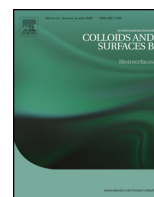




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Cytotoxic effects of platinum nanoparticles obtained from pomegranate extract by the green synthesis method on the MCF-7 cell line

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ABSTRACT

The study utilizes monodisperse platinum nanoparticles (Pt NPs) biosynthesized from *Punica granatum* crusts as anti-tumor agents on the human breast cancer cell line, MCF-7. The obtained Pt NPs were fully characterized using the UV–vis spectrum (UV–vis), transmission electron microscopy (TEM), X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM) and Fourier transformation infrared spectroscopy (FTIR). Effectiveness of the Pt NPs was determined by cell viability, propidium iodide staining test, flow cytometry and comet tests on the MCF-7 cancer cell line. Cell survival percentage was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The biosynthesized monodisperse platinum nanoparticles inhibited MCF-7 proliferation with an IC₅₀ of 17.84 µg/ml after 48 h of incubation. Propidium iodide staining demonstrated that the monodisperse Pt NPs induced apoptosis by means of molecular DNA fragmentation.

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1. Introduction

Nanobiotechnology, a new area of nanoscience, utilizes nanobased-systems for diverse interdisciplinary medical biology applications [1–26]. This fast-developing field of nanoscience has raised the possibility of using therapeutic nanoparticles in the diagnosis and treatment of human cancers [1]. According to worldwide statistics produced by the International Agency for Research on Cancer (IARC), approximately 7.6 million fatalities occur due to cancer, and 12.7 million new cancer cases are diagnosed [2]. Breast cancer is the most commonly diagnosed cancer type in women worldwide.

Nanoparticles will soon have an important place in nanoscience because of their environmentally safer biosynthesis and their potential to develop reliable biomedical applications [15]. Noble metal nanoparticles such as gold, silver, palladium, and platinum have a wide range of applications, including material science, chemistry, medicine and pharmaceuticals [29–35]. Among them, platinum has some specific properties such as high surface area

and good resistance to corrosion and chemical attacks. Up to now, several methods were investigated for the synthesis of Pt NPs such as chemical precipitation, vapor deposition and sol–gel route. However, these methods have some limitations, including using unsafe and toxic chemicals, multi-step preparation process, high cost and high energy requirements. To overcome these limitations, more recently, plant-mediated synthesis of noble metal nanoparticles, specifically Pt NPs, have been gaining attention due to their non-toxic, simple usage and eco-friendly nature. For this purpose, recently, several plant extracts were applied to produce Pt NPs, including *Diopyroski kaki*, *Cacumen platycladi*, *Quercus glauca*, *Prunus x yedoensis* tree gum, water hyacinth, *Azadirachta indica* and *Cochlospermum gossypium* [32,33]. The utilization of plants for the synthesis of Pt NPs rely on the fact that the process is faster, easier, eco-friendly, reliable and cost effective and forms more stable synthesized particles than other, classical methods [20]. Among these plants, *Punica granatum* has a wide distribution in Turkey. *P. granatum* shows anti-inflammatory, anti-bacterial [21] and anti-diabetic activity [22] and contains anti-inflammatory agents [23], antioxidants [24] and anti-HIV components [25]. Additionally, its leaves and flowers cause snake toxin neutralization [26] and larvicidal activities [27]. Recently, Arulvasu et al. stated that *P. granatum* shows cytotoxic activity in a human breast cancer cell line [28]. By

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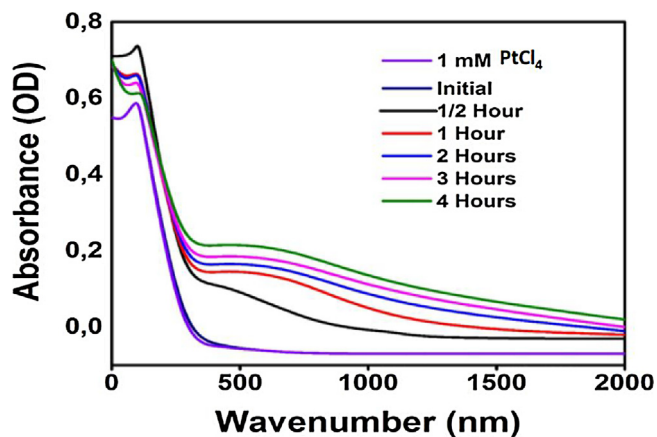


Fig. 1. UV spectral peaks of synthesized Pt NPs at different hours. (For interpretation of the references to colors in the figure legend, the reader is referred to the web version of the article.).

the reference of this evidence, we have examined the green synthesis of monodisperse Pt NPs from *P. granatum* crusts using an ethanol/water extraction protocol in an ultrasonic condition and investigated its in vitro cytotoxic effects against the MCF-7 cell line.

2. Materials and methods

2.1. Preparation of *P. granatum* extracts

P. granatum was collected from pomegranate orchards in the local surroundings of Bartın, Turkey. Fresh crusts were dried by shading and powdered at room temperature to extract its active

ingredients. Briefly, 15 g of pomegranate powder was weighed and placed in 250 mL of ethanol/water solvent for 4 h with a Soxhlet brand extruder. The mixture was filtered and obtained extract was left to cool down to 4 °C.

2.2. Green synthesis of platinum nanoparticles

Biological synthesis of Pt NPs was performed by using the following procedure. Briefly, 10 mM PtCl₄ solution was added to 220 mL pomegranate extract and executed in ultrasonic conditions using an ultrasonic tip sonicator. The solution was magnetically stirred at room temperature for 24 h. A color change was observed from yellow to brown, which indicated the formation of platinum nanoparticles. The obtained Pt NPs was purified by centrifugation at 4 °C for 30 min at 15,000 rpm, which was repeated two times. Next, the final pellet obtained was washed three times with ethanol and dried in a vacuum oven.

3. Results and discussion

3.1. Synthesis and characterization of biosynthesized monodisperse Pt NPs

The present study investigates the synthesis of uniformly dispersed Pt NPs from the ethanol/water extract of *P. granatum* crusts and their in vitro cytotoxic effects against a human breast cancer cell line (MCF-7). In previous studies, pomegranate plants have been shown to contain essential oils and resins with distinct phyto-molecule odor [34]. On the other hand, they also contain various alkaloids, glycosides, flavonoids, phenolic compounds, reducing sugars, resins and tannins [19,31]. After the completion of Pt NP synthesis, Pt NPs were kept for 4 h to evaluate and observe the formation of intense yellow-brown color. The color change at different

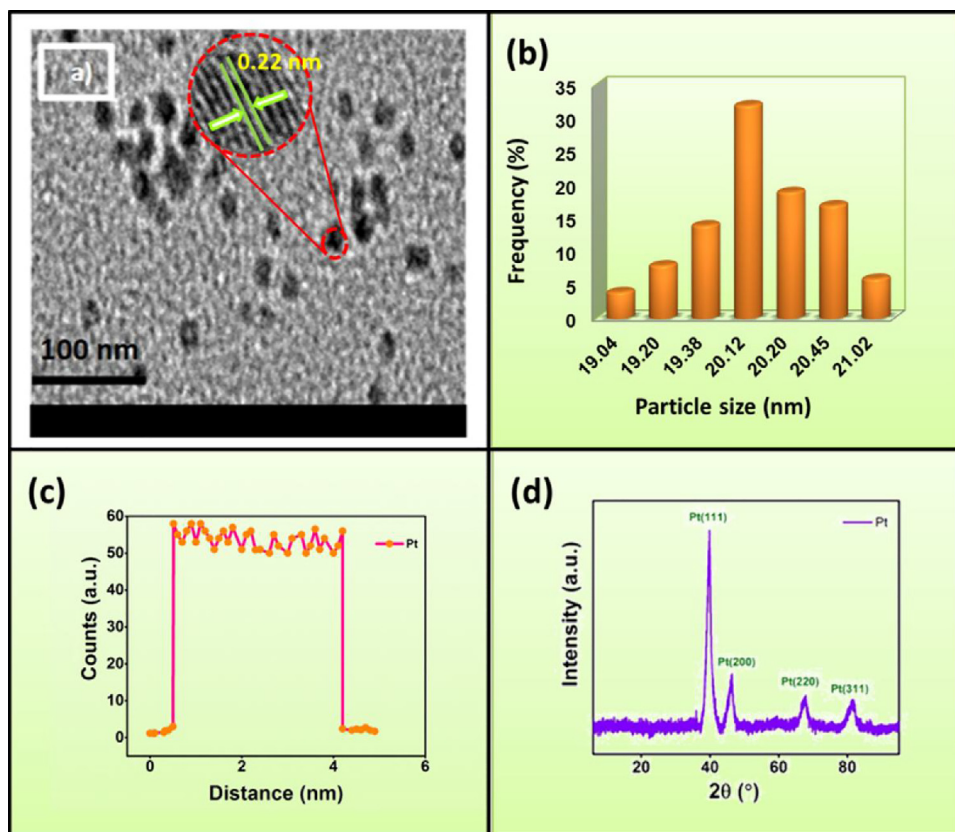


Fig. 2. (a) Representative TEM image and HRTEM image, (b) particle size histogram (c) EELS line profile, and (d) XRD of Pt NPs.

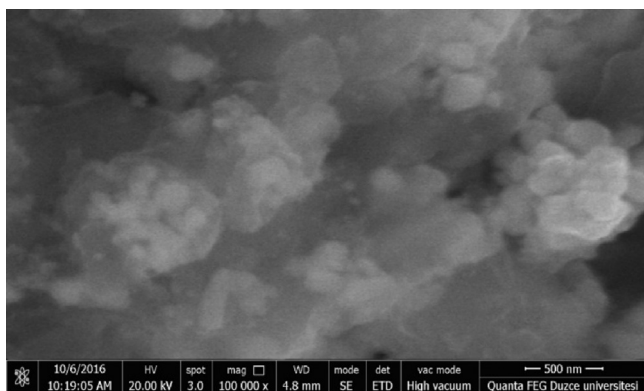


Fig. 3. FESEM analysis of Pt NPs confirming the cubical and spherical shaped particles.

time points was confirmed within 4 h by obtaining UV spectral data with the supporting spectral band peak at different hours as shown in Fig. 1.

The newly biosynthesized monodisperse Pt NPs have been characterized by using several different techniques such as XRD, TEM, HRTEM and EELS. The TEM measurements were applied to determine the particle morphology and size of the prepared Pt NPs. As illustrated in Fig. 2a and b, the particles have a spherical shape, and the particle size is well distributed with an average particle size of 20.12 nm. The atomic lattice fringes of the prepared Pt nanoparticles were observed to be 0.22 nm, which is exactly same as the nominal Pt value in the literature [36,37]. In addition, the EELS line profile also indicates the existence of Pt nanoparticles in prepared composites as seen in Fig. 2c. In previous studies, TEM images for biosynthesized platinum nanoparticles have been reported to have spherical shapes with sizes 20–100 nm [36,37]. Furthermore, the recorded XRD pattern for biosynthetic platinum nanoparticles clearly shows intense peaks. The XRD results of this study clearly show that platinum nanoparticle crystals were formed by the reduction of Pt^{+4} ions using *P. granatum* leaf extraction (Fig. 2d). As shown in Fig. 2d, the obtained Pt NPs are crystalline and show four distinct diffraction peaks at 39.9, 46.2, 67.3 and 82.1°, which could be assigned to the (111), (200), (220) and (311) planes of face-centered cubic Pt NPs, respectively. The XRD data were in good agreement with the results of Dasdelen et al. [36] and Karatepe et al. (2016) [37]. On the other hand, the field emission scanning electron microscopy (FESEM) was used to determine the in-depth morphology and size of synthesized Pt NPs. The images showed the presence of cubes and spherical structures, which are in good agreement with the TEM results (Fig. 3).

3.2. Cell viability test

Biosynthesized Pt NPs from the pomegranate extract were evaluated for cell viability at different concentrations in a human breast adenocarcinoma cell line (MCF-7) using the MTT assay. As presented in Fig. 4, the biosynthesized Pt NPs showed a dose-dependent decrease in cell viability at different concentrations (2.5, 5, 10, 20, 40 and 50 $\mu\text{g}/\text{ml}$) by showing a significant difference between the control and treated groups. Moreover, increasing the period of incubation time (from day 1 to day 3) further reduced cell viability, which demonstrates the efficiency of the biosynthesized Pt NPs. A decrease of cell viability by almost 80% was observed for 2.5 $\mu\text{g}/\text{ml}$ to 50 $\mu\text{g}/\text{ml}$ of Pt NPs at 72 h. The results indicate that the breast adenocarcinoma cell line MCF-7 is very sensitive to biosynthesized Pt NPs (Fig. 4).

3.3. Cytomorphology observations

A Carl Zeiss light inverted microscope was used for monitoring microscopic observations. Using this method, the untreated MCF-7 cells were compared with MCF-7 cells treated with two different concentrations of biosynthesized Pt NPs (25 and 100 $\mu\text{g}/\text{ml}$) as shown in Fig. 5. The images show that the control cell shapes were round and polygonal and that they formed irregular confluent aggregates. On the other hand, cells treated with biosynthesized Pt NPs had narrow and spherical shapes, and cell diffusion plates were restricted when compared to those of the control. Cell diffusion improved when the concentration increased from 25 $\mu\text{g}/\text{ml}$ to 100 $\mu\text{g}/\text{ml}$ (Fig. 5c). The obtained results for biosynthesized Pt NPs are compatible with data from the literature, which show the successful design of our current work [32,33].

3.4. Propidium iodide staining

Nuclear densification and apoptotic changes were examined by the propidium iodide staining method to ensure the anti-proliferative effect of biosynthesized Pt NPs from the *P. granatum* crust extract. In the control cells, there were only few cells that positively responded to propidium iodide (Fig. 6a). In the case of cells treated with 25 $\mu\text{g}/\text{ml}$ and the maximum concentration of biosynthesized Pt NPs with 48-h exposure times, a gradual front increase was observed in the number of cells that positively responded to propidium iodide (Fig. 6b and c). [38]. These data display that monodisperse biosynthesized Pt NPs scan the number of apoptotic cells in MCF-7 cell line, which suggests that this biosynthesized Pt NPs could induce cell apoptosis (Fig. 6).

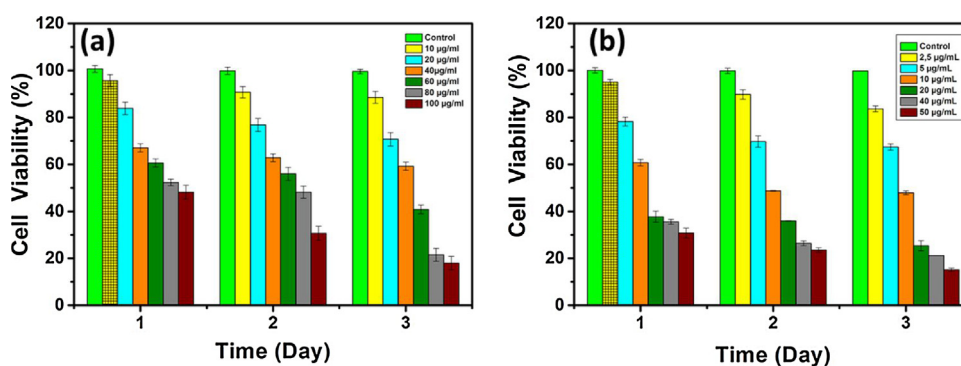


Fig. 4. In vitro cytotoxicity of (a) pomegranate peel and (b) Pt NPs against MCF-7 cell line. Data represented are mean \pm SD of five identical experiments made in three replicates.

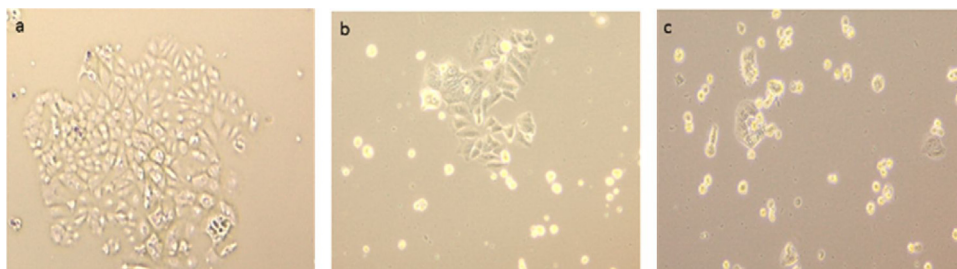


Fig. 5. Morphology of control and Pt NPs treated MCF-7 breast cancerous cells (40 × Magnification) (a) Control; (b) IC₅₀ molarity (25 µg/ml); (c) Maximum molarity (100 µg/ml).

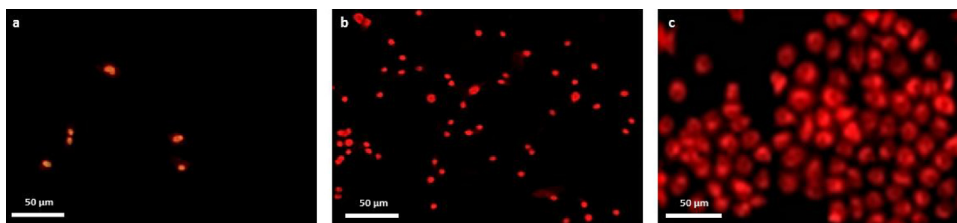


Fig. 6. Propidium iodide staining of MCF-7 cells in the reference and Pt NP-treated groups (20 × Magnification) (a) Control; (b) IC₅₀ molarity (25 µg/ml); (c) Maximum molarity (100 µg/ml).

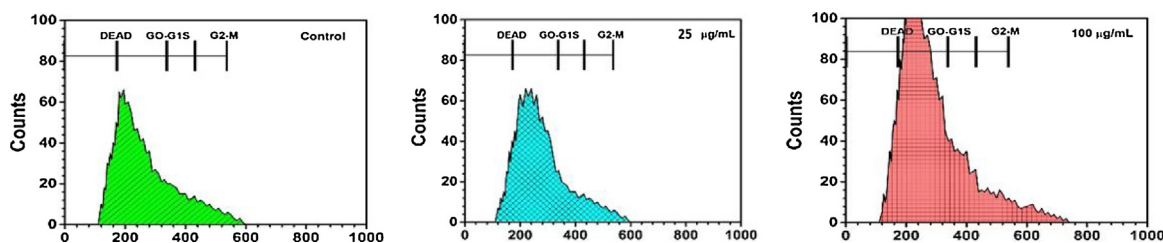


Fig. 7. Analysis of treated-MCF-7 cells in their cell cycle. Demonstrated cell population representative histograms in their DNA content, defined by propidium iodide staining. (a) Control; (b) IC₅₀ molarity (25 µg/ml); (c) Maximum molarity (100 µg/ml).

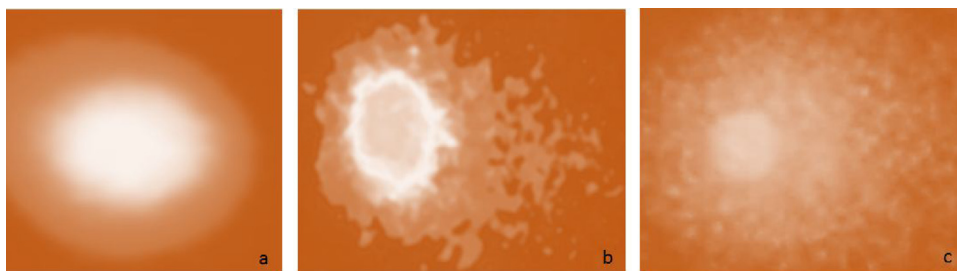


Fig. 8. Analysis of apoptosis-inducing effect of Pt NPs on MCF-7 cells by comet assay assessment. (a) Control; (b) IC₅₀ molarity (20 µg/ml); (c) Maximum molarity (100 µg/ml).

3.5. Pt NPs intervene MCF-7 cell cycle control

Flow cytometry was performed to investigate the role of oxidative stress in inducing DNA damage and the cell cycle distribution in MCF-7 cell lines for the biosynthesized Pt NPs. As shown in Fig. 7, incubation of Pt NPs with MCF-7 cells for 48 h significantly reduced DNA content by showing apoptosis in the part of G0/G1 region, indicating that G1 phase cells were lost by programmed cell death [39].

3.6. Comet assay

The apoptosis-inducing effect and DNA damage analysis of biologically expressed monodisperse Pt NPs on MCF-7 cells was performed by comet assay (Fig. 8). Any DNA damage observed

was as shown in the cell nuclei of the control group (Fig. 8a), and the nuclei were slightly changed as biosynthesized Pt NPs were applied at the concentration of 25 µg/ml (Fig. 8b). However, significant change was observed when the concentration of Pt NPs was increased to 100 µg/ml (Fig. 8c). Previous studies have found that Pt NPs increase DNA tail extension in a comet analysis as a measure of DNA breakage, as well as existence of alkaline labile regions [40,41]. It has also been reported that the stimulation of DNA single strand breaks is frequently used to predict the degree of oxidative depredation of tumor cells [42–45].

4. Conclusion

In conclusion, the Pt NPs were successfully biosynthesized from *P. granatum* crust extracts and fully characterized by using several

methods such as TEM, HRTEM, FESEM, EELS, XRD and UV–vis techniques. Cell viability test, morphology studies, propidium iodide staining test, flow cytometry and comet tests were performed to show the efficiency of biosynthesized Pt NPs on the human breast adenocarcinoma cell line MCF-7. The prepared Pt NPs showed an efficient anti-proliferation effect on the MCF-7 breast cancer cell line and induced apoptosis. The results demonstrate that Pt NPs induce apoptosis through G0/G1 cell cycle arrest. It is clear that DNA damage results from the indexed oxidative pressure in the Pt-NP-transacted cells. As a result, we can comment that biologically synthesized platinum nanoparticles can be used for the treatment of cancer cells and can be further exploited for the administration of drugs via conjugation with metabolites.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.colsurfb.2017.12.042>.

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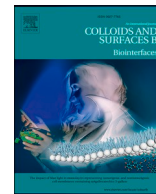
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Corrigendum to “Cytotoxic effects of platinum nanoparticles obtained from pomegranate extract by the green synthesis method on the MCF-7 cell line” [Colloids Surf. B: Biointerfaces 163 (2018) 119–124]

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The authors regret that Fig. 2 has been published erroneously. Therefore, the authors would like to replace it with the correct one given below. In current version, the mean particle size of prepared nanoparticle is about 6 nm as shown in TEM image. This does not alter the discussion. The authors confirm that this change does not affect the

originality and importance of the scientific findings reported in the paper. The authors would like to apologise for any inconvenience caused.

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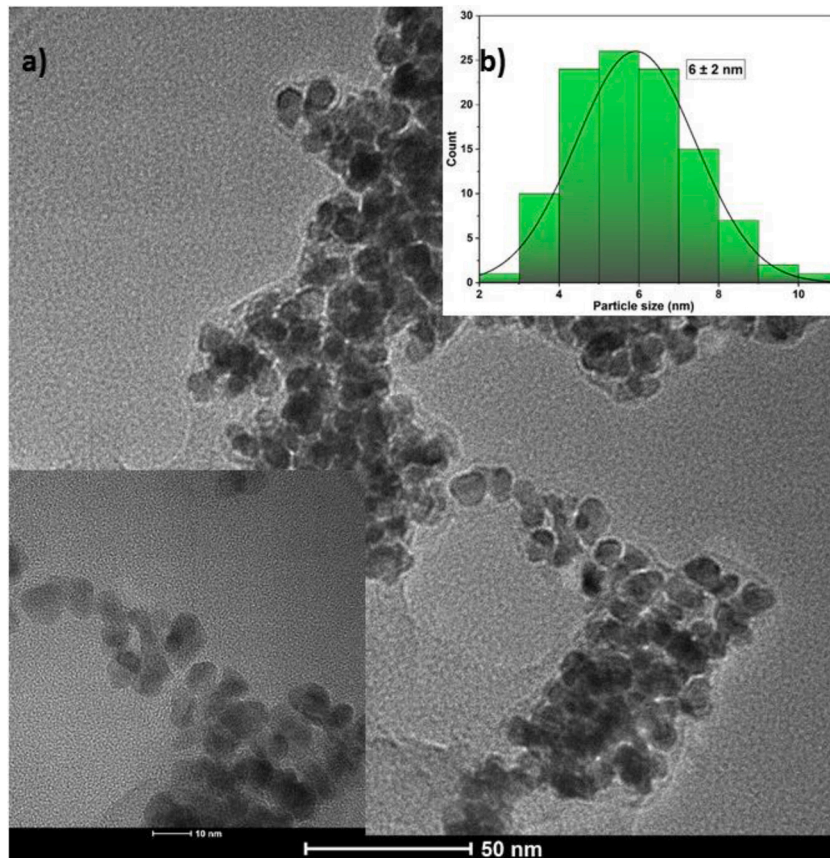


Fig. 2. (a) Representative TEM image and HRTEM image, (b) Particle size histogram of Pt NPs.