RESEARCH ARTICLE

Study of Platinum Nanoparticles with Methotrexate as Drug Delivery System for Cancer Therapy on MCF7

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ABSTRACT

The present study aims to innovative nanotechnology that can overcome multidrug resistance (MDR), raise drug localization, and efficiency. This is done by applying a nanotechnology technique to cancer cells, which lead up to a new prospect for developing treatment methods on cancer cells. Platinum nanoparticles (PtNPs) was synthesized by the chemical approach in a range of different temperatures (180- 200-220-240-260°C), the characteristics of Pt NPs were measured using atomic force microscopy (AFM), UV-visible spectroscopy, zeta potential and dynamic light scattering (DLS). The cytotoxicity of Pt NPs was tested in vitro by applying it on MCF7 cell lines first as an anticancer agent, second as a drug delivery system by combining the nanoparticle with Methotrexate together, and third as an applied Methotrexate alone on cell lines. The effect of Pt NPs and Methotrexate was measured by MCF7 assay to evaluate their drug delivery activity and anticancer activity. The result of the MCF7 assay test shows a great potential of Pt NPs with a high inhibition rate of cancer cell growth.

Keywords: Drug Delivery System, Methotrexate, Platinum nanoparticles.

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INTRODUCTION

Cancer is one of the leading causes of morbidity and mortality worldwide, and it is expected to become the major cause of death in the coming decades.[1] Cancer is defined as a multifactorial disease involving a malignant growth of tissue (malignant tumor) that possesses no physiological function, and arises from an uncontrolled, usually fast, cellular proliferation.[2] The tumor can expand locally in the same tissue by cellular invasion and systemically to other organs, a process known as metastasis. In cancer, the cellular mechanisms that regulate gene expression and cell proliferation are altered, mostly due to mutations of the genetic material or other epigenetic modifications. The cell type and these alterations are what will mainly determine a tumor's growth rate and metastatic potential, and consequently, severity. However, other factors, such as the patient hormone profile or immune system characteristics can be determinant in the individual clinical development of cancer, increasing its intricacy and pledging for personalized treatments.[3] Besides, the considerable progress made in understanding the biological and molecular basis of cancer during the past 50 years has not been translated into a notable improvement of its incidence and mortality,[4] neither in the control of treatment-limiting side effects, also contributing to improper treatment compliance.[5] Therefore, efficient cancer therapies still remain elusive. Ideally, cancer treatments aim to entirely eliminate all tumor cells, minimizing side effects on the rest of the organism. Surgery, radiotherapy, and chemotherapy have been the main treatment approaches used in the past decades. Today, along with them, other forms of therapy as hormone therapy, immunotherapy, photodynamic therapy and targeted therapies complete the catalog of treatment modalities used in the clinic to fight cancer.

Nanoparticles (NPs) for cancer therapeutics are rapidly evolving and are being introduced in an attempt to overcome several limitations of conventional small-molecule chemotherapeutics. Though chemotherapy is successful to some extent in certain cancers, it has several limitations.[6,9]

Platinum nanoparticle's anticancer agents represent one of the great success stories in the field of medicinal inorganic chemistry; they highlight the confluence of serendipity and rational design in drug development; Platinum complexes remain among the most widely used anticancer chemotherapeutics.[7,8]

In the present work, the synthesis of platinum nanoparticles then select the suitable type of cell to correspond chemotherapy, applying nanoparticles, and chemotherapy and combine Pt NPs with chemotherapy on the cell line. Evaluating the results by MCF7 assay and studying the morphology of the cancer cells also done.

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Experimental Procedure

Synthesis of Platinum nanoparticles

Platinum nanoparticles were synthesized by chemical reaction at different specimens of Pt NPs were prepared in a high-temperature range (180, 200, 220, 240, 260°C refers to sample 1 to 5 respectively) by using Hexachloroplatinic acid (H2PtCl6) with high purity (99.99%) and molecular weight (517.90g/mol) dissolved in distilled water then add 0.05 mL to 15mL reducing agent (ethylene glycol) (62.07g/mol) mixed with 0.5g of polyvinylpyrrolidone (PVP) (40000g/mol) that stabilize the nanoparticles.

Synthesis of Human Breast Cancer cell line (MCF7)

In this study we use the MCF-7 cell line which is human cell line derived from breast carcinoma, the steps of MCF-7 cell line preparation as follow:

- The growth medium was poured, and the cell sheet was washed once by adding (2mL) of a trypsin-overseen solution, after that the flask rocked gently. Part of it will pour again to obtain about (1mm) of a trypsin-overseen solution, which covers the cell surface, then the cell incubated for 1–2min at 37°C until the cells had separated from its flask.
- Complete media were added to the flask, and the cells were re-incubated at 37°C for 24h, to be ready for applying the viability test. Figure 1 demonstrates the MCF-7 cell culture.

UV-Vis spectra (DR 500 UV-Visible Spectrophotometer) used to detect the spectrum of the prepared Pt. The AFM (Augestrom advance inc., USA) was used to determine the particle size and the homogeneity of prepared Pt NP and dynamic light scattering (DLS) was used effect of diameter. Finally, the Zeta potential (Brookhaven, USA) was used to study the stability of Pt NP.

RESULTS AND DISCUSSION

Platinum Nanoparticle Analysis

UV-visible spectroscopy

Figure 2 shows the UV-vis spectrums of the prepared Pt NPs. There is one peak that refers to Nanosphere Pt located at 256 nm. The curve refers to the arrangement of the diameters of the nanoparticles and the width of the curve related to the variety of diameters.

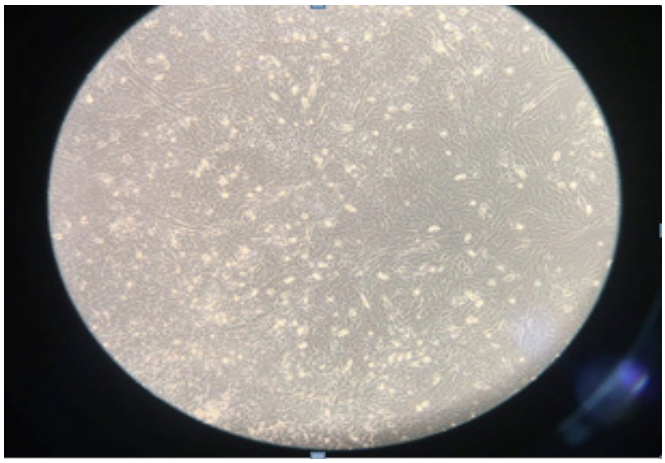

Figure 1: Demonstrate the Human Breast Cancer cell line (MCF7)

Zeta potential

It is an abbreviation for the electro-kinetic potential in colloidal systems. It's the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle.

Guidelines are classified NP-dispersions with zeta potential rates of [± (0–10mV) ± (10–20mV) ± (20–30 mV) and (± 30mV)] as highly unstable, relatively stable, moderately stable and highly stable respectively of Pt NPs with different prepared temperature. The samples 1 showed the stability was from (-44.22mV) to (-50.74mV), which means that the Pt NPs are highly stable with a negative magnitude, which confirmed that the PH is basic, as shown in Figure 3. The zeta potential

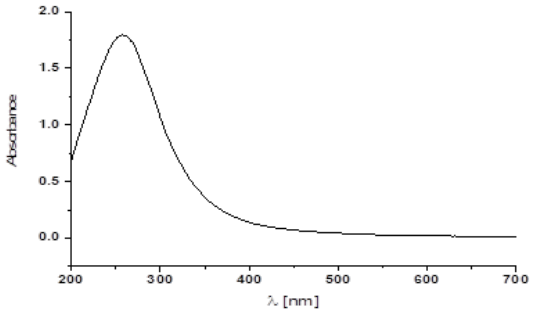

Figure 2: UV-Visible Spectrum of Platen Nanoparticle

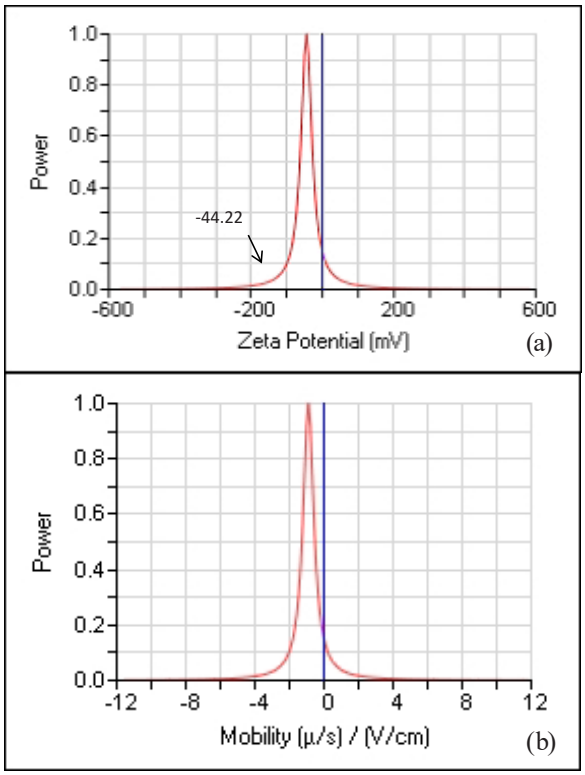

Figure 3: a) Zeta potential and b) Mobility of Platinum nanoparticles for sample 1.

variance with pH and becomes more positive and negative in magnitude with acidic pH and basic pH, respectively. The samples show a negative magnitude, which means that the pH is basic.

Dynamic light scattering (DLS)

The DLS was recorded for Pt NP and the result showed in Figure 4. The grain size distribution ranged from 10 nm to 200 nm, while the highest number was 55 nm, as shown in Figure 4.

Atomic force microscopy (AFM)

Figure 5 shows the two and 3D dimensions of samples. It shows the chart of granulite cumulating destitution explains the distribution of particles according to the diameter, which that shows that the synthesized Pt NPs were spherical in shape and average size (8nm).

Results of Cytotoxic effect of Platinum nanoparticles

This result manifest the effect of Platinum nanoparticle and methotrexate on cancer cells by using MTT assay as follows:

Platinum nanoparticles effects on cancer cells

During applying the Platinum nanoparticles alone to the MCF7 cell line, different inhibition rate was demonstrated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT assay). As shown in Table 1: Inhibition Rate percent five Samples with two different concentrations of Platinum Nanoparticle.

From the results of Table 2, the cytotoxic effect was observed for Pt NPs at different concentrations that were applied to the MCF7 cell line alone. The less concentration gives high inhabitation rate of Pt NPs; this can be attributed to small particle size and a large distribution surface of Pt NP that promotion to act on the MCF7 cell line leads to a high Inhibition rate. Figure 6 illustrates the mean values of inhibition rate percentage (IR %) of five different samples of platinum nanoparticles.

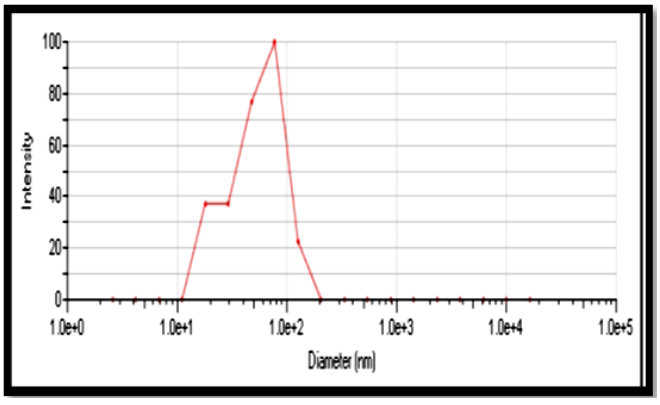

Figure 4: Dynamic light scattering of platinum nanoparticle

Table 1: Inhibition Rate (%) of Different Specimens of Platinum Nanoparticles

Concentration mg/ml	*Inhibition rate %*				
	Sample 1	*Sample 2*	*Sample 3*	*Sample 4*	*Sample 5*
0.0386	49.03	61.21	51.92	23.07	50.96
0.0193	57.05	65.38	65.06	65.38	65.7

Methotrexate effect on cancer cells

Applying the chemotherapy (methotrexate) alone on the MCF7 cell line with different concentrations gives the following growth inhibition effect as mentioned in shown in Table 2,

Table 2 shows the mean values of inhibition rate percentage (IR %) Induced by different concentrations of Methotrexate (anticancer drug) it acts on the MCF7 cell line. It works by killing or damaging the cancer cells so that they cannot be

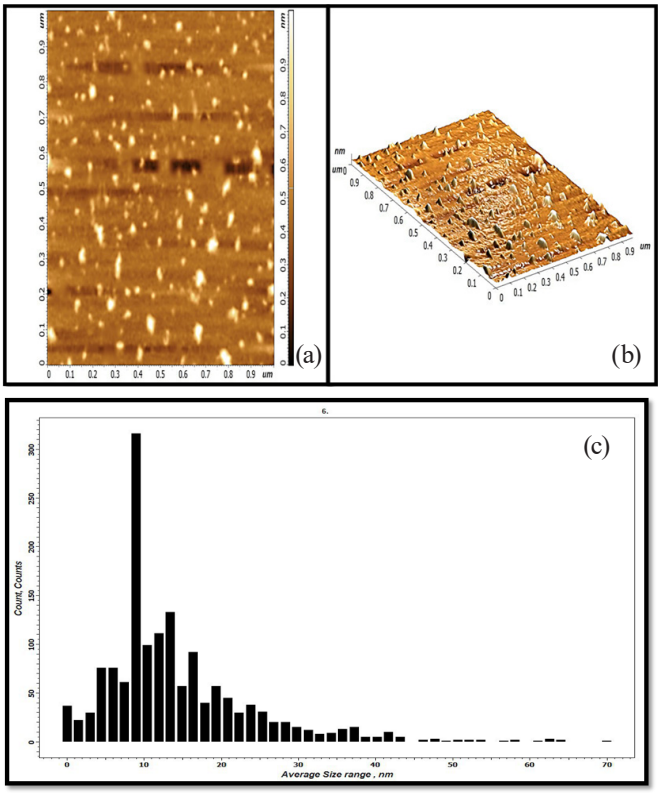

Figure 5: AFM results of platinum nanoparticles, a) 2-D, b) 3-D, c) distribution of grain size of Pt NP.

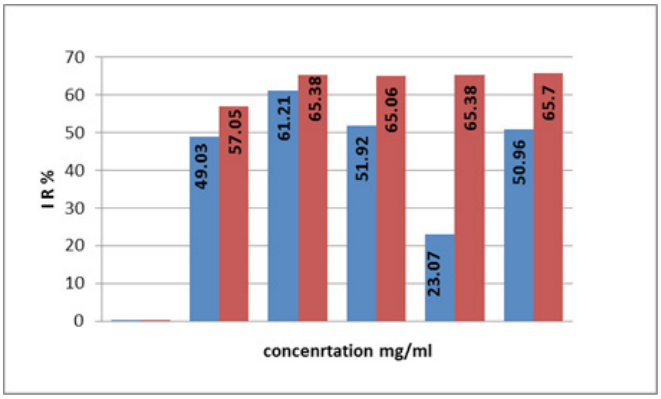

Figure 6: Chart for the Mean Values of Inhibition Rate Percentage (IR %) Induced by Five Different Samples of Platinum Nanoparticles

Table 2: Inhibition rate (%) of different concentrations of methotrexate

Concentration mg/mL	*Inhibition rate %*
5	40.38
2.5	44.23
1.25	58.97
0.625	54.48

spread and make more cancer cells. The MTT assay results show the higher concentration of the anticancer drug gives a higher inhibition rate. Also, Figure 7 illustrate the mean values of inhibition rate percentage (IR %) of methotrexate.

The platinum nanoparticles with methotrexate effect on cancer cells

Both Pt NP and methotrexate, which were applied on MCF7 cell line with different concentrations, and the result is shown in Table 3.

It has shown from Table 3 that the inhabitation rate of Pt NPs at temperature 180 and 200°C (samples 1 and 2) combined with methotrexate after applied on the MCF7 cell line acts as drug delivery. It also gives a high inhibition rate comparing with the Pt NPs prepared in the temperature range of 200 and 240°C (samples 3 and 4), which provide an indication of the strong binding of the Pt NPs with the anticancer drug.

CELL MORPHOLOGY RESULTS

The morphological images of the previous results are demonstrated in this section as follows:

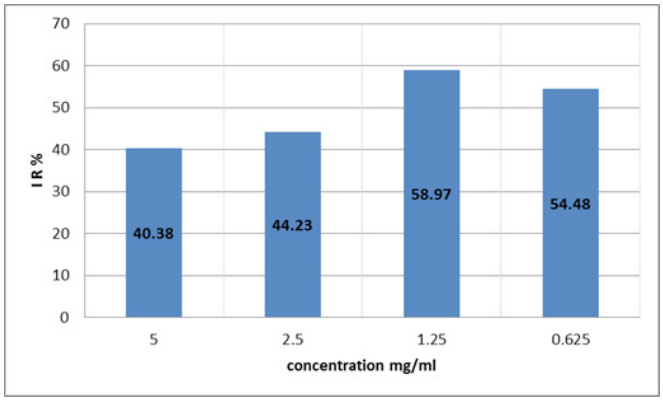

Figure 7: Chart for the Mean Values of Inhibition Rate Percentage (IR %) Induced by Different Concentrations of Methotrexate

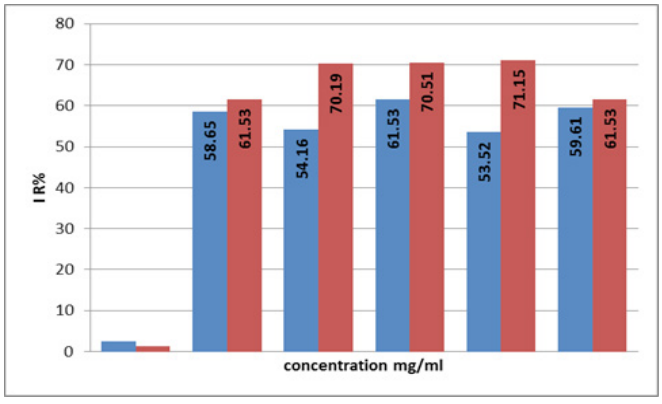

Figure 8: Chart for the Mean Values of Inhibition Rate Percentage (IR %)

Cell Morphology of Platinum Nanoparticles Results

After incubation for 24h at 37°C, Platinum nanoparticles with the MCF7 cell line at different concentrations (0.0386, 0.0193 mg/ml) were examined under a microscope to see the changes that occur on the cells that are shown in Figure 9. The changes

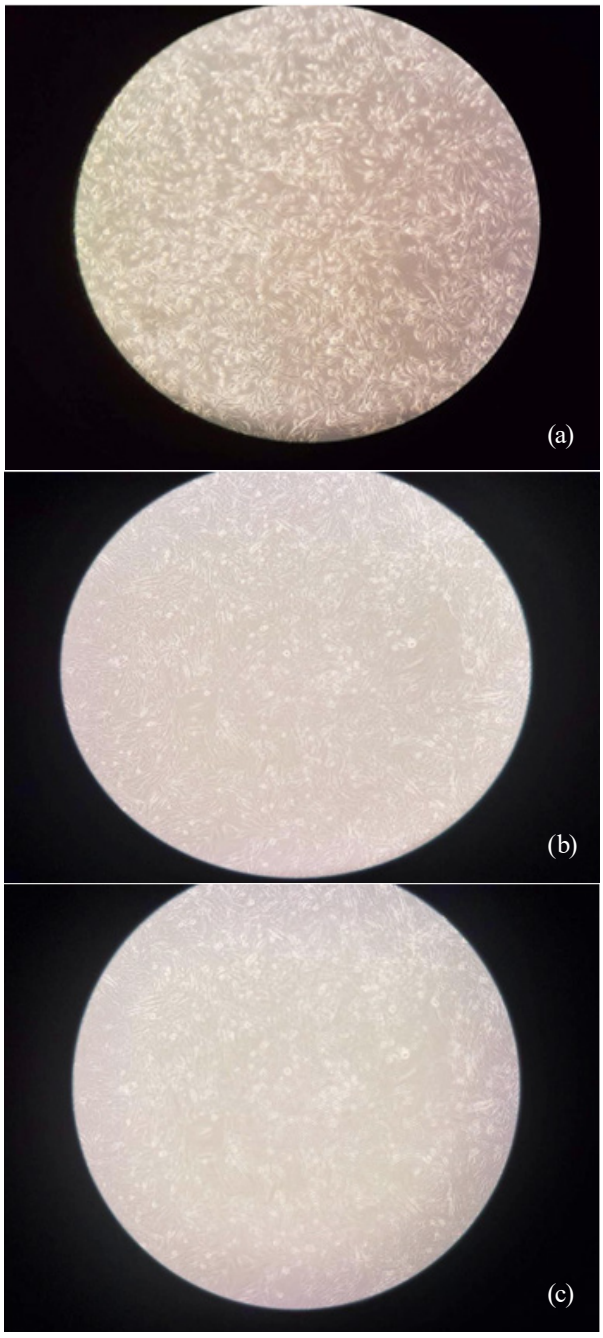

Figure 9: Microscopic Changes in Cell After Applying Pt NP : (a) cell control, (b) cell exposed of 0.0386mg/ml Pt NP, (c) cell exposed of 0.0193 mg/ml Pt NP

Table 3: Inhibition Rate(%) of Different Specimens of platinum Nanoparticles with Methotrexate

Concentration	*Inhibition rate %*				
mg/ml	*Specimen1 + M*	*Specimen2 +M*	*Specimen3+M*	*Specimen4 +M*	*Specimen5+M*
2.5386	58.65	54.16	61.53	53.52	59.61
1.2693	61.53	70.19	70.51	71.15	61.53

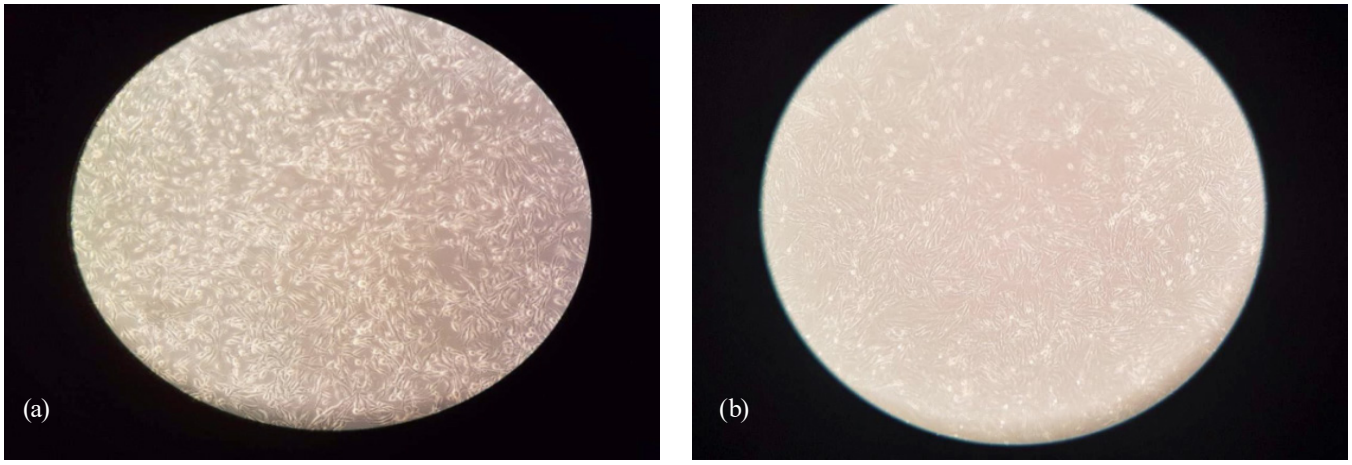

Figure 10: changes in cell morphology after applying methotrexate, (a) MCF7 cell line does not rate with Methotrexate, (b) MCF7cell line after exposing to Methotrexate (1.25mg/mL)

in the cell morphology after adding Pt NP with decreasing concentrations give higher inhibition rate of cell growth, cell shrinkage, and changes in cell number according to the applied sizes and concentrations of Pt NP.

Cell changes result in damage by using Methotrexate

By adding the methotrexate alone to MCF7 cell and incubated for 24h at 37°C, cells were examined under an optical microscope, and the result is shown in Figure 10. Figure 10 shows the variation of cell morphological after adding methotrexate (1.25 mg/mL). It also showed a decrease in the number of the cell compared with cell control. The increasing of drug concentrations manifested increasing of inhibition rate but at the same time cause more damage to the healthy tissue that surrounds the cancer cells, as shown in Figure 10.

CONCLUSION

Pt NPs were successfully synthesized chemically at a range of temperatures from 180 to 260°C. The Pt NP is proved by UV-Vis spectroscopy, zeta potential, Dynamic Light Scattering (DLS), and atomic force microscopy. The effect of adding Pt NP on the MCF7 cell line has increased inhibition rate with decreasing concentrations of Pt NP. Comparing these results with ranges of inhibition rate when adding different concentrations of methotrexate on the MCF7 cell line confirmed that the Pt NP could be used as an anticancer drug. In the next step, the Pt NP was combined with methotrexate and adding to the MCF7 cell line with different concentrations. Results has shown a higher Rinhibition rate comparing with the inhibition rate of methotrexate alone; this result proved that the Pt NP could be used as drug delivery system for loading the chemotherapy and delivering it to cancer cells, which maximize the number of cell death and minimize damage of healthy tissues that surround the cancer cells.

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