Chemoradiotherapeutic Wrinkled Mesoporous Silica Nanoparticles for use in Cancer Therapy

UTD AUTHOR(S): Imalka Munaweera and Kenneth J. Balkus

©2014 The Authors

Creative Commons 3.0 Attribution License

Chemoradiotherapeutic wrinkled mesoporous silica nanoparticles for use in cancer therapy

Imalka Munaweera,1 Bhuvaneswari Koneru,2 Yi Shi,2 Anthony J. Di Pasqua,2,a and Kenneth J. Balkus, Jr.1,a
1Department of Chemistry, University of Texas at Dallas, 800 West Campbell Rd., Richardson, Texas 75080, USA
2Department of Pharmaceutical Sciences, University of North Texas System College of Pharmacy, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, Texas 76107, USA

(Received 13 August 2014; accepted 12 October 2014; published online 30 October 2014)

Over the last decade, the development and application of nanotechnology in cancer detection, diagnosis, and therapy have been widely reported. Engineering of vehicles for the simultaneous delivery of chemo- and radiotherapeutics increases the effectiveness of the therapy and reduces the dosage of each individual drug required to produce an observable therapeutic response. We here developed a novel chemoradiotherapeutic 1,2-dioleoyl-sn-glycero-3-phosphocholine lipid coated/uncoated platinum drug loaded, holmium-containing, wrinkled mesoporous silica nanoparticle. The materials were characterized with TEM, FTIR, 1H NMR, energy dispersive x-ray, inductively coupled plasma-mass spectrometry, and zeta potential measurements. In vitro platinum drug release from both lipid coated and uncoated chemoradiotherapeutic wrinkled mesoporous silica are reported. Various kinetic models were used to analyze the release kinetics. The radioactivity of the chemoradiotherapeutic nanocarriers was measured after neutron-activation. © 2014 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution 3.0 Unported License. [http://dx.doi.org/10.1063/1.4899118]

Over the last two decades, nanoparticle based drug delivery systems have provided unique approaches for cancer treatment. Early clinical results suggest that nanoparticle based treatments can show enhanced efficacy, while simultaneously reducing side effects by localizing in tumors. Different types of nanoparticle delivery systems have been reported for cancer chemotherapy and radiotherapy. Polymeric nanoparticles,1 dendrimers,2 nanoshells,3,4 liposomes,5,6 micelles,7,8 and magnetic particles9–11 have been widely reported as drug delivery systems for cancer therapy. Different anticancer drugs and radiotherapeutic materials have been anchored to nanocarriers for cancer therapy. The combination of chemotherapy and radiotherapy can improve survival rates. Jung et al. reported taxanes incorporated in polymeric nanoparticles (PNP) for chemoradiation therapy of non-small cell lung cancer.12 Wang et al. developed a lipid–polymer hybrid NP for prostate cancer that is capable of delivering both chemotherapy and radiotherapy.13 These nanoparticles can encapsulate chemotherapeutics up to 9% of the nanoparticle weight and radiotherapeutics of 100 mCi of radioisotope per gram.13 Cisplatin (CDDP)-loaded magnetic nanoparticles (MNP) in combination with chemoradiotherapy showed that CDDP-loaded MNP alone or in combination with radiotherapy can effectively inhibit the growth of nasopharyngeal carcinoma in nude mice without increasing the toxicity.14 Werner et al. also reported folate-targeted chemoradiotherapeutic nanoparticles encapsulating paclitaxel (Ptxl) and Y90 for ovarian cancer.15 They demonstrated that the folate-targeted nanoparticle Ptxl Y90 is an effective biologically targeted chemoradiotherapy for ovarian cancer.15

---

1Authors to whom correspondence should be addressed. Electronic addresses: Balkus@utdallas.edu, Tel.: (972) 883-2659, Fax: (972) 883-2925 and Anthony.DiPasqua@unthsc.edu, Tel.: (817) 735-2144, Fax: (817) 735-2603

This article is copyrighted as indicated in the article. Reuse of AIP content is subject to the terms at: http://aplmaterials.aip.org/about/rights_and_permissions
Materials developed for radiotherapy should be safe for clinical practice. An attractive and safe method for producing materials for radiotherapy is to incorporate stable isotopes within suitable carriers and then subsequently irradiate within a neutron flux. This avoids handling large amounts of hazardous radioactive material and allows for the preparation of a stable isotope carrier without the constraints of short isotope half-lives. Various materials have been reported as carriers for stable isotopes for the production of radiotherapeutic particulates. For example, glass microspheres containing $^{90}$Y and polymeric microparticles with $^{166}$Ho and $^{166}$Ho in mesoporous silica nanoparticles (MSNs) have been reported. $^{166}$Ho emits high-energy $\beta^-$-particles with a maximum energy of 1.84 MeV, with a half-life of 26.8 h, which is suitable for use in radionuclide therapy. Also, $^{166}$Ho emits 81-keV $\gamma$-rays (6.6% photon yield), which can be used to quantify and image the biodistribution of the particles. The penetration range of $^{166}$Ho into the soft tissues is 1.23 mm on average, with a maximum of 8.6 mm. When $^{166}$Ho is injected into the tumor, 90% of the energy is deposited within the range of 2.1 mm and the remaining 10% is deposited within the range of 2.1–8.6 mm, suggesting that it could be used to irradiate the tumor only, while preserving the surrounding normal tissue. Thus, $^{165}$Ho is an attractive radionuclide for radiotherapeutic applications. A novel chemoradiotherapeutic 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) lipid coated/uncoated platinum drug loaded, holmium-containing, wrinkled mesoporous silica nanoparticle has been prepared ($^{165}$Ho-MS np) (Fig. 1), which after neutron-activation is radioactive ($^{166}$Ho-MS np). $^{165}$Ho was incorporated into the walls of the mesoporous silica nanoparticles and the platinum drugs were then anchored through hydrogen bonding to the silica surface.

FIG. 1. DOPC lipid coated platinum drug loaded holmium-165-containing, wrinkled mesoporous silica nanoparticle.
Platinum (Pt)-based drugs have been reported to be effective for the treatment of cancer. Some of the most commonly employed chemotherapeutic agents include cisplatin, carboplatin, and oxaliplatin, which can form adducts with genomic DNA, ultimately leading to cell apoptosis. In vitro studies have shown that the Pt-based anticancer drugs cisplatin, carboplatin, and oxaliplatin are able to act as sensitizers in radiotherapy, which make tumor cells more sensitive to radiation therapy. Mesoporous silica was selected as a carrier material to incorporate a high dosage of anticancer platinum drugs with the $^{165}$Ho complexes in the pore walls.

Mesoporous silica nanoparticles have been reported as promising drug vehicles due to the mesopore structure, chemical stability, surface functionality, and biocompatibility. An advantage of the mesopore structure is tunable pore sizes to accommodate different drug molecules. Also, mesoporous silica nanoparticles have well-defined surface properties that allow easy functionalization of the silanol-containing surface for controlled drug loading and release. Surface functionalization is important for loading in different types of drugs and to develop chemical links with stimuli-responsive, luminescent, or capping materials for multifunctional properties.

Lipid coated nanocarriers are also widely used in target drug delivery. Lipid layers have potential to mimic cell membranes in the study of transportation mechanisms through membrane channels or drug effects on membrane stability. Also lipids can enhance the biocompatibility of small particles for medical purposes such as drug delivery. Amorphous mesoporous silica nanoparticles coated with lipid bilayers have been used as in vitro carriers for small drugs and DNA delivery. In this paper, chemoradiotherapeutic mesoporous silica nanoparticles were coated with DOPC lipid and in vitro platinum drug release was studied, where various kinetic models were used to describe the release kinetics. The radioactivity of the chemoradiotherapeutic nanocarriers was analyzed after neutron-activation.

Urea, cetylpyridinium bromide, cyclohexane, 1-propanol, tetraethyl orthosilicate (TEOS), holmium (III) chloride hexahydrate, DOPC, and cisplatin were all purchased from Aldrich Chemical Co. The 3-(trimethoxysilylpropyl)diethylene triamine (SiDETA) was purchased from Gelest, Inc. Carboplatin was purchased from Strem Chemicals, Inc. Oxaliplatin, chloroform, and diethylether were purchased from Fisher Scientific. All reagents were used as received.

Holmium-165 chelated 3-(trimethoxysilylpropyl)diethylene triamine complex ($^{165}$Ho-SiDETA) was prepared to synthesize the $^{165}$Ho-MS np. First, holmium (III) chloride hexahydrate (0.66 mmol, 0.2529 g) was dissolved in 5 ml of deionized water. Subsequently, 3-(trimethoxysilylpropyl)diethylene triamine (2 mmol, 0.5308 g) was added to the above solution and stirred for 2 h to form $^{165}$Ho-SiDETA. Cetylpyridinium bromide (1.3 mmol, 0.5000 g) and urea (5.0 mmol, 0.3000 g) were mixed in deionized water (10 ml) in another flask. Then, cyclohexane (15 ml) and iso-propanol (0.46 ml) were added to the solution. With vigorous stirring, TEOS (6 mmol) and above prepared $^{165}$Ho-SiDETA complex were added dropwise to the reaction mixture. After vigorous stirring for 30 min at room temperature, the reaction mixture was heated up to 70 °C, and this state was maintained for 21 h. The reaction mixture was then centrifuged and washed with acetone and water three times. Subsequently, the mixture was re-dispersed in ethanol (50 ml) and stirred for 24 h at 70 °C. The resulting products were dried at 100 °C for 24 h.

$^{165}$Ho-MS np (30 mg) was added to a solution containing one of the platinum drugs (5 mg) in 10 ml of deionized water with stirring. At different time intervals, the mixture was centrifuged and aliquots of platinum drug solution were collected and analyzed using UV-vis spectroscopy to calculate the amount of drug loaded. The Pt drug loaded nanoparticles were then collected by centrifugation. The products were dried at 80 °C for 8 h. The dried $^{165}$Ho-MS-Pt nanoparticles ($^{165}$Ho-MS-cisplatin, $^{165}$Ho-MS-carboplatin, and $^{165}$Ho-MS-oxaliplatin) were digested in hydrofluoric acid at 80 °C overnight. The solution was then evaporated and the materials were digested further in nitric acid at 70 °C for 4 h, and Pt and holmium contents were analyzed using inductively coupled plasma-mass spectrometry with a NexION 300D ICP-MS, PerkinElmer.

DOPC lipid (10 mg) was dissolved in 5 ml of chloroform and $^{165}$Ho-MS-Pt was added to a DOPC solution and stirred for 3 h. The solvent was evaporated under a flow of argon gas. Then, 5 ml of heated water was added to the dried powder and stirred for another hour. The DOPC coated $^{165}$Ho-MS-Pt was collected by centrifugation and washed using diethyl ether to remove excess DOPC. The product was...
then dried at 80 °C for 5 h, digested as earlier described, and the Pt and Ho contents were analyzed using ICP-MS.

$^{165}$Ho-MS-Pt nanoparticles (30 mg each) were individually dispersed in 50 ml of simulated body fluid (SBF, pH 7.3) and kept at 37 °C. A 3 ml of aliquot was withdrawn after centrifugation of the suspension at each specified time period and was replaced with an equal volume of fresh SBF. The concentrations of the released Pt drugs were measured using UV-vis spectroscopy. The same procedure was followed to study the release of platinum drugs from DOPC-$^{165}$Ho-MS-Pt.

Mesoporous silica nanoparticles containing either holmium or holmium in combination with a platinum drug or platinum drug and DOPC (approximately 5 mg of each sample) were neutron-activated in a 1 MW TRIGA Mark I nuclear reactor at the Texas A&M Nuclear Science Center at a thermal neutron flux of approximately $3.5 \times 10^{12}$ neutrons/cm$^2$ s for 1 h. Radioactivity was determined by quantifying the photons emitted using a gamma spectrometer (Canberra Industries HPGe Model GC3518). Radioactivities were calculated directly after neutron-activation.

The $^{165}$Ho-MS nanoparticles were analyzed by transmission electron microscopy (TEM) using a JEOL 2100 analytical TEM with an accelerating voltage of 200 kV. Energy Dispersive X-ray Spectroscopy (EDX) analysis of carbon coated samples was carried out using a Zeiss-LEO model 1530 SEM. The zeta potential and dynamic light scattering measurements of nanoparticles were determined using Malvern Zetasizer Nano ZS. Fourier Transform Infrared Spectroscopy (FTIR) analysis was carried out using a Nicolet 380 spectrometer. $^1$H-NMR spectra were recorded on a 500 MHz Bruker spectrometer using the residual proton resonance of deuterated chloroform ($\text{CDCl}_3$). UV–vis spectroscopy of platinum drug samples was carried out using a Shimadzu UV-1601PC spectrometer.

Holmium-165 was chelated to SiDETA in order to synthesize $^{165}$Ho-MS nanoparticles. Fig. 2 shows the FTIR spectrum of SiDETA and $^{165}$Ho-SiDETA. The sharp peak between 1150 and 1000 cm$^{-1}$ confirms the formation of siloxane bonds and Si-O-Si stretching vibrations. The bands at 3300, 3370, 1590, and 789 cm$^{-1}$ correspond to the symmetric stretching, deformation, and wagg- ing vibration of N-H, respectively. Peaks at 2930 and 2880 cm$^{-1}$ are due to the vibrations of the methylene groups in SiDETA. The characteristic amine stretching mode of $^{165}$Ho-SiDETA shifted to a lower wavenumber as compared to the free SiDETA (3300 to 3170 cm$^{-1}$ and 3370 to 3270 cm$^{-1}$) (Fig. 2). This shift is due to the chelation of holmium ions by the DETA. Similar shifts towards lower frequencies have been reported for metal chelated polyamidoamine (PAMAM) dendrimers and poly(acrylo-amidino diethylenediamine) chromate complexes. Tridentate N donor diethylenetriamine lanthanide ion complexes have also been reported. The presence of only three

![FIG. 2. FTIR spectra of (a) Si-DETA and (b) $^{165}$Ho-SiDETA.](image-url)
bands in the NH$_2$ stretching region indicates that all NH$_2$ groups are coordinated. Fig. 2(b) shows the presence of three bands in the NH$_2$ stretching region and indicates that all NH$_2$ groups are coordinated to Ho$_{3}^+$. Thus, an iso-structural complex is proposed for Ho$_{3}^+$ chelated by SiDETA as shown in Fig. 1. There are no characteristic shifts in N-H wagging and deformation band at 789 and 1590 cm$^{-1}$. This may be due to presence of some free SiDETA in the pore walls.

The $^{165}$Ho-SiDETA complex was characterized by $^1$H NMR. Fig. 3 shows the $^1$H NMR spectra of SiDETA and the Ho$_{3}^+$-chelated SiDETA, $^{165}$Ho-SiDETA. An upfield chemical shift and broadening of the peaks are observed in the $^1$H NMR spectrum of the holmium ion chelated by SiDETA as compared to the free SiDETA. A similar up field shift and line broadening were reported for lanthanide ions chelated by 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine diethylenetriaminepentaacetic acid. 46

Wrinkle structured $^{165}$Ho-MS nanoparticles were synthesized by modifying a reported procedure. 30 The synthesis of wrinkle, radial, and fibrous mesoporous silica nanoparticles using cetylpyridinium bromide has been reported by different groups. 30,47 However, this is the first time that holmium has been incorporated in to the mesoporous silica nanoparticles. These wrinkled nanoparticles have high surface areas due the fibrous morphology. 47 Also, the radial pore structure makes it possible to obtain a high concentration of highly dispersed and easily accessible adsorbed molecules. 47 The wrinkled mesoporous silica nanoparticles also possess high thermal stability and high mechanical stability. 47 The mechanism of formation of these nanoparticles involves a micro emulsion formed using cetylpyridinium bromide (CPB) as the template and urea in a cyclohexane, iso-propanol, and water mixture. 47 The silica precursors (TEOS and $^{165}$Ho-SiDETA) are hydrolyzed by urea, followed by assembly of the hydrolyzed, negatively charged silicates between the self-assembled template molecules. Finally, condensation of the self-assembled silicate within the micelles yields the fibrous silica nanospheres. To remove the surfactant, the mixture was extracted in ethanol (50 ml) five times and stirred for 24 h at 70 °C. Removal of the surfactant was determined by FTIR. Fig. S1(a) shows the presence of peaks at 2911 and 2848 cm$^{-1}$ corresponding to the CH stretching modes of the CPB surfactant (Fig. S1(c)) before extraction. These characteristic peaks are no longer evident in Fig. S1(b) for Ho-MS after extraction and this is consistent with the removal of the surfactant template (Figs. S1(a)–S1(c) are in the supplementary material). 48 Fig. S2 shows the formation of $^{165}$Ho-MS nps at different reaction times. Figs. S2(a)–S2(c) show the wrinkled structure formed at 70 °C, with further heating better spherical wrinkled shapes form. After 21 h reaction time, best wrinkled $^{165}$Ho-MS nanoparticles (Fig. S2(d)) were observed (Figs. S2(a)–S2(d) are in the supplementary material). 48

The morphology and particle size of the $^{165}$Ho-MS nanoparticles after template removed were analyzed by TEM as shown in Fig. 4(a). The average particle size of $^{165}$Ho-MS np is 82 nm as shown

![FIG. 3. $^1$H NMR spectra of (a) Si-DETA and (b) $^{165}$Ho-SiDETA.](image-url)
The average pore size of the $^{165}$Ho-MS np is 10 nm. EDX (Fig. S3) (Fig. S3 is in the supplementary material) further confirms the presence of the holmium in the template free $^{165}$Ho-MS np. The percent of Ho in $^{165}$Ho-MS was determined to be 29.1% (w/w).

The amount of cisplatin drug adsorbed (w/w) by the $^{165}$Ho-MS np with time is shown in Fig. S4 (Fig. S4 is in the supplementary material). After 24 h, the maximum drug loading of 28% (w/w) was achieved at room temperature. A 20% (w/w) maximum drug loading after 24 h stirring time was reported for carbamazepine loaded SBA-15 mesoporous silica.

The FTIR spectra in Figs. 5(a-3), (b-3), and 5(c-3) show the successful loading of platinum drugs in to $^{165}$Ho-MS np. The FTIR spectrum of cisplatin (Fig. 5(a-2)) shows the characteristic amine stretching and bending mode at 3330 and 3260 cm$^{-1}$, and the symmetric amine bending mode at 1340 cm$^{-1}$. Fig. 5(b-2) shows the characteristic asymmetric amine stretching bands at 3260 and 3160 cm$^{-1}$. The red shift of the characteristic amine stretching and bending modes of oxaliplatin (Fig. 5(c-2)) and the amine stretching and wagging mode of cisplatin (Fig. 5(b-3)), and the amine stretching and the degenerate deformation vibrations of carboplatin (Fig. 5(c-1)) are ascribed to the hydrogen bonding of the platinum drug molecules with surface hydroxyl and free amine groups of the $^{165}$Ho-MS nanoparticles.

The FTIR spectrum of cisplatin (Fig. 5(a-2)) shows the characteristic amine stretching mode at 3330 and 3260 cm$^{-1}$, the asymmetric amine bending mode at 1580 cm$^{-1}$, and the symmetric amine bending mode at 1340 cm$^{-1}$. Fig. 5(b-2) shows the characteristic asymmetric amine stretching bands at 3260 and 3160 cm$^{-1}$, the degenerate deformation vibrational mode of the amine at 1600 cm$^{-1}$ for carboplatin.

The red shift of the characteristic amine stretching and bending modes of cisplatin (Fig. 5(a-3)), the amine stretching and the degenerate deformation vibrations of carboplatin (Fig. 5(b-3)), and the amine stretching and wagging mode of oxaliplatin (Fig. 5(c-3)) are ascribed to the hydrogen bonding of the platinum drug molecules with surface hydroxyl and free amine groups of the $^{165}$Ho-MS nanoparticles.

EDX (Figs. S5–S7) (Figs. S5–S7 are in the supplementary material) further confirms the presence of platinum in $^{165}$Ho-MS. The weight percent of Ho and Pt, determined using ICP-MS is shown in Table I. The variation of the weight percentages of Ho in the platinum drug loaded $^{165}$Ho-MS np is due to the different loading of platinum drugs.

Relatively high loading of holmium has been reported in various carriers. For example, a lipophilic acetylacetonate complex of $^{165}$Ho ($^{165}$Ho(AcAc)$_3$) was incorporated in commercial MCM-41 mesoporous silica nanoparticles (80-100 nm) and subsequently irradiated in a neutron flux to produce particles containing $^{165}$Ho. The holmium content in these nanoparticles was 17.8% + 1.4%. Holmium-166 containing mesoporous silica nanoparticles containing 20.8% + 1.9% was also reported for the treatment of non-small cell lung cancer.

Hamoudeh et al. reported $^{165}$Ho(AcAc)$_3$ encapsulated in poly-l-lactide (PLLA) nanoparticles with a holmium content of 18% w/w. Cacaina et al. reported SiO$_2$ xerogels containing holmium with 15.8 (w/w). Lu et al. also reported 20% (w/w) $^{165}$holmium loaded mesoporous silica nanoparticles for treatment of peritoneal tumor
FIG. 5. FTIR spectra of ((a-1), (b-1), (c-1)) Ho-MS np, (a-2) cisplatin, (b-2) carboplatin, (c-2) oxaliplatin, (a-3) Ho-MS-cisplatin, (b-3) Ho-MS-carboplatin, (c-3) Ho-MS-oxaliplatin.

The holmium content in our $^{165}$Ho-MS nanoparticles is higher than most reported holmium containing particles. This is an advantage since higher the amount of holmium, lower the dose of material is required for radiotherapy. Also for the mesoporous silica containing holmium particles reported to date, the Ho$^{3+}$ is only weakly adsorbed to the pore surface. In contrast, Ho$^{3+}$ in $^{165}$Ho-MS, the system is part of the pore walls and not blocking the pores. This is an advantage since higher amounts of drugs like cisplatin can be loaded inside the mesoporous silica.

The $^{165}$Ho-MS and $^{165}$Ho-MS-Pt nanoparticles were neutron-activated in a thermal neutron flux of approximately $3.5 \times 10^{12}$ neutrons/cm$^2$ s for 1 h. Radioactivities of 213.6, 189.7, 218.6, and...
TABLE I. Weight percentage of Ho and Pt in $^{165}$Ho-MS np carrying cisplatin, carboplatin, and oxaliplatin.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wt. % Pt</th>
<th>Wt. % Ho</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{165}$Ho-MS-cisplatin</td>
<td>14.6</td>
<td>21.8</td>
</tr>
<tr>
<td>$^{166}$Ho-MS-carboplatin</td>
<td>11.7</td>
<td>31.7</td>
</tr>
<tr>
<td>$^{165}$Ho-MS-oxaliplatin</td>
<td>16.1</td>
<td>27.4</td>
</tr>
</tbody>
</table>

150.1 $\mu$Ci/mg were obtained for $^{166}$Ho-MS, $^{166}$Ho-MS-cisplatin, $^{166}$Ho-MS-carboplatin, and $^{166}$Ho-MS-oxaliplatin, respectively. Previously, an acetylacetonate complex of holmium was incorporated with MCM-41 mesoporous silica nanoparticles, and these exhibited a radioactivity of 327.1 $\mu$Ci/mg after neutron-activation in a thermal neutron flux of approximately $5.5 \times 10^{12}$ neutrons/cm$^2$ s for 2.2 h.\textsuperscript{21} After administration of 110 $\mu$Ci of these $^{166}$Ho nanoparticles via intraperitoneal injection, tumors decreased in size in ovarian tumor-bearing mice; the total absorbed radiation dose in each tumor was estimated to be 135 Gy.\textsuperscript{21} In humans, total doses of 45 to 80 Gy or higher are used.\textsuperscript{59} Other examples of holmium nanoparticles exist. Holmium acetylacetonate encapsulated in PLLA nanoparticles were irradiated in a neutron flux of $1.1 \times 10^{13}$ neutrons/cm$^2$ s for 1 h, yielding a specific activity of approximately 74.85 $\mu$Ci/mg which was suitable for intratumoral administration by the targeted multitherapy (TMT) technique.\textsuperscript{58} Bult \textit{et al.} reported holmium acetylacetonate nanoparticles for solid malignancies with a radioactivity of 324 $\mu$Ci/mg after activation in a neutron flux of approximately $5 \times 10^{12}$ neutrons/cm$^2$ s for 1 h.\textsuperscript{18} The radioactivities obtained here for the $^{166}$Ho-MS nanoparticles are comparable to that of the previously reported nanoparticles; thus, a therapeutic level can be achieved.

Although, the mesoporous silica nanoparticles suspend in aqueous solutions, they will eventually settle. Therefore, the nanoparticles were coated with a lipid to keep them in solution. A TEM image of a DOPC lipid coated cisplatin loaded $^{165}$Ho-MS nanoparticle is shown in Fig. 6. Average particle size of the lipid coated $^{165}$Ho-MS-Pt np is 95 nm. The increase in the size of the DOPC-$^{165}$Ho-MS-Pt np is due to the lipid coating around the $^{165}$Ho-MS np. The thickness of the lipid coating is about 7-10 nm. Further, Fig. 6 shows that lipid is adhered in to the surface of the platinum drug loaded $^{165}$Ho-MS np. ICP-MS showed that DOPC-$^{165}$Ho-MS-cisplatin contained 8.1% w/w Pt and 13.5% w/w Ho, DOPC-$^{165}$Ho-MS-carboplatin contained 8.3% w/w Pt and 18.5% w/w Ho, and DOPC-$^{165}$Ho-MS-oxaliplatin contained 10.0% w/w Pt and 14.0% w/w Ho.

Furthermore, $^{165}$Ho-MS- and DOPC-$^{165}$Ho-MS- np were suspended in simulated body fluid and characterized using dynamic light scattering (DLS). Fig. S8 (Fig. S8 is in the supplementary material)\textsuperscript{48} shows the average sizes of the $^{165}$Ho-MS- and DOPC-$^{165}$Ho-MS- np are 252 ± 82 nm and 180 ± 35 nm, respectively. According to TEM analysis, the average size of the $^{165}$Ho-MS particles is 82 nm. Therefore, the DLS results show some aggregation of nanoparticles due to the electrostatic interaction between nanoparticles. This aggregation decreases with the lipid coating on the nanoparticles. No settling of the nanoparticles was observed even after five days.

FIG. 6. (a) Schematic diagram and (b) TEM image of DOPC lipid coated cisplatin loaded $^{165}$Ho-MS np.
FIG. 7. Zeta potential measurements of (a) $^{165}$Ho-MS np, (b) $^{165}$Ho-MS-cisplatin np, (c) $^{165}$Ho-MS-carboplatin np, (d) $^{165}$Ho-MS-oxaliplatin np.

A plot of the zeta potential for $^{165}$Ho-MS-Pt and DOPC-$^{165}$Ho-MS-Pt at different pH values is shown in Fig. 7 and Fig. S9 (Fig. S9 is in the supplementary material). The isoelectric point of Ho-MS is 9.4, and the isoelectric point of cisplatin, carboplatin, and oxaliplatin loaded $^{165}$Ho-MS are 10.4, 10.2, 9.6, respectively. The isoelectric point of DOPC coated cisplatin, carboplatin, and oxaliplatin loaded $^{165}$Ho-MS are 6.6, 6.7, and 7.0, respectively. When the pH value of the solution is less than the isoelectric point, the $^{165}$Ho-MS and $^{165}$Ho-MS-Pt are positively charged, and they are negatively charged when the pH is above the isoelectric point. The zeta potential of $^{165}$Ho-MS in neutral aqueous media was determined to be 20 mV. A positive potential is due to the chelated holmium ions and free amine groups in $^{165}$Ho-MS. Positive zeta potential value has been reported for aminated mesoporous silica nanoparticles. The zeta potentials of $^{165}$Ho-MS-cisplatin, $^{165}$Ho-MS-carboplatin and $^{165}$Ho-MS-oxaliplatin were 15, 17, and 25 mV, respectively. In contrast, the zeta potentials of DOPC coated $^{165}$Ho-MS-cisplatin, $^{165}$Ho-MS-carboplatin, and $^{165}$Ho-MS-oxaliplatin were $-10$, $-8$, and $-9$, respectively. Similar results were reported for DOPC coated calcium phosphate nanoparticles (CaP), where the zeta potential was $-11$ mV, which was close to that (5 mV) of pure DOPC liposomes. These results indicate that the surface of the platinum drug loaded $^{165}$Ho-MS nanoparticles was covered by DOPC lipid layer. Zeta potential values for all systems were above $+15$ and below $-15$ mV at pH 7.2-7.4 (pH of simulated body fluid) which inhibit aggregation of nanoparticles.

DOPC-$^{165}$Ho-MS-Pt was neutron-activated for 1 h at a thermal neutron flux of approximately $3.5 \times 10^{12}$ neutrons/cm$^2$ s. Radioactivities of 151.9, 170.4, and 137.4 $\mu$Ci/mg were obtained for DOPC-$^{165}$Ho-MS-cisplatin, DOPC-$^{165}$Ho-MS-carboplatin, and DOPC-$^{165}$Ho-MS-oxaliplatin, respectively.

The amount of Pt complexes released from the mesoporous nanoparticles over time was measured by UV-Vis spectroscopy. A plot of release of platinum drugs versus time from the nanoparticles is shown in Fig. 8. Here, 56% of cisplatin, 68% of carboplatin and 35% of oxaliplatin were released after 1 h from platinum loaded $^{165}$Ho-MS samples (Figs. 8(a-1), (b-1), and (c-1)). From DOPC lipid coated platinum loaded $^{165}$Ho-MS samples, 48% of cisplatin, 38% of carboplatin, and 25% of oxaliplatin were released after 1 h (Figs. 8(a-2), (b-2), and (c-2)). Thereafter, a period of controlled platinum release occurred, reaching a value of 99% after 2400 min, for both lipid coated and uncoated platinum loaded $^{165}$Ho-MS samples. The initial burst release is attributed to the immediate dissolution and release of the portion of platinum drug molecules located on and near the surface of the nanoparticles. Similar burst releases have been reported for different systems such as coumarin derivative loaded MCM-41, trypsin loaded mesoporous silica nanoparticles, and protamine capped mesoporous silica nanoparticles.

The lipid coated nanoparticles show (Figs. 8(a-2), (b-2), and (c-3)) significantly slower release of the platinum drugs compared with the uncoated samples. The slow release is due to the lipid envelope surrounding the $^{165}$Ho-MS nanoparticles. The lipid coating also prevented the burst release of...
FIG. 8. *In vitro* release kinetics of encapsulated Pt(IV) compound (a-1) $^{165}$Ho-MS-cisplatin, (a-2) DOPC-$^{165}$Ho-MS-cisplatin, (b-1) $^{165}$Ho-MS-carboplatin, (b-2) DOPC-$^{165}$Ho-MS-carboplatin, (c-1) $^{165}$Ho-MS-oxaliplatin, (c-2) DOPC-$^{165}$Ho-MS-oxaliplatin in SBF (pH 7.3).

platinum drug from the surface of $^{165}$Ho-MS nanoparticles. With time, the lipid layer swells and solvent enters the pores of the $^{165}$Ho-MS and the platinum molecules diffuse out of the pores. A similar phenomenon was reported for trypsin release from poly(ethylene glycol) (PEG)-coated MSNs.63

To further analyze the *in vitro* release data, various kinetic models were used to describe the release kinetics. The zero-order rate describes systems where the drug release rate is independent of concentration. A first-order describes the release from a system where the release rate is concentration...
dependent. The Higuchi model describes the release of drugs from an insoluble matrix as a square root of a time dependent process based on Fickian diffusion. The Hixson-Crowell cube root law describes the release from systems where there is a change in surface area and diameter of particles or tablets.68 The linear plots of cumulative % drug release vs. time (zero-order kinetic model), log cumulative % drug remaining vs. time (first-order kinetic model), log cumulative % drug release vs. square root of time (Higuchi model), log cumulative % drug release vs. log time (Korsmeyer-Peppas model), and cube root of drug % remaining in matrix vs. time (Hixson-Crowell model), were plotted and compared.

Table II shows the $R^2$ values of linear plots of different kinetic models for platinum drugs release from $^{165}$Ho-MS-Pt and DOPC-$^{165}$Ho-MS-Pt. According to the $R^2$ values, the best linearity can be found in Higuchi model ($R^2$ for $^{165}$Ho-MS-Pt = 0.9822, 0.984, and 0.9813 and $R^2$ for DOPC-$^{165}$Ho-MS-Pt = 0.9837, 0.983, and 0.9877) indicating the fraction of drug release is proportional to the square root of the time. This equation has been applied to diffusion controlled release from a porous matrix, from which a drug is leached by a bathing fluid that penetrates the matrix through pores and capillaries.63

All of the above data suggest that the platinum loaded lipid coated and uncoated novel wrinkled $^{165}$Ho-MS nanoparticles are capable of providing both chemotherapy and radiotherapy. This chemoradiotherapeutic system is initially not radioactive and safe to handle. Upon neutron-activation, the $^{165}$Ho converts into radioactive $^{166}$Ho and emits $\beta^-$-particles that are sufficient to damage DNA in cancer cells and $\gamma$-photons that allow for imaging and quantifying dose. Also, the $^{165}$Ho-MS nanoparticles release platinum drugs molecules under clinically relevant conditions.

Platinum drugs and radioactive $^{166}$Ho have been combined in a wrinkled mesoporous silica nanoparticle for the first time. The synthesized $^{165}$Ho-MS has 29.1% w/w holmium and radioactivity of 213.6 $\mu$Ci/mg after neutron-activation for 1 h in a thermal neutron flux of $3.5 \times 10^{12}$ neutrons/cm$^2$.s. Platinum drug loading of 14.6, 11.7, and 16.1% (w/w) was achieved for cisplatin, carboplatin, and oxaliplatin, respectively. In vitro platinum drug release studies showed controlled release of platinum drugs from the nanoparticles up to 2400 min for both lipid coated and uncoated systems. The lipid coated drug nanocarriers show a slower release of platinum drugs as compared to the uncoated drug nanocarriers. The Higuchi model best describes the kinetic data for platinum drug release for both lipid coated and uncoated systems. These results imply that both $^{165}$Ho-MS-Pt and DOPC-$^{165}$Ho-MS-Pt are promising candidates for combined chemo and radiotherapy.

This work was financially supported by the Texas Medical Research Collaborative (TxAIRC) and the Robert A. Welch Foundation—AT1153.


### Table II. $R^2$ values of linear plots of different kinetic models for drug release from $^{165}$Ho-MS-Pt and DOPC-$^{165}$Ho-MS-Pt.

<table>
<thead>
<tr>
<th>Kinetic model</th>
<th>$^{165}$Ho-MS-cisplatin</th>
<th>$^{165}$Ho-MS-carboplatin</th>
<th>$^{165}$Ho-MS-oxaliplatin</th>
<th>DOPC-$^{165}$Ho-MS-cisplatin</th>
<th>DOPC-$^{165}$Ho-MS-carboplatin</th>
<th>DOPC-$^{165}$Ho-MS-oxaliplatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero order</td>
<td>0.9615</td>
<td>0.7827</td>
<td>0.9615</td>
<td>0.9559</td>
<td>0.8989</td>
<td>0.9615</td>
</tr>
<tr>
<td>First order</td>
<td>0.9791</td>
<td>0.9310</td>
<td>0.9258</td>
<td>0.9785</td>
<td>0.7784</td>
<td>0.9258</td>
</tr>
<tr>
<td>Higuchi</td>
<td>0.9822</td>
<td>0.984</td>
<td>0.9813</td>
<td>0.9837</td>
<td>0.983</td>
<td>0.9877</td>
</tr>
<tr>
<td>Korsmeyer-Peppas</td>
<td>0.9558</td>
<td>0.9487</td>
<td>0.9239</td>
<td>0.9485</td>
<td>0.7171</td>
<td>0.9239</td>
</tr>
<tr>
<td>Hixson-Crowell</td>
<td>0.9745</td>
<td>0.9064</td>
<td>0.9417</td>
<td>0.9736</td>
<td>0.9064</td>
<td>0.9417</td>
</tr>
</tbody>
</table>


