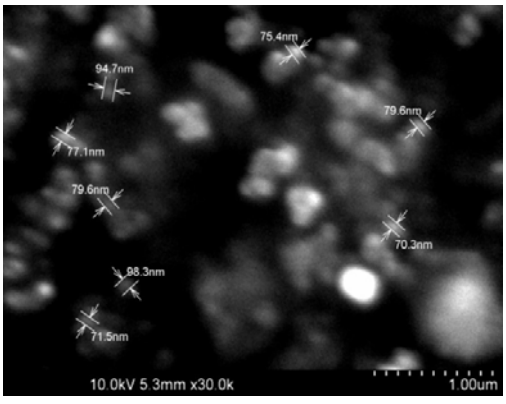
FIG. shows the UV-Visible ***(UV Double Beam Spectrophotometer, Shimadzu, Japan)*** absorption spectra of different sensitizers used in this study. A band due to the >C=N chromophore in the spectrum of the compound at 365 nm shifts to a higher wavelength. Such a shift in n-π * transition band is probably 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.

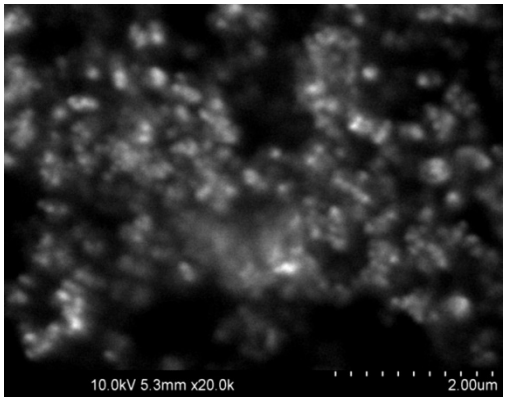
FTIR



The FTIR spectrum of prepared dried Nanoparticles 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 compound

SEM



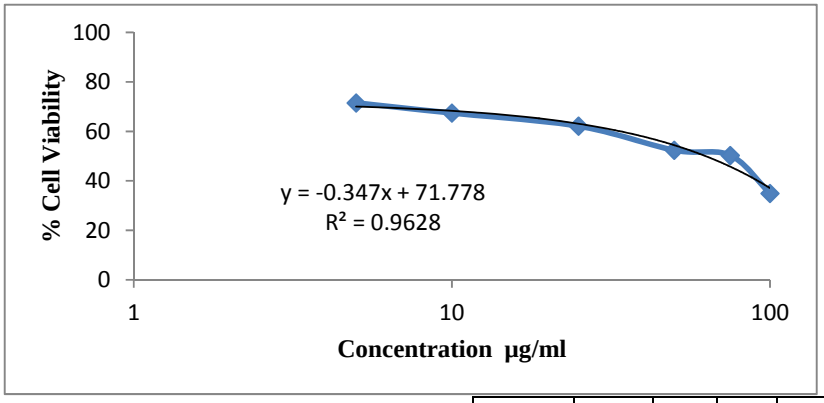


The nano particles are formed as shown by the scanning electron microscopy, SEM (*FEI Quanta 200 Hv model*) images of Pt and Pd complexes in the range between 75-95nm.

Antitumor activity towards Breast cancer cell lines

The anticancer activity of the synthesized palladium (II) complexes were evaluated by the "MTT cell proliferation assay" . 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.

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



Cytotoxic effect of the Pd complexes on MCF 7 cell line

Concentration (µg/ml)	Absorbance at 570nm			Average	Average-Blank	%Viability	IC_{50} (µg/ml)
100	0.834	0.837	0.839	0.836	0.82214	34.947	62.73
75	1.196	1.198	1.199	1.197	1.1824	50.306	
50	1.245	1.246	1.248	1.246	1.23114	52.391	
25	1.472	1.476	1.479	1.475	1.46004	62.134	
10	1.597	1.599	1.601	1.599	1.5844	67.41	
5	1.693	1.696	1.699	1.696	1.6814	71.536	
Untreated	2.365	2.365	2.366	2.365	2.35004	100	
Blank	0.003	0.038	0.003	0.0146	0		
Cisplatin				3.106		4.75	4.489

Table : Cytotoxic properties of Pd complex on MCF 7 cell line

Conclusion

During the course of our screening programme for antifungal and anti bacterial activities, Pd 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. Palladium (II) complexes have been proved potent against cytotoxicity as percent inhibition is 62.73, revealed by the graph and the table.

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