NanoAnalysis
An International Journal
Available online at: www.jnanoanalysis.net or www.jnanoanalysis.com

Hollow Mesoporous Silica Nanoparticles (HMSNs) Synthesis and in vitro Evaluation of Cisplatin Delivery

Zohreh Jomeh Farsangi[a], Seyed Mehdi Rezayat[a*], Ali Beitollahi[b], Saeed Sarkar[a], Mahmoudreza Jaafari[c], Amir Amani[a]

[a] School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Keshavarz Blvd., Tehran, Iran, 1417755469
[b] Center of Excellence for advanced Materials and processing, Iran University of Science and Technology, Narmak, Tehran, Iran, 16846-13114
[c] Nanotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

Abstract
Cisplatin continues to be a first line chemotherapy agent alone or in combination with other cytotoxic agents or radiotherapy. Dose-limiting side effects, intrinsic and acquired resistance are the main reasons for inventing and developing new ways of delivering cisplatin. Biocompatible hollow porous materials offer high void volume, shell porosity, low density and controllable size which make them promising platforms for efficient drug delivery systems. In this study, hollow mesoporous silica nanoparticles (HMSNs) were successfully synthesized by a hard-templating method. Further, the synthesized HMSNs were studied by characterizing their morphology, nanostructure, specific surface area, particle size distribution and chemical composition using FESEM, HRTEM, N_2-Sorption and FTIR techniques, respectively. The high specific surface area (1201 m^2g^{-1}) of the prepared HMSNs resulted in relatively high loading capacity of cisplatin (35 wt.%). Furthermore, release test performed at pH value of 7.4 showed a sustained release pattern. The cytotoxicity of the formulated drug was also examined in c26 colon carcinoma cell lines by MTT assay. The drug loaded HMSNs showed a lower toxicity than free drug due to the sustained drug release.

Keywords: Cisplatin; Hollow mesoporous silica; drug delivery; sustained release.

1. Introduction

Cisplatin is one of the major genotoxic agents for treating almost 50% of cancers including ovarian, bladder, breast, testicular, head and neck, liver, small-cell and non-small-cell lung cancers (1, 2). However, the clinical practicality of cisplatin is restricted due to faint selectivity for tumor against normal tissue that leads to dose-limiting systemic toxicity such as acute nephrotoxicity, gastrointestinal toxicity, asthenia, peripheral neuropathy, and ototoxicity (3-5). The other compressing therapeutic ratio by tumors is intrinsic or acquire resistance during the course of treatment (6). Nanoparticulate drug delivery systems offer several advantages to overcome these limitations. They can target solid tumors via passive extravasation of drug-loaded nanocarriers through tumor endothelium due to enhanced permeability and retention effect (EPR), arising from the leaky neovasculature and the deficient lymphatic drainage in tumor tissue (7). Research in recent decades has resulted in the development of a diversity of nanoparticles (NPs) for cisplatin delivery including organic (polymeric NPs, liposomes, Polymeric micelles, dendrimers, and solid lipid NPs) and inorganic magnetic NPs, carbon nanotubes, gold NPs, and

* Corresponding Author Email: rezayat@tums.ac.ir

mesoporous silica NPs (8-13). Hollow mesoporous silica nanoparticles (HMSNs) with unique morphology, high specific surface area, large void space, low density and excellent biocompatibility has been emerged as an encouraging platform for various field applications such as catalysis, adsorption, storage and controlled drug delivery (14, 15). The integration of the hollow architecture with the well tuning mesoporous silica shell result in a more efficient diffusion and a relatively high storage volume and these advantageous features make the structure an attractive nanocontainer for drug storage and delivery for biomedical applications (16). Generally, the main two strategies for synthesis of HMSNs include soft and hard-templating routes according to the utilized template for the creation of the hollow interiors (17). In soft-templating approach, both the hollow structure and the mesopores are generated simultaneously via the self-assembly between the organic surfactant templates and the precursor molecules (18). In hard-templating method, however, the specially prepared core templates such as latex nanoparticles, metallic nanobeads, silica and carbon nanospheres are used. These hard templates are then removed through calcination, etching or dissolution, after the generation of the mesoporous silica shell. In this technique, the size of the void space, the shell thickness, the morphology and the monodispersity of the particles are very tunable (17). Arean *et al* (19) claimed cisplatin loadings of 8.7 (±0.7), 16.4 (±4.6) and 14.7 (±0.6) mg per gram of sample for mesoporous silica nanoparticles of MCM-41, NH_2-MCM-41 and COOH-MCM-41, respectively. Jinlou Gu et al (20) reported synthesis and functionalization of MSNs with different amounts of COOH groups for cisplatin loading. The amount of the loaded cisplatin in their three samples were 5.9, 8.7, and 11.3 wt.%, respectively. Hanwen *et al* (21) designed a novel co-delivery system of cisplatin and doxorubicin based on poly(acrylic acid) modified mesoporous silica nanoparticles. In our previous work (22), we have reported a novel one-pot controllable synthesis method for COOH functionalized HMSNs to evaluate the cisplatin loading and the controlled release (20). This study is devoted to the synthesis and use of HMSNs as a carrier for cisplatin loading and evaluation of the drug release profile in order to design an efficient nanocarrier for drug delivery purposes.

2. Materials and Methods

2.1 Materials

Cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS, ≥99.0%), O-Phenylenediamine (OPDA), potassium persulfate (KPS), dimethylformamide (DMF) were purchased from Sigma Aldrich. Analytically pure styrene (>99.0%) washed through an inhibitor remover column, tetrahydrofuran (THF), ethanol and ammonia solution (25%) were purchased from Merck. Sodium bicarbonate ($NaHCO_3$), sodium dodecyl sulfate (SDS), tetra hydro furan (THF), hydrochloric acid (HCl) were purchased from Merck. All other chemicals and reagents used were of analytical grade. Deionized water was prepared using a Milli-Q integral pure and ultrapure water purification system.

2.2 Synthesis of Polystyrene latex nanoparticles

The PS latex nanospheres with the size around 100 nm in diameter were prepared through conventional emulsion polymerization. In this regard, 0.375 g of SDS and 0.1 g $NaHCO_3$ were dissolved in 84 ml of de-ionized water in a 500 ml glass round-bottom flask. The resulted mixture was stirred strongly for about 30 min under a dynamic nitrogen atmosphere at room temperature and then heated to 70 °C. Subsequently, 1 ml of KPS aqueous solution (0.1 M) was added to the reaction mixture. Further, the reaction was performed for 12 h which led to latex nanospheres formation.

2.3 Synthesis of HMSNs

To obtain monodisperse HMSNs with rationally soft surface in a typical synthesis, 0.80 of CTAB was dissolved in a mixture of 80.00 g H_2O, 50.00 g ethanol. Then 10 ml of PS 9 wt.% was added drop-wise to the CTAB solution at room temperature under vigorous stirring. The resulted milky suspension was stirred for 30 min followed by sonicating for 15 min. Subsequently, 4 g TEOS was injected to the suspension with the rate of 1 ml/h. The mixture was kept at room temperature for 48 h before the mesoporous silica coated latex was collected by centrifugation at 10,000 rpm. Finally, the precipitate was washed three times with ethanol and water by centrifugation and dispersed in a mixture of 200 ml THF and concentrated HCl (2 ml 37%) for simultaneously removing the CTAB surfactant and the PS template under reflux condition at 80 °C for 12 h. The final suspension was centrifuged, and the resulted precipitate

washed with water and ethanol three times and dried under vacuum overnight.

2.4 Loading the cisplatin Anticancer Drug

To assay the cisplatin loading efficacy, a solution of (40.00 mg) cisplatin in 40 ml water-DMSO (1:1, v/v) was prepared. Then dried HMSNs (20.00 mg) were added to the above cisplatin solution and stirred at dark for 48 h at 37 °C. The resulted suspensions were centrifuged at 10,000 rpm for 30 min. The cisplatin loaded HMSNs were washed with 20 ml of EtOH and DI water and dried in a vacuum oven overnight. The original and residual cisplatin content was measured in supernatant and washing supernatant using UV spectroscopy.

2.5 In Vitro Release of cisplatin

To evaluate the *in vitro* release of the drug, we used a dialysis bag diffusion technique. The as prepared cisplatin-HMSNs samples were re-dispersed in PBS buffer at pH value of 7.4, placed into pre-treated dialysis bag with the molecular weight cut off of 14,000 and immersed in PBS buffer solution at dark with gentle shaking. The samples were collected at fixed time intervals followed by measuring the concentration of cisplatin by UV-Vis spectroscopy at the wavelength of 706 nm. The spectrophotometry measurements were achieved using a mixture of 1.40 mg/ml of OPDA solution, phosphate buffer pH 6.8, and DMF as a blank solution. Each sample must be heated for 10 min at 100 °C before adding the DMF. The samples cooled down before the UV-Vis measurements to determine the amount of residual cisplatin and compared to the created standard curve. The amount of cisplatin loaded in the HMSNs was estimated by subtracting the unloaded value from that initially added.

2.6 In vitro Cell Viability Assay

The in vitro cytotoxicity of HMSNs and cisplatin-HMSNs against a *c26* colon carcinoma cell line were assessed by 3-[4,5-dimethylthiazol-2-yl] -2,5-diphenyltetrazolium bromide (MTT) test. The murine colon carcinoma cell line *C26* was cultured in RPMI medium in 5 % CO_2 at 37 °C. Cells were harvested from sub-confluent cultures using trypsin, and were re-suspended in fresh complete medium. 4000 cells were seeded in 96-well plates for 24 h. Each plate also included untreated *c26* cells as positive controls, and blank wells with medium free of cells. After overnight incubation of plates (37 °C, 5% CO_2), the medium was removed carefully and exposed to the fresh medium (200 µl), containing up to 100 µl of serial concentration of drug formulations. After incubation at 37 °C, 5% CO_2 for 48 h, the medium was removed carefully and replaced by 100 µl FCS free cell cultured medium containing 10 µl of MTT solution. The insoluble formazan produced by living cells was dissolved by adding 200 µl DMSO and its optical density (OD) was read with a multi-well scanning spectrophotometer (at 570 nm wavelength).

2.7 Microstructural and Physical Characterization of HMSNs

The microstructure and morphology were distinguished (characterized) utilizing field emission scanning electron microscopy (FE-SEM) with a SIGMA VP ZEISS instrument operated at 20 kV and high resolution transmission electron microscope (HRTEM) with a HF3300 Hitachi TEM operated at 200 kV. The Nitrogen adsorption-desorption isotherms were obtained according to Brunauer-Emmett-Teller (BET) theory using a Belsorp pore analyzer at 77 K. The pore size distributions and pore volumes were extracted using the Barrett–Joyner–Halenda (BJH) model. The particle size distribution was measured by Scatteroscope I nanoparticle size analyzer. Fourier transformed infrared (FTIR) spectra were obtained by a FTIR-Shimadzu S8400. Cisplatin concentration measurements were performed by an Agilent Cary 60 UV-Vis spectrophotometer at 706 nm wavelength.

3. Results and Discussion

In this study, we used a hard-templating method to prepare well dispersed and uniform HMSNs. This synthesis method involves PS nanospheres as hard templates, CTAB as the mesostructure directing agent and TEOS as the silica precursor. In appropriate water/EtOH ratio and optimum CTAB concentration, PS nanospheres were completely covered by CTAB micelles. The hydrolysis and condensation of TEOS takes place around CTAB micelles deposited as a layer around PS templates. Spherical, uniform, and monodisperse HMSNs can be achieved by controlling the rate of the reaction.

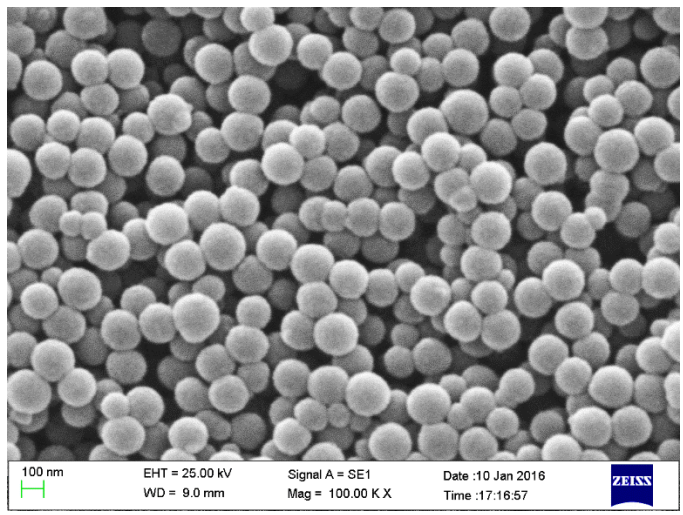

Figure 1. FESEM-image of HMSNs of the size between 100 to 120 nm

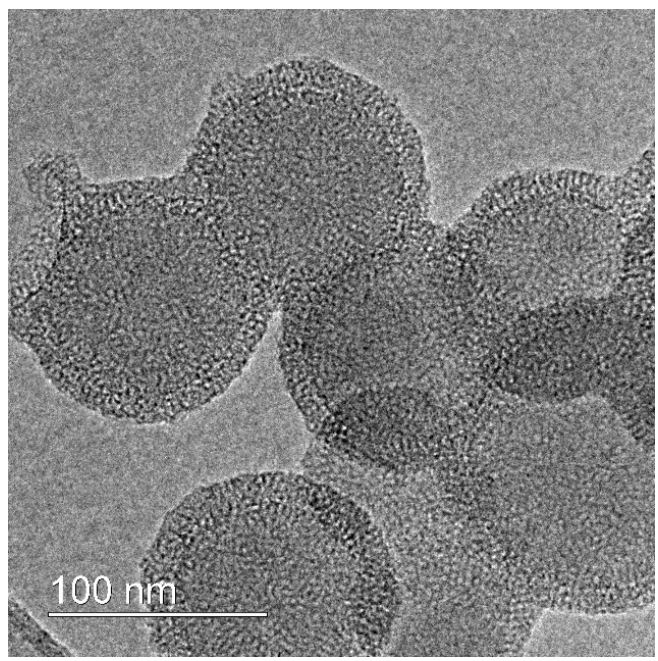

Figure 2. HRTEM-image of HMSNs

Further, PS and CTAB could be simultaneously removed by refluxing in a mixture of THF and HCl. The spherical morphology, uniformity and mono-dispersity of the as-prepared HMSNs are clearly seen in FESEM-image shown in figure 1.

The HRTEM-image, presented in figure 2, clearly shows the synthesized HMSNs of the size between 100 to 120 nm, the hollow cores, the uniform silica shells of about 15 nm thickness and the worm-like microporous structure of the silica shell. Figure 3 shows the FTIR spectra of PS and HMSNs with and without templates. The common spectral bands around 808, 960, 1105 cm^{-1} are owing to symmetric stretching vibration, stretching and asymmetric vibrations of Si-OH, respectively. The broad band around 3480 cm^{-1} is due to silanol groups and also absorbed water. Considering these spectra, the C-H vibration bands at about 2850 and 2930 cm^{-1}, the C-H deformation bands around 1470 cm^{-1}, also the characteristic band of C-H, C-C and C-aromatic group between 1500 and 1600 cm^{-1} were almost disappeared confirming the removal of the CTAB and PS after THF treatment (23).

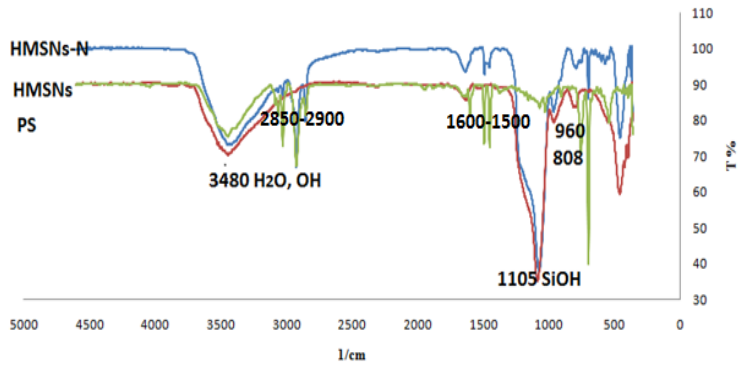

Figure 3. FTIR spectra of PS and HMSNs before and after template extraction

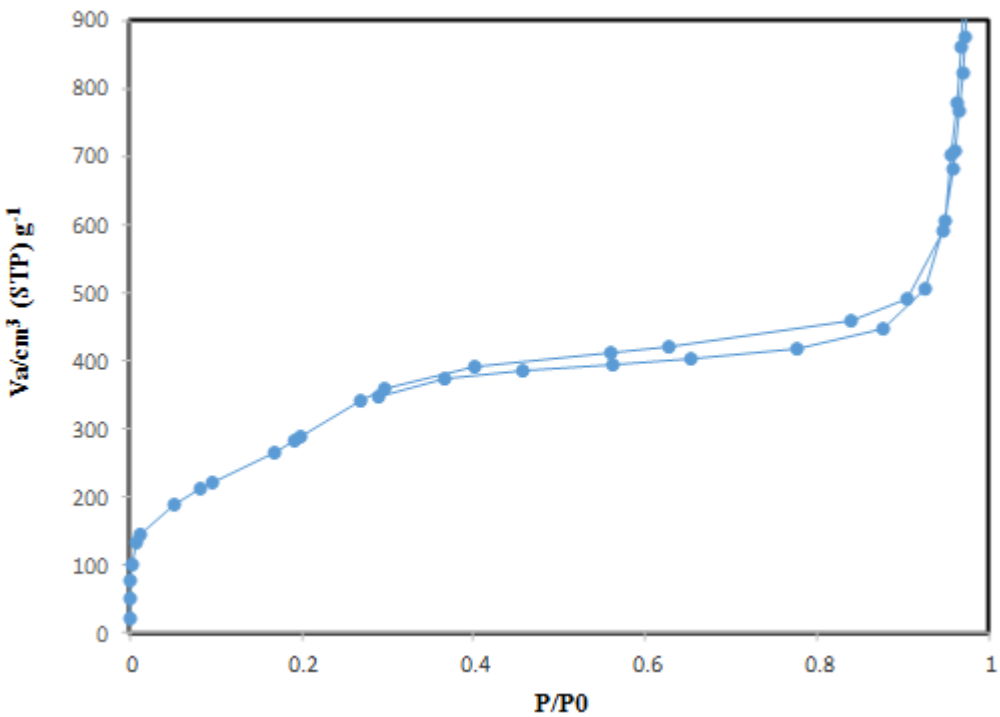

Figure 4. N_2 adsorption - desorption isotherms of HMSNs

As presented in Figure 4, the HMSNs show type IV isotherms with a specific surface area of 1201 $m^2 g^{-1}$ and a total pore volume of 3.48 $cm^3 g^{-1}$. This relatively high specific surface area can be used to accommodate the drug molecules. The average pore diameter is 2 nm in regard to Barrett-joyner-Halenda (BJH) curve.

In this study, we used a simple and cost-effective colorimetric-based selective derivatization method for measuring cisplatin concentrations (24). Derivatization of cisplatin with o-phenylendiamine (OPDA) at pH=6.8 and 90 °C resulted in a maximum absorption at 706 nm in the UV-vis spectra. Utilizing this method, precise measurement of the cisplatin loading and release with a relatively high accuracy in the 1-10 µg/ml range could be achieved. The loading capacity was calculated using the following equation:

Loading capacity:

$$(wt.\%) = \frac{Mass\ of\ the\ drug\ in\ HMSNs}{Initial\ Mass\ of\ the\ HMSNs}$$

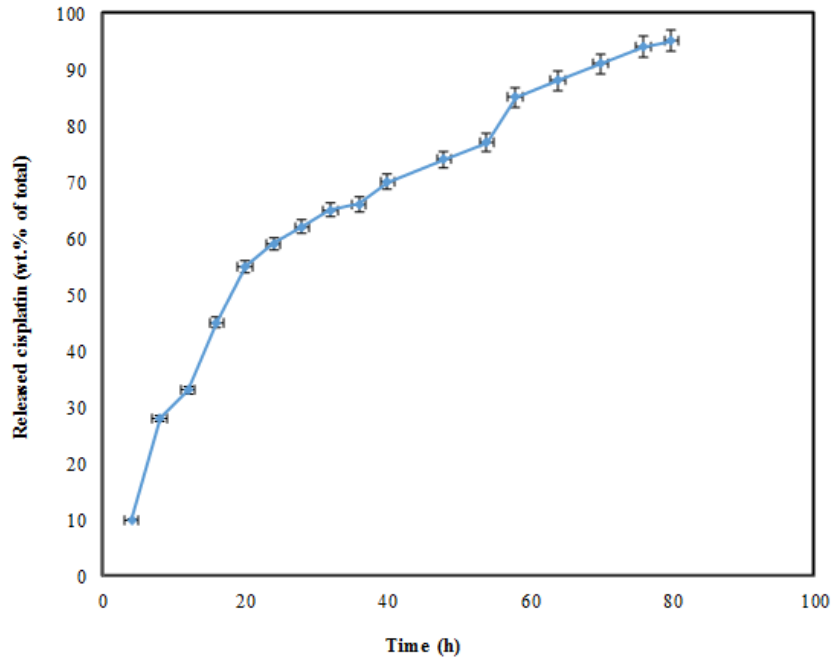

Figure 5. The time dependent release behavior of cisplatin- HMSNs.

As we expected, compared to mesoporous silica nanoparticles (MSNs), HMSNs can load quite high amounts of the drug, due to their hollow core. In this study, cisplatin loading capacity up to almost 35 wt.% was achieved. Compared to the loading values reported in the literature (19, 20), the achieved loading value is significantly higher.

To estimate the cisplatin release profile, the cisplatin loaded samples were immersed in PBS buffer with pH value of 7.4, at 37 °C. Figure 5 shows the time-dependent release behavior of cisplatin-HMSNs. As seen, the overall release pattern is sustained. However, a fast release occurred in the first 20 h, but it became slower to the end of the assay. After 20 h, almost 55 wt.% of the drug was released.

The cytotoxicity results are shown in figure 6. The cisplatin-HMSNs delivery system shows a lower toxicity than the free drug due to sustained drug release, which provides enough time for the nanocarrier to accumulate in tumor tissue by EPR effect. The estimated IC50 values were 0.3 and 1.6 µg/ml for free cisplatin and formulated cisplatin-HMSNs, respectively.

The percentage of cytotoxicity was calculated according to the following formula:

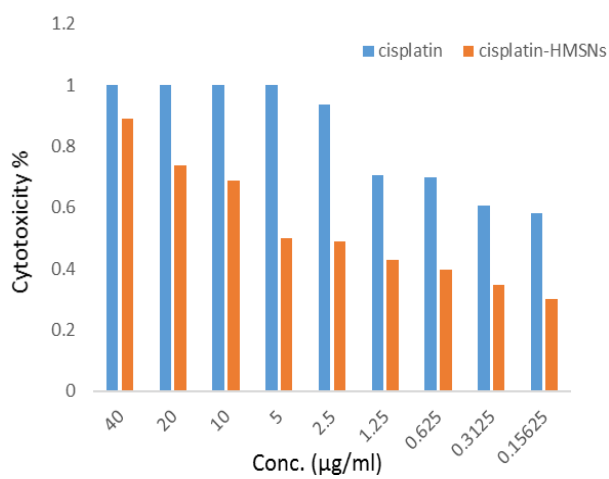

Figure 6. The cytotoxicity results of cisplatin-HMSNs in 24h.

$$\%\ \text{Cytotoxicity} = 100 * \left(1 - \frac{\text{Ad} - \text{Ab}}{\text{Ac} - \text{Ab}}\right)$$

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

Ad = mean absorbance of drug treated cells
Ab = mean absorbance of blank
Ac = mean absorbance of positive control cells

4. Conclusions

In this study, we have reported a sustained drug delivery system based on HMSNs synthesized by a hard-templating procedure. The HMSNs exhibited relatively narrow size distribution, good morphological uniformity, and a high loading capacity of the cisplatin anticancer drug. This drug delivery carrier synthesis and design procedure offers some advantageous features including; a very low density carrier with high loading capacity (35 wt.%) and high loading efficiency, a sustained release of cisplatin (around 90% over 80 h), and an enhanced therapeutic efficacy by decreasing the necessary dose. We propose that this synthesis procedure has a highly controllable number of structural parameters (hard template size, shell thickness, functionality) that can precisely tailor the drug loading and release behavior of a broad range of the therapeutic agents.

References

1.Ali, I., et al., *Platinum compounds: a hope for future cancer chemotherapy.* Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 2013. 13(2): p. 296-306.
2.Wheate, N.J., et al., *The status of platinum anticancer drugs in the clinic and in clinical trials.* Dalton transactions, 2010. 39(35): p. 8113-8127.
3.Hartmann, J.T. and H.-P. Lipp, *Toxicity of platinum compounds.* Expert opinion on pharmacotherapy, 2003. 4(6): p. 889-901.
4.Sastry, J. and S.J. Kellie, *Severe neurotoxicity, ototoxicity and nephrotoxicity following high-dose cisplatin and amifostine.* Pediatric hematology and oncology, 2005. 22(5): p. 441-445.
5.Hanigan, M.H. and P. Devarajan, *Cisplatin nephrotoxicity: molecular mechanisms.* Cancer therapy, 2003. 1: p. 47.
6.Marrache, S., R.K. Pathak, and S. Dhar, *Detouring of cisplatin to access mitochondrial genome for overcoming resistance.* Proceedings of the National Academy of Sciences, 2014. 111(29): p. 10444-10449.
7.Maeda, H., *The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting.* Advances in enzyme regulation, 2001. 41(1): p. 189-207.
8.Dhar, S., et al., *Targeted delivery of cisplatin to prostate cancer cells by aptamer functionalized Pt (IV) prodrug-PLGA–PEG nanoparticles.* Proceedings of the National Academy of Sciences, 2008. 105(45): p. 17356-17361.
9.Nishiyama, N., et al., *Novel cisplatin-incorporated polymeric micelles can eradicate solid tumors in mice.* Cancer research, 2003. 63(24): p. 8977-8983.
10.Pisani, M.J., et al., *Anionic PAMAM dendrimers as drug delivery vehicles for transition metal-based anticancer drugs.* Journal of inorganic biochemistry, 2009. 103(3): p. 373-380.
11.Krieger, M.L., et al., *Overcoming cisplatin resistance of ovarian cancer cells by targeted liposomes in vitro.* International journal of pharmaceutics, 2010. 389(1): p. 10-17.
12.Thierry, B., et al., *Multifunctional core–shell magnetic cisplatin nanocarriers.* Chemical Communications, 2009(47): p. 7348-7350.
13.Dhar, S., et al., *Polyvalent oligonucleotide gold nanoparticle conjugates as delivery vehicles for platinum (IV) warheads.* Journal of the American Chemical Society, 2009. 131(41): p. 14652-14653.
14.Slowing, I.I., et al., *Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carriers.* Advanced drug delivery reviews, 2008. 60(11): p. 1278-1288.
15.Chen, Y., H.-R. Chen, and J.-L. Shi, *Construction of homogenous/heterogeneous hollow mesoporous silica nanostructures by silica-etching chemistry: principles, synthesis, and applications.* Accounts of chemical research, 2013. 47(1): p. 125-137.
16.Li, Y. and J. Shi, *Hollow-Structured Mesoporous Materials: Chemical Synthesis, Functionalization and Applications.* Advanced Materials, 2014. 26(20): p. 3176-3205.

17.Chen, Y., H. Chen, and J. Shi, *Engineering of hollow mesoporous nanoparticles for biomedical applications.* Advanced Porous Materials, 2013. 1(1): p. 34-62.
18.Shi, J., *On the synergetic catalytic effect in heterogeneous nanocomposite catalysts.* Chemical reviews, 2012. 113(3): p. 2139-2181.
19.Arean, C., et al., *Effect of amine and carboxyl functionalization of sub-micrometric MCM-41 spheres on controlled release of cisplatin.* Ceramics International, 2013. 39(7): p. 7407-7414.
20.Gu, J., et al., *One-pot synthesis of mesoporous silica nanocarriers with tunable particle sizes and pendent carboxylic groups for cisplatin delivery.* Langmuir, 2012. 29(1): p. 403-410.
21.Li, H., et al., *Cisplatin and doxorubicin dual-loaded mesoporous silica nanoparticles for controlled drug delivery.* RSC Advances, 2016. 6(96): p. 94160-94169.
22.Farsangi, Z.J., et al., *One-pot controllable synthesis of carboxylic group functionalized hollow mesoporous silica nanospheres for efficient cisplatin delivery.* RSC Advances, 2016. 6(72): p. 67592-67598.
23.Jinlou, G., et al., *Surface Modification-complexation Strategy for Cisplatin Loading in Mesoporous Nanopaticles.* J. Phys. Chem. Lett., 2010. 1: p. 3446-3450.
24.Mohit. Basotra, Sachin. Kumar. Singh, and M. Gulati., *Development and Validation of a Simple and Sensitive Spectrometric Method for Estimation of Cisplatin Hydrochloride in Tablet*