

ORIGINAL ARTICLE

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Evaluation of synthesized platinum nanoparticles on the MCF-7 and HepG-2 cancer cell lines

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Abstract

Platinum nanoparticles (PNPs) were synthesized by chemical reduction of potassium hexachloroplatinate (IV) with trisodium citrate under vigorous stirring and addition of sodium dodecyl sulfate as stabilizer reagent. Reducing agent was chosen depending on the oxidation reactions and potential values of the chemical materials used in the experiment. The aim of this study is to investigate the effects of PNPs on the different cancer cell lines and cytotoxicity study of this nanomaterial. The morphology of PNPs was investigated by scanning electron microscope (XL30, Philips Electronics, Amsterdam, The Netherlands) with the ability to perform elemental analysis by EDX. Malvern Zetasizer 3000 HSA (Malvern Instruments, Worcestershire, UK) was used to determine the distribution of particle size and zeta potential of PNPs. The cytotoxicity property of the nanoparticles was evaluated by MTT assay on MCF-7 and HepG-2 cell lines, and the cytotoxic concentration 50% values were determined for 24 h.

Keywords: Platinum nanoparticles, Potassium hexachloroplatinate, Chemical reduction, MTT assay, MCF-7, HepG-2

Background

In recent years, the interest in the synthesis and properties of metal nanoparticles has been increasing because of their unique properties and promising applications as catalysts, ferrofluids, and semiconductors [1,2]. Nanotechnology is the most promising field for generating new applications in medicine. However, only few nanoproducts are currently in use for medical purposes [3]. The most prominent nanoproduct is PNPs. PNPs are generally smaller than 100 nm and contain 20 to 15,000 platinum atoms. Nanoparticles are often in the range 1 to 100 nm, and this is the size as that of human proteins. Metal nanoparticles possess a very high surface-to-volume ratio. In biology and biochemistry, nanoparticles have attracted much attention. Especially, PNPs with size in the range of 10 to 50 nm are most attractive for practical reasons [2-6]. Cancer killed 7.9 million people worldwide in 2007 [7]. Breast cancer is one of the leading causes of women mortality worldwide. Chemotherapy is the only option for treating the malignant breast cancer and condition to increase the life

span of the patient [8]. Successful chemotherapy of cancer depends on the delivery of sufficient concentrations of an effective drug to tumor cells without causing intolerable toxicity to the patient. Platinum-based drugs are traditional cancer drugs used in chemotherapy to kill cancer cells. Consequently, an effective synthetic technique is required to produce nanoparticles with controlled shape and small size within a few Angström standard deviations. The usual synthetic technique for making such nanoparticles involves chemical or electrochemical reduction of metal ions in the presence of a stabilizer such as linear polymers [9-11] and ligands [12-16] which prevent the nanoparticles from aggregation and allow isolation of the nanoparticles. To control the particle size and shape, various reductants, stabilizers, solvents etc., have been utilized in nanoparticle preparation. The control of particle size and morphology has been extensively studied using different stabilizers [17-20]. MCF-7 and HepG-2 cells are a suitable *in vitro* model system for the study of polarized human hepatocytes. MCF-7 cells are useful for *in vitro* breast cancer studies because the cell line has retained several ideal characteristics particular to the mammary epithelium [21]. These include the ability for MCF-7 cells to process estrogen, in the form of estradiol, via estrogen receptors

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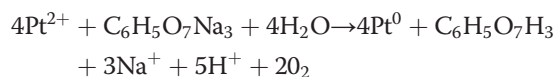
in the cell cytoplasm. This makes the MCF-7 cell line an estrogen receptor positive control cell line [22]. Because of their high degree of morphological and functional differentiation *in vitro*, HepG-2 cells are a suitable model to study the intracellular trafficking and dynamics of bile canalicular and sinusoidal membrane proteins and lipids in human hepatocytes *in vitro*. In this research, we want to synthesize stable PNPs by use of K_2PtCl_6 and a weak reductant agent such as trisodium citrate then evaluate them on the cell lines to determine cytotoxic concentration 50% (CC50).

Methods

Preparation of PNPs

Potassium hexachloroplatinate (IV) and trisodium citrate of analytical grade purity were used as starting materials without further purification. Platinum nanoparticles were obtained by chemical reduction of K_2PtCl_6 (approximately %38Pt, Sigma-Aldrich, MO, USA) with trisodium citrate (Sigma-Aldrich). All solutions of reacting materials were prepared in distilled water. In typical experiment, 50 ml of 0.001 M K_2PtCl_6 was heated to boiling point. To this solution, 5 ml of 1% trisodium citrate was added drop by drop. The solution was stirred by magnetic stirrer (RH-KT/C, IKA, Selangor, Malaysia) during the experiment. The temperature was controlled using a water bath (Ultratemp FP35-HC, Julabo GmbH, Seelbach, Germany). The solution was heated until color change is evident (bright yellow to black). Then, it was removed from the heating element and stirred until cooled to room temperature. Platinum precipitate was separated by centrifuge (NF 615, Nuve, Ankara, Turkey) at 3,000 rpm for 5 min. The precipitate formed by the metallic nanoparticles was washed several times [23] with deionized water and acetone, then put in acetone as a suspension. Then, the acetone and water in liquid phase was vaporized in an oven at 110°C.

Mechanism of reaction could be expressed as follows [5,6]:



Characterization methods

Morphological analysis and the measurement of the nanoparticle size were performed by scanning electron microscope (SEM). Malvern Zetasizer 3000 HSA (Malvern Instruments Ltd., Worcestershire, UK) was also used to determine the size and zeta potential of the platinum nanoparticles. This instrument allows the measurement of particle size distributions in the range above 2 nm and can also be used to measure the distribution of zeta potentials for dispersed particles in a similar size range. The zeta potential is determined by measuring the electrophoretic particle velocity in an

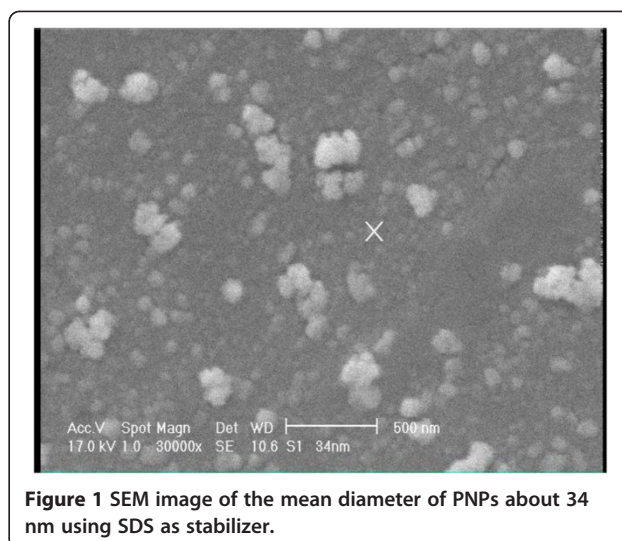


Figure 1 SEM image of the mean diameter of PNPs about 34 nm using SDS as stabilizer.

electrical field. During the particle movement, the diffuse layer is shorn off; hence, the particle obtains a charge due to the loss of the counter ions in the diffuse layer. This potential at the plane of shear is called the zeta potential. As this electric potential approaches zero, particles tend to aggregate.

Cell line and cell culture

MCF-7 and HepG-2 cell lines were obtained from the National Cell Bank of Iran. The cells were cultured in DMEM containing 10% FBS, 2 mM glutamine, antibiotics (penicillin G, 60 mg/l; streptomycin, 100 mg/l; amphotericin B, 50 µl/l) under a humid atmosphere (37°C, 5% CO₂).

Determination of cell viability and CC50

The cytotoxicity effect of PNPs on MCF-7 and HepG-2 cells was determined by MTT (Sigma, USA) assay. Briefly, 10⁴ cells/well were treated with various concentrations of PNPs 0, 1, 2, and 4 mg/ml. After the 24-h incubation, the cells were washed twice with phosphate buffered saline (PBS), and MTT (0.5 mg/ml PBS) was added to each well and incubated at 37°C for 3 h. The formazan crystals that formed were dissolved by adding

Table 1 Elemental analysis of PNPs by EDX ZAF quantification

Element	Wt%	At
F (k)	0.15	1.40
Na (k)	0.41	3.11
K (k)	1.90	8.47
Pt (L)	97.53	87.02
Total	100.00	100.00

k and L refer to level energy of X-ray that reflected from the nanoparticle surface.

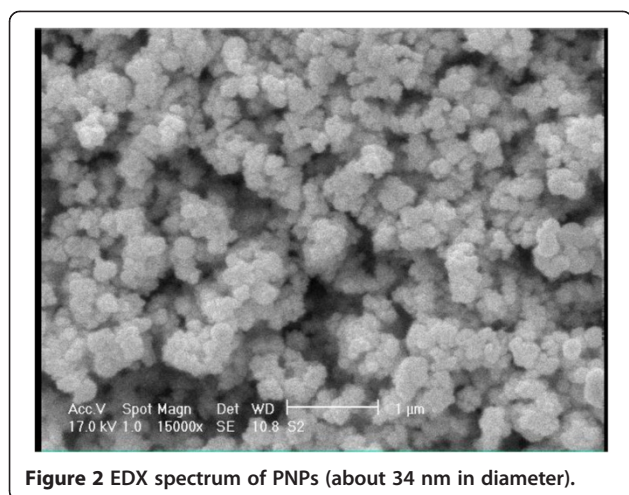


Figure 2 EDX spectrum of PNPs (about 34 nm in diameter).

dimethyl sulfoxide (100 µl/well), and the absorbance was read at 570 nm using a microplate scanning spectrophotometer (ELISA reader, Organon Teknika, Netherlands). The toxicity level was calculated using the following formula [24]:

$$\text{Cytotoxicity \%} = 1 - \frac{\text{Mean absorbance of toxicant}}{\text{Mean absorbance of negative control}} \times 100$$

$$\text{Viability \%} = 100 - \text{Cytotoxicity \%}$$

The CC50 values of PNPs on MCF-7 and HepG-2 cells at 24 h were determined. CC50 was determined by probit analysis using the Pharm PCS (Pharmacologic Calculation System) statistical package (Springer-Verlag, USA).

Statistical analysis

The results were expressed as mean ± SD. Statistical significances of difference throughout this study were calculated using a Student *t* test and by one-way variance analysis.

Results and discussion

Synthesis and characterization of PNPs

The particle size of the PNPs was measured by SEM. The average size of the particles is about 34 nm as shown in Figure 1. This means that PNPs have typical nanomaterial characteristics and can be efficiently taken up by cells. The results observed with the SEM show that the particles in PNPs are spheroid, their surface is smooth, and they are dispersed evenly without adhesion. SEM micrographs of the platinum particles were obtained in 25°C. In addition to the SEM images, we collected EDX spectra of the PNPs to evaluate their elemental composition. Table 1 presents a representative spectrum showing well-resolved peaks for both platinum (L α and L β at 9 and 11 keV, respectively) and potassium (K α at 3.5 keV) shown in Figure 2. The observed potassium in the EDX spectra is due to the presence of a small number of potassium from incomplete K₂PtCl₆. We observed no peaks that were unambiguously attributable to PNPs, which were expected given the low atomic numbers of the constituent elements. Chemical reduction techniques have been extensively investigated in the preparation of nanoparticles because these methods can be implemented under simple and mild conditions, and can be used to prepare nanoparticles on a large scale. A weak reductant will result in slow reaction for the formation of metal atoms, which may be favorable for the size and morphology control of the metal nanoparticles obtained. Hence, we utilized a weak reductant, trisodium citrate, to synthesize the noble PNPs in this work.

Zeta potential measurement and stability of PNPs

The dispersion of PNPs is easy to aggregate due to their high specific surface areas (Figure 3). It was expected that the storage stability of PNPs could be improved after coating with SDS. To explore the reason about the improved storage stability of metallic nanoparticles, their

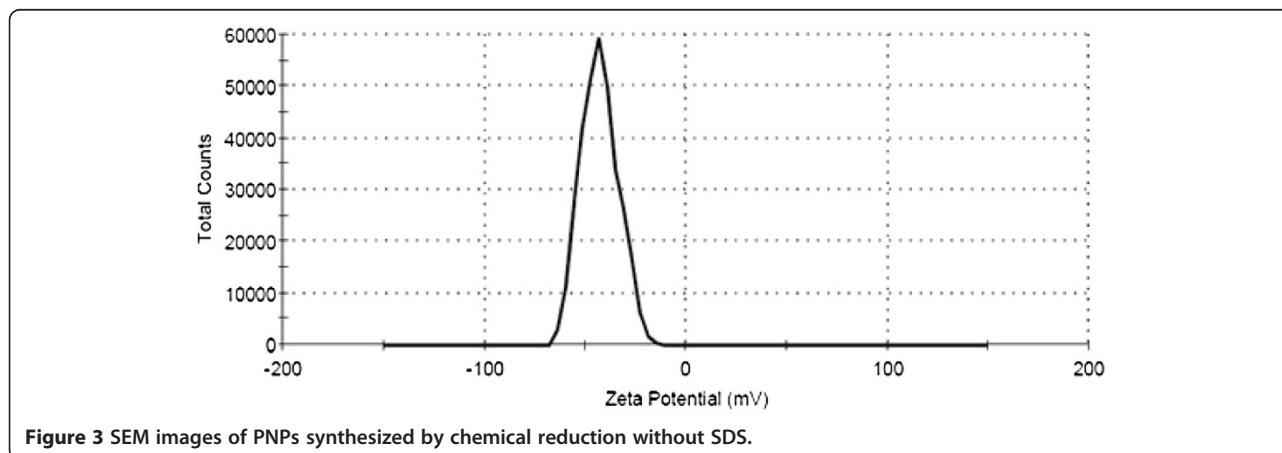


Figure 3 SEM images of PNPs synthesized by chemical reduction without SDS.

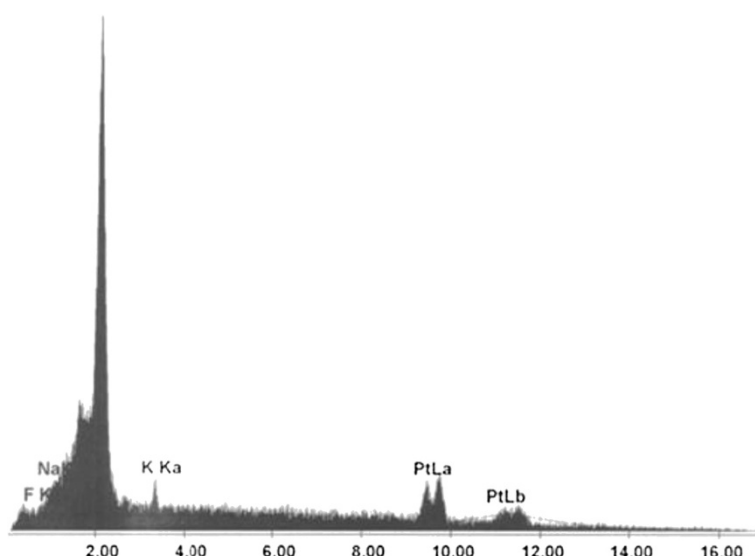


Figure 4 Zeta potential of PNPs. This potential was about -42.8 mV.

zeta potentials were measured. Zeta potential measurement gives an idea of the surface charge associated with the particles. The particle charge is one of the factors in determining the physical stability of emulsions and suspensions. If all the particles in suspension have a large negative or positive zeta potential, then they will tend to repel each other and there is no tendency to flocculate. However, if the particles have low zeta potential values, then there is no force to prevent the particles from coming together and to flocculate. The general dividing line between stable and unstable suspensions is generally taken at about either $+30$ or -30 mV. Particles with zeta potentials more positive than $+30$ mV or more negative than -30 mV are normally considered stable. As can be seen from the SEM micrographs and zeta potentials, there is stability on platinum particles obtained in this work. When the particle size decreases, the absolute value of zeta potential increases. The zeta potential of PNPs was measured (Figure 4). The zeta potentials of PNPs resulted in strong charge repulsive forces, which prevented aggregation among PNPs.

Results of MTT assay

Different concentrations of PNPs 0, 1, 2, 4, and 8 mg/ml at 24 h have cytotoxicity effects on MCF-7 and HepG-2 cell lines. Compared to the controls, different doses of PNPs decreased the MCF-7 and HepG-2 cells, respectively: for 1 mg/ml, 13.124% (difference not significant $P > 0.05$) and 4.524% (difference not significant $P > 0.05$); for 2 mg/ml, 25.184% (difference not significant $P > 0.05$) and 34.97% ($P < 0.001$); for 4 mg/ml, 37.24% (difference not significant $P > 0.05$) and 67.854% ($P < 0.001$); for 8 mg/ml, 53.5% ($P < 0.05$) and 91.8% ($P < 0.001$) (see Figure 5). The

IC_{50} of the PNPs after 24 h for MCF-7 and HepG-2 cell lines was calculated as 6.8294 and 2.9044 mg/ml, respectively ($P < 0.05$). There was a significant difference between the PNP effect on growth depression of MCF-7 and HepG-2 cells ($P < 0.05$).

Conclusion

PNPs with a mean size of 34 nm were synthesized by a salt reduction reaction having high stability. The major advantage of using trisodium citrate as a reducing agent is that an improved control over not only particle size but also morphology distribution can be achieved through the choice of the stabilizing agent. Aside from

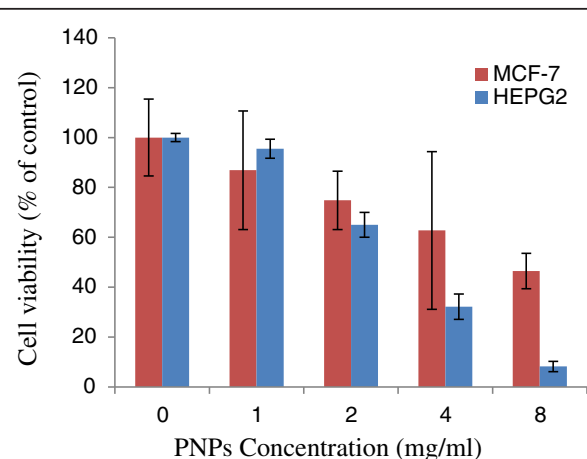


Figure 5 Results of MTT test on MCF-7 and HepG-2 under various concentrations of PNPs. Results are expressed as a percentage of viability compared to control and are presented as mean \pm SD from at least three independent experiments.

PNPs, various other transition metals have been used in anticancer drugs. PNPs may display multiple functional groups at the surface, which can be hydrophilic, lipophilic, and chemically reactive. Some of the platinum compounds are used as very effective anticancer agents. This property is associated with the inhibition of DNA replication and mitosis by the addition of PNPs DNA strand [25]. The purpose of this study is to assess the biological assay of PNPs on cancerous cell lines. In 2010, Porcel *et al.* showed that the addition of PNPs enhances strongly the lethality of damage and, thus, the biological efficiency of radiations [26]. The results demonstrated concentration-dependent toxicity for the cells tested, and CC50 was determined to be 2.904 and 6.829 mg/ml in HepG-2 and MCF-7, respectively.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

HM, AnA, and AzA carried out the designing, synthesizing, and characterizing the nanoparticles. MJM and HES carried out the MTT assay. MRM, SJ, and MC participated in the design of the study and performed the statistical analysis. All authors read and approved the final manuscript.

Acknowledgments

The authors are grateful to the Department of Pilot Nano Biotechnology, Pasteur Institute of Iran for the financial support for this research.

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Received: 24 August 2011 Accepted: 8 October 2012

Published: 26 April 2013

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doi:10.1186/2228-5326-3-28

Cite this article as: Mohammadi *et al.*: Evaluation of synthesized platinum nanoparticles on the MCF-7 and HepG-2 cancer cell lines. *International Nano Letters* 2013 **3**:28.