



Polypropylene/Pt Nanocomposite: Synthesis, Characterization and Study the Cytotoxic Effects

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Abstract

This paper presents the synthesis of a polypropylene/Platinum [PP/Pt] nanocomposite. The nanocomposites was characterized using different techniques: atomic force microscopy (AFM), surface morphology was evaluated by (SEM), (EDS),(XRD) and also the (FTIR) technique to find functional groups. The study showed that the [PP/Pt] nanocomposite had a fatal effect on both strains of bacteria used, as well as on the growth of fungi. The compound tested showed antioxidant properties moderate activity was found. The mentioned material were evaluated in normal cell line HdFn (Human Dermal Fibroblasts, neonatal) and breast cancer MCF-7 cancer cell line, by MTT assay for study cytotoxic effects, morphological changes, all experiments were conducted on cell lines by using the materials alone. The results of the MTT assay showed that the nanocomposite [PP/ Pt] had a toxic effect on the cell lines, and the toxicity of the materials was concentration-dependent manner where that have higher toxicity on the MCF-7 cell lines than HdFn. When there appeared a significant increase in the concentration of nanocomposite [PP/Pt] nanocomposite have an inhibitory effect on both breast cancer and normal cell lines. Conclude both materials have genotoxic effects on normal and cancer cell lines.

Keywords: Polymer composite, Polypropylene, Platinum, SEM, Antioxidant activity and anticancer activity.

1. Introduction

With the advancement of science and technology in terms of research and development in the field of nano-technology, it is considered one of the most important areas in technical disciplines. This appears to include polymer science and technology, and in this field covers a wide range of topics[1]. In the past decades, the nature of nanomaterials has been widely reported in the scientific-literature to improve their properties even in compounds with low nanoparticle content.

Therefore, in nanotechnology polymer nanocomposites are solid materials consisting of mixture of more than one substance that are separated in a phase, where the dispersed phase in most composites is one or more of the nanophase. Similarly, materials when they measure their size can be referred to as nanoscale, which means that at least one of the three-external dimensions ranges from about 1 nm to about 100 nm [2].

Recently, various metallic nanoparticles such as Au, Ag, Pd, Cu, Pt, TiO₂ and ZnO (NPs), it is widely used as an alternative to developing new treatment methods for human use [3,4]. Just as AgNPs have shown antibacterial activity and have been employed in varied fields such as catheters and biomedical implants, we see PtNPs as well show antibacterial activity [5]. However, silver nanoparticles exhibited high toxicity in vivo [6]. Compounds containing platinum have been used in the treatment of cancer, clinically used. We are seeing more reports on the anticancer activity of PtNPs, but little is known about its antifungal and antibacterial activity compared to copper and silver nanoparticles. In recent years, many have reported that platinum nanoparticles with sizes less than 3 nanometers, it has strong bacteriostatic toxicity against bacteria [*P. aeruginosa*] gram-negative [7].

In this work we tried to detect the antifungal activity, antibacterial, anticancer and antioxidant activity of platinum (NPs), so we made an uncomplicated method for platinum synthesis by tungsten oxide, and some antibacterial tests were done in the laboratory. Establishment of the direct inhibitory effect of nanocomposite on cultures of reference strains of both selected bacteria as gram negative [*Escherichia Coli* (-)] and [*Staphylococcus aureus* (+)] gram positive, by disc diffusion method, As well as fungal cultures [*Candida albicans*], antioxidant and cancer outcomes were also discussed.

2. Experimental

2.1 Materials and methods

Polypropylene [PP] purity 100 % were supplied by SABIC, WO₃ Tungsten oxide supplied by Hongwunew material, H₂PtCl₆ Chloroplatinic acid solution, NaBH₄ Sodium borohydride supplied from BDH and L.lysine from Spectrum.

2.2 Synthesis of Platinum (NPs)

Typically, 0.30 g of the previously prepared WO₃ nanorods were dispersed on it in 100 ml of deionized water with constant stirring, and then add by adding 8 ml of H₂PtCl₆ and 12 ml of 0.01 M L.lysine. Display the suspension for ultrasound to 60 minutes, then 5 ml of 0.03 M sodium borohydride solution was added to reduce H₂PtCl₆ to Pt metal nanoparticles. The gray precipitate was collected by 3000 r/min centrifugation. Washed with distilled water, ethanol, and transferred to the oven for drying at 80 C, the product was calcined at 300 C for 1 hour to remove all the lysine. The change from white to gray color of the product indicates that Pt NPs were obtained using WO₃ nanorods [8] **Figure (1)**.



Figure 1: Platinum (NPs)

2.3 Preparation of Nanocomposite

The composites was prepared by dissolved 0.5 g of PP in 15 ml xylene at a temperature of 90 C The metal Platinum nanoparticles 0.1 g were added to the polymer and stirred for 1 hour on a hot plate until a homogeneous mixture was obtained, the mixture was placed in a Petri dish for dried in air [9].

3. Results and Discussion

3.1 AFM of metal nanoparticles Pt (NPs)

The images and their results of AFM for nanoparticles synthesized were shown in **Figure (2)**. For Pt (NPs) grooves are not homogenous as depicted by the three-dimensional image, which is mainly due to agglomeration of nanoparticles [10], and showed that the diameter of the particles was average value 24.01 nm median

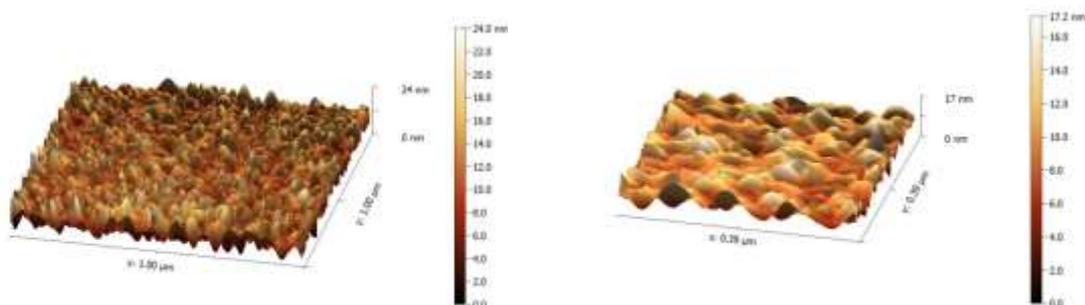


Figure 2: AFM images of Pt (NPs)

3.2 SEM/EDX of [PP/Pt] nanocomposite

The SEM images for [PP/Pt] composite in **Figure (3)** shows fine particles and the mapping spectrum explaining, the nano-granular morphology produced by the aggregation of globular structures was evident on the SEM in the form of balls, as well as the components, the EDX spectrum indicated a peak for a Pt 2.00 % and carbon at 98.00 percent.

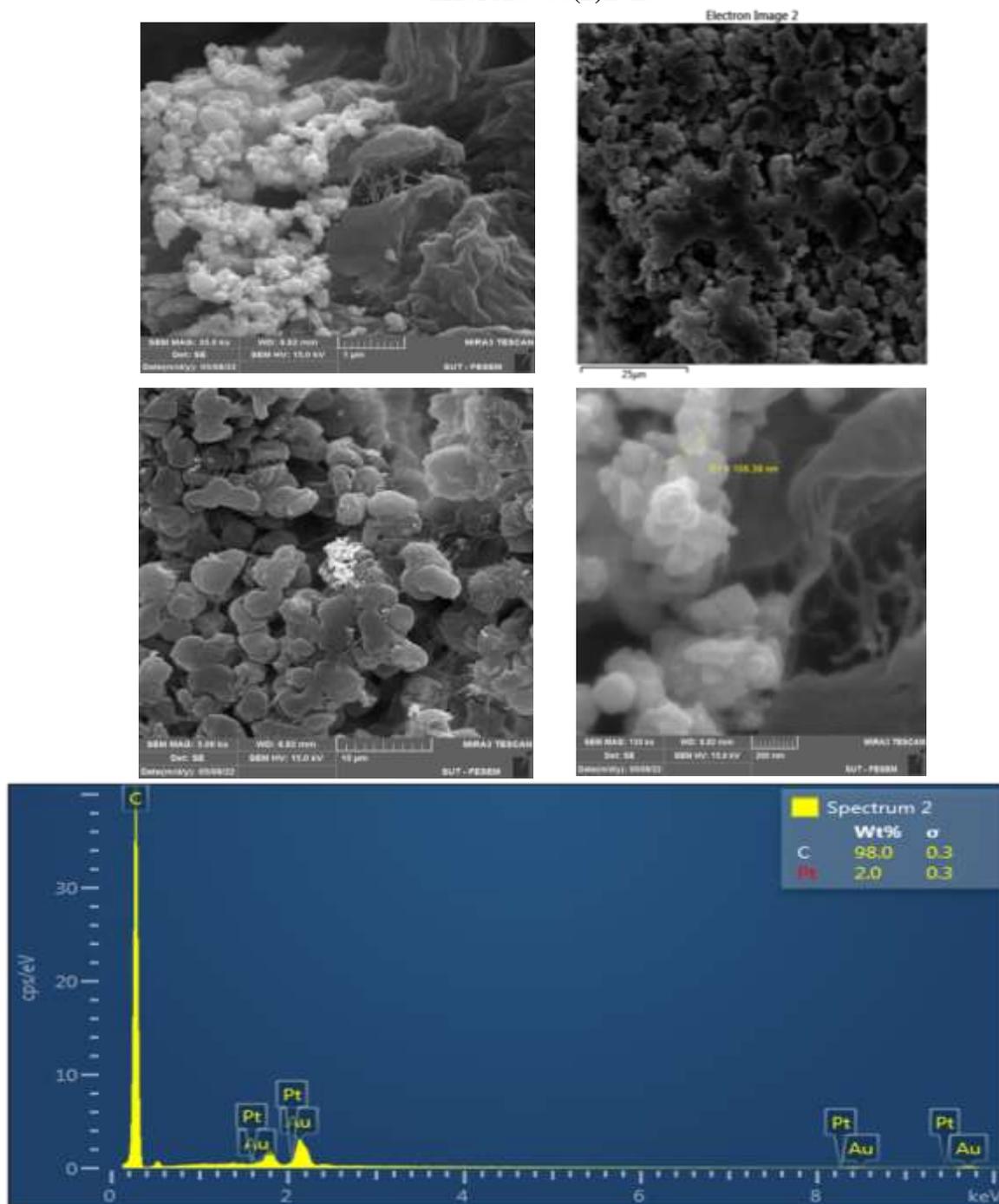


Figure 3: SEM images & EDS for [PP/Pt] nanocomposite

3.3 Fourier Transform Infrared Spectroscopy (FTIR) of [PP/Pt] nanocomposite

The FTIR spectrum of [PP/Pt] nanoparticle composite is reported in **Figure (4)**. The bands at (3446.56 and 3427.27) cm^{-1} are indicated to the stretching vibrations of (-OH) [11] Interlayer or absorbed water while the bands at (2960.53, 2921.96, 2839.02) cm^{-1} indicated to stretching vibration of C-H. Furthermore the band at (1460.01) cm^{-1} is indicated of CH_2 deformation, A strong absorption area appears at band (1379.01) cm^{-1} is indicated of symmetric CH_3 deformation. Also, the bands at (1168.78, 999.06, 973.99, 842.83) cm^{-1} is indicated isotactic polypropylene band, the other bands in the range from (649.97 to 1510.16) cm^{-1} are the finger print region for Pt [12].

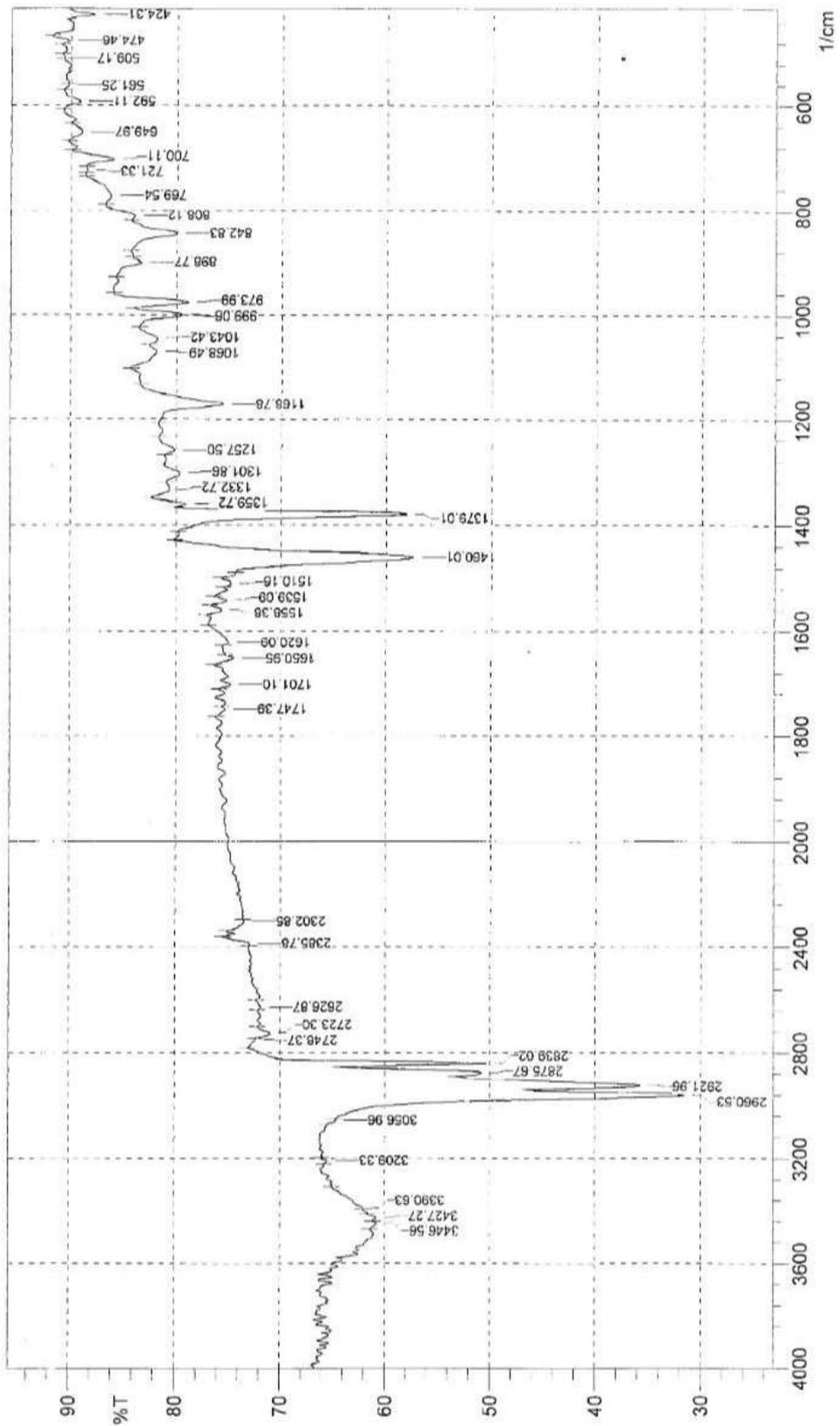


Figure 4: FT-IR spectrum of [PP/Pt] composite

3.4 X-Ray diffraction of [PP/Pt] nanocomposite

Figure (5) revealed the XRD pattern for the [PP/Pt]. This figure illustrates peaks compared with ICDD card no.96.101.1104, corresponding to platinum phases crystallize in the Cubic

appeared at $2\theta = 39.5567^\circ$, 45.9666° and 66.8750° indexed to (111), (200) and (220) planes respectively [13].

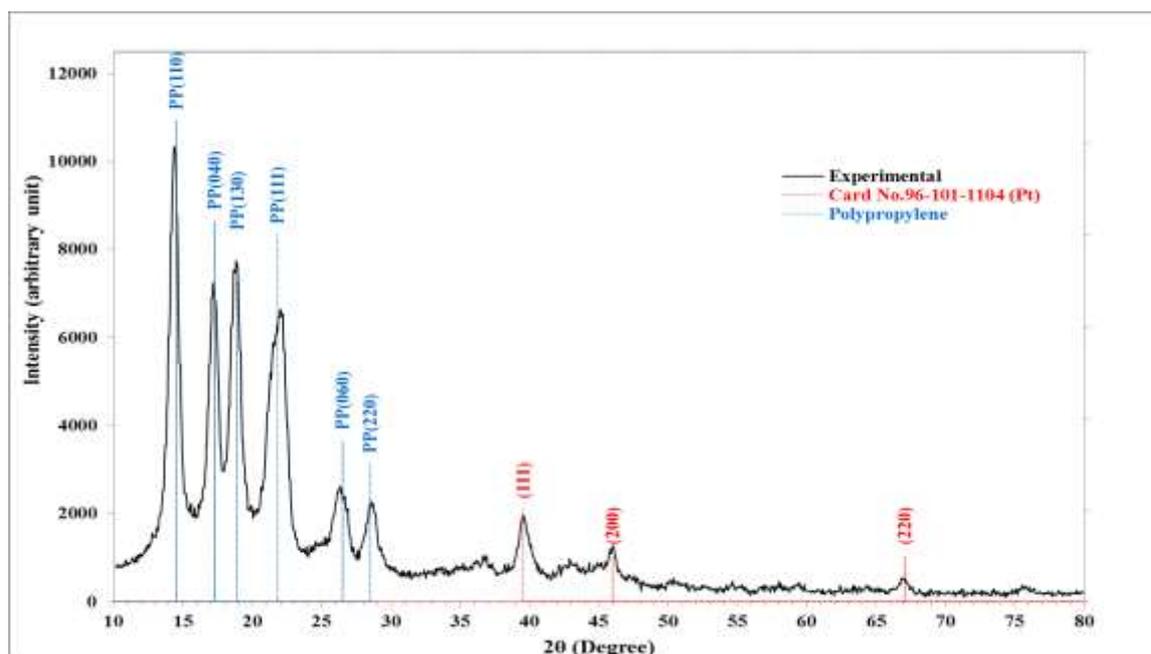


Figure 5: X-ray diffraction pattern of (PP/Pt)

3.5 Antibacterial activity

Antibacterial tests were performed in the central environmental laboratory. The straight inhibitory effect [PP/Pt] nanocomposite was established on cultures of reference strains about 0.1 ml from both selected bacteria gram negative as [*E-Coli*(-)] and gram positive as [*S- aureus*(+)], by method of spreading the disc into Petri dishes with a diameter of (20) ml. Petri dishes were placed inverted and kept in an incubator at constant temperature 37 C for (18-24) hours (depending on the type of micro-organism tested). The diameter of the bacterial growth inhibition zones was measured by platinum nanocomposite from the edge of the film to the end of the absence-zone [14]. And exhibited an activity as shown in **Table (1)**.

Table 1: show the antibacterial and antifungal activity of [PP/Pt] nanocomposite

nanocomposite	[<i>E. Coli</i> (-)]	[<i>S. aureus</i> (+)]	<i>candida albicans</i>
[PP/Pt]	30mm	22mm	40mm

3.6 Antifungal activity

In vitro assay was performed on type of growth medium PDA, the straight inhibitory effect [PP/Pt] nanocomposite was about 0.1 ml from fungus (*candida albicans*) by disk diffusion method in (20) ml on Petri dishes, were placed inverted and kept in an incubator at constant temperature 28°C for 72 hours . Using the platinum nanocomposite, the diameter of the petri dish containing the fungi was measured, the growth inhibition region of the fungi was calculated from the edge of the film to the end of the absence region the measurement table (1) [15].

3.7 Antioxidant activity

DPPH (1,1-Diphenyl-2-picryl-hydrazyl): DPPH (4 mg) was dissolved in (100 ml) of methanol, and by shielding the aluminum foil test tubes. The solution was kept shielded away from light. Various concentrations (6.25, 12.5, 25, 50, 100) ppm were prepared from (1-5), by dissolving 1 mg of the compound and dissolving it with 10 ml of methanol to prepare 100 ppm, this concentration was then diluted to prepare the other concentration. As well as ascorbic acid similar concentrations of the prepared compounds were prepared.

A radical scavenging effect of the stable free-radical DPPH, and the process was appraised for the antioxidant efficacy of the plant methanol extract and Normal vitamin C. In a test tube, 1 ml of the diluted or normal solution (100, 50, 25, 12.5, 6.25 ppm) was applied to 1 ml of DPPH solution. The absorbance of each solvent was measured with a spectrophotometer at 517 nm after that incubation at 37 C for 1 h was done. Triplicate measurements were made by the following equation (1) which able to determine the potential to scavenge DPPH [16].

$$I\% = [\text{Abs blank} - \text{Abs sample}] / \text{Abs blank} \times 100 \dots\dots\dots(1)$$

The PP/Pt composite showed antioxidant activity against DPPH free radical and gave acceptable scavenging percentage **Figure (6)**. and then. The values of inhibitory concentrations IC_{50} were recorded and tabulated in **Table (2)**. In this paper, antioxidant activity dependent on IC_{50} , values was applied, that is mean, strong antioxidant activity if was ($IC_{50}=10$ to $50 \mu\text{g/ml}$), but it is intermediate antioxidant activity if was ($IC_{50}=50$ to $100 \mu\text{g/ml}$), and weak antioxidant activity if was ($IC_{50} >100 \mu\text{g/ml}$). Therefore, it gives intermediate antioxidant activity, (comes from the IC_{50} range values published by Phongpaichit).

Table 2: DPPH radical scavenging activity for PP/Pt composition with ascorbic acid

	Scavenging %					IC_{50} values
	[6.25 $\mu\text{g/ml}$]	[12.5 $\mu\text{g/ml}$]	[25 $\mu\text{g/ml}$]	[50 $\mu\text{g/ml}$]	[100 $\mu\text{g/ml}$]	
PP/Pt	3.84	24.61	25.49	49.89	62.41	71.88
Ascorbic acid	51.40	57.4	61.1	64.7	93.2	1.4

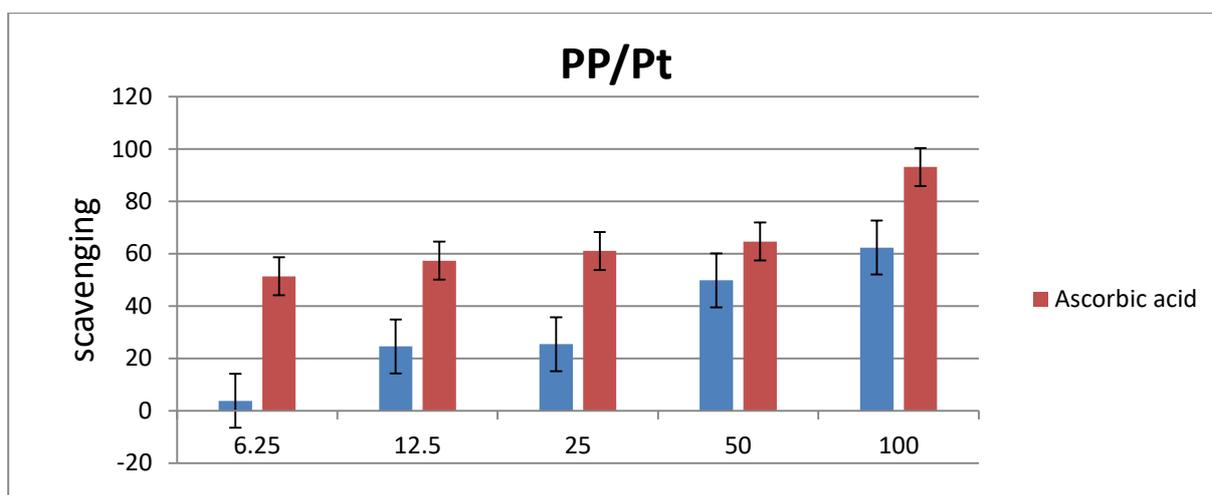


Figure 6: Antioxidant activity of PP/Pt

3.8 Anticancer activity

In vitro cytotoxicity of the [PP/Pt] was evaluated against two cell lines, the cell-lines, breast cancer MCF-7 cancer cell-line and normal cell-line HdFn (Human Dermal Fibroblasts, neonatal), were obtained from the tissue culture Laboratory in the Biotechnology Research Centre organization in Al- Nahrain University of different concentrations (15.60, 31.20, 62.50, 125.00, 250.00 and 500.00 $\mu\text{g}/\text{mL}$) was completed using MTT 3-(dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide ready to use kit.

A-Kit Contents (MTT solution 1ml \times 10 vials, solubilization solution 50 ml \times 2 bottle)

1- Cell Line protocol

The vessel was inverted with gentle rocking and the monolayer was completely covered, then placed in the incubator at 37° for 100 s. The cell layer separates from the vessel that contains it. Then 15-20 ml of new RPMI medium is added and the cells are dispersed from the surface into the growth medium by a pipette. After that, Cells are placed at the required concentrations in culture dishes or plates and transferred to an incubator at 37 C and 5 % carbon dioxide, the concentration of E-cells is checked electronically using a haemocytometer, the cells are counted and the formula of the equation (2) is applied [17].

$$\text{Total cells / ml} = \text{count of cells} \times \text{volume of sample or the dilution factor} \times 10^4 \dots \dots (2)$$

2- MTT Assay

Tumor cells (1×10^4 – 1×10^6 cell /ml) they were cultured in 96 flat titer micro-plates, with volume of 200 μL complete culture medium per well. Microplate surrounded by sterile-parafilm and gently shaken. Transfer to an incubator at 37 C, 5 % carbon dioxide for one day. Then the medium and two serial dilutions of the desired concentrations are removed from ADI enzyme (15.60, 31.20, 62.50, 125.00, 250.00 and 500.00 $\mu\text{g}/\text{ml}$) are added to the wells, each concentration is used in triplicate to avoid errors and controls also (cells-treated with serum free medium). The plates are incubated for a day at 37 C and 5 % carbon dioxide, MTT solution was added in a volume of 10 μl to each well of the plate. Return to the incubator at 37 C and 5 % carbon dioxide for four

hours. After the incubation is over, media is removed and 100 μl solubilization- solution is added to each well for five minutes.

By using an ELISA device for measurement, at wavelength 575 nm. The data is subjected to analysis and statistics to obtain the values of the concentrations of compounds required to reduce cell viability by 50 percent for each cell-line [18].

3.8.1 Cytotoxicity of PP/ Pt nanocomposite

The MTT assay was performed to evaluate the toxic effect of [PP/Pt] nanocomposite on cells. On the breast cancer cell-line MCF-7 compared with the normal cell-line HdFn (Human Dermal Fibroblasts, neonatal) MTT assay was performed to calculate cell viability and rate of inhibition using six different concentrations and three copies for each concentration. It is also natural to compare them. The toxic effect of [PP/Pt] on cells was shown at the six concentrations from 15.60 to 500.00 $\mu\text{g/ml}$ on MCF-7 and HdFn. As shown in **Table (3)** there was a decrease in the capacity and viability (Mean)of the cell when the concentration of [PP/Pt] was increased.

Table 3: Cytotoxicity effect of [PP/Pt] on MCF-7 and HdFn cells

Concen.	MCF-7		HdFn	
	Mean	mistake percentage	Mean	mistake percentage
500.00	62.19	± 2.14	73.30	± 0.88
250.00	75.77	± 2.64	83.26	± 0.97
125.00	87.62	± 1.22	88.95	± 5.05
62.50	92.90	± 0.93	95.56	± 1.43
31.20	96.30	± 1.95	95.29	± 1.00
15.60	96.30	± 1.10	96.33	± 0.68

Noted by 500.00 $\mu\text{g/ml}$ the decreasing in MCF-7 cell viability (62.19 ± 2.14) while the highest viability of cell MCF-7 at 15.60 $\mu\text{g/ml}$ reached to (96.30 ± 1.10) and the value of the toxic activity on the cells IC_{50} was 306.70 $\mu\text{g/ml}$, while on the normal cell-line HDFn, an IC_{50} value equal to 362.60 $\mu\text{g/ml}$ was obtained from the effect of [PP/Pt]. **Figure (7)** shows this.

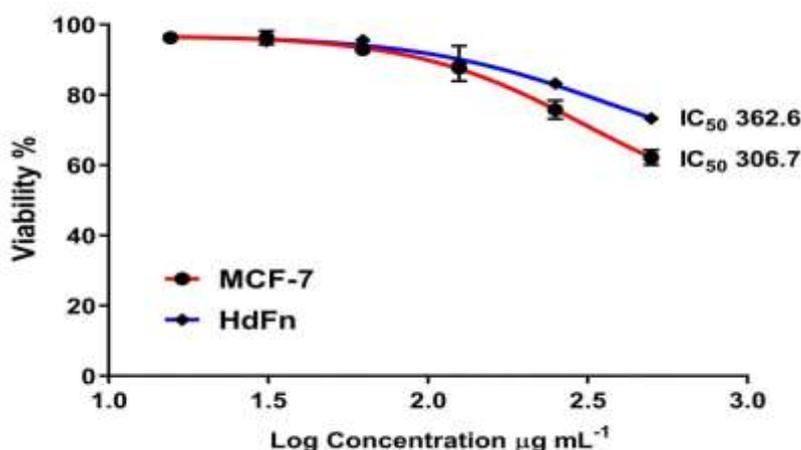


Figure 7: Cytotoxicity effect of [PP/Pt] on MCF-7 and HdFn cells

4. Conclusion

The Pt (NPs) was synthesized by using chloroplatinic acid solution and tungsten oxide nanorods, and then the nanocomposite was prepared by mixing it with polypropylene dissolved in xylene. The results of the measurements of the AFM technique, showed that the composite was synthesized as nanoparticles, where the dimensions and particle sizes of this nanocomposite were less than 100 nm, it was also observed that the grooves in the nanostructures are not homogeneous by the 3D images. Also, the SEM technique demonstrated the formation of metallic nanoparticles as a coating layer over and between polypropylene. EDX, FTIR and XRD were performed, Pt nanoparticles come in Cubic shape. The anti-bacterial and anti-fungal activity were investigated, knowing the growth retardation area of microorganisms. The antioxidant activity against free radicals was measured and gave a good scavenging ratio. For synthesized nanocomposite studied the cytotoxicity of had been against breast cancer (MCF-7) and shown to give acceptable results.

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