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3 **Loading of Cisplatin into Mesoporous Silica Nanoparticles: Effect of Surface**
4 **Functionalization**
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Abstract

Cisplatin (CDDP) plays a crucial role in the treatment of various malignant tumors. However, its clinical efficacy and applicability are restricted by issues of toxicity and resistance. Here, for drug delivery purposes, the outer surface of MCM-41 silica nanoparticles (MSNs) were functionalized with polyethylene glycol (PEG, $M_w = 10,000$ g/mol) or low molecular weight ($M_w = 1,800$ g/mol) branched polyethyleneimine (PEI). Given the strong affinity of sulfur for platinum, thiol-functionalized MSNs were synthesized for comparison by co-condensation with (3-mercaptopropyl)triethoxysilane (MPTES). CDDP loading was performed either by adsorption or impregnation in aqueous media without the use of dimethylsulfoxide (DMSO) as a solubilizer. CDDP loading capacities obtained by impregnation were higher than those obtained by adsorption and varied from 3.9 to 16.1 wt%, depending on the functional group. Loaded nanomaterials were characterized by SEM, STEM-HAADF and Raman spectroscopy. Depending upon the functional groups, platinum-based species were either dispersed in the nanomaterials as nanocrystals or uniformly distributed as molecular species. The spectral signature of CDDP was strongly modified when platinum species were homogeneously distributed within the nanomaterials. Preliminary drug release studies performed at 37°C showed that the behavior of the CDDP loaded MSNs strongly depends on the nature of the present functional groups. Among the functionalization routes investigated in this paper, PEI-based functionalization showed the most promising results for further applications in controlled drug release with an absence of burst release and a sustained release over 72 h.

Keywords: mesoporous silica nanomaterials, co-condensation approach, post-synthesis grafting, cisplatin

1. Introduction

Since its accidental discovery by Rosenberg *et al.* in 1969, cisplatin (*cis*-diaminedichloroplatinum(II), CDDP) is one of the most widely used and effective cytotoxic agents in the treatment of almost 50 % of cancers^{1–3}. It is currently used in combination with other drugs, against cancers of the head and neck, ovaries, esophagus, stomach, colon, testis, cervix and uterus, and as second line chemotherapy against most other advanced cancers, such as pancreas, liver, kidney, prostate, glioblastomas and metastatic melanomas⁴. However, the efficacy and usage of cisplatin are restricted due to the emergence of intrinsic and acquired resistance, and side-effects which include neuro- and nephrotoxicity, which are closely related to its lack of tumor selectivity⁵.

In order to provide a higher accumulation of platinum drugs in cancer cells and to minimize the side effects, numerous organic nanometer-sized cisplatin delivery systems have been developed, including polymeric micelles⁶, liposomes⁷ and cisplatin pro-drug nanoparticles⁸. In addition, different inorganic materials⁹ with unique and interesting properties such as gold nanoparticles¹⁰, iron oxide nanomaterials¹¹ and carbon nanotubes¹² have been explored for delivering platinum-based drugs. Over the last two decades, mesoporous silica nanoparticles (MSNs) have attracted extensive attention to overcome challenges in current cancer treatments due to their unique physico-chemical properties such as biocompatibility, ordered pore network, chemical stability, tunable pore diameters (2 nm to 10 nm), high pore volume (~1 cm³/g) and high surface area (800 m²/g)^{13,14}. Moreover, these materials can be functionalized either inside or outside the pore channels by using a co-condensation approach or a post-synthesis modification with various organic linkers, including mercapto groups, thereby simplifying the design of controlled-release mechanisms and improving the solubility of poorly water-soluble drugs^{15–20}.

To increase the biocompatibility and reduce undesired interactions between the biological environment and the nanocarriers, the functionalization of the outer MSNs surface with poly(ethylene glycol) (PEG) has been investigated by covalent grafting^{21,22}. This approach

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3 has been highly used to avoid clearance of the nanoparticles by 'first-pass' organs, such as
4 lung and kidney, prolong their blood circulation half-lives and consequently, increase their
5 accumulation in the targeted tissues. PEG modification also increases the dispersion and
6 thus the stability of the nanoparticles under physiological conditions²³. Dispersion of
7 nanoparticles through electrostatic repulsion can also be achieved by grafting the
8 nanoparticle surface with charged polymers to achieve sufficient electrokinetic potential to
9 obtain repulsion at the desired conditions. Among them, polyethyleneimine (PEI) has been
10 one of the most widely used cationic dispersant and gene transfection agent due to its
11 strong DNA condensation capacity and intrinsic endosomolytic activity^{24–28}. Although
12 inefficient for gene delivery but less toxic than high molecular weight PEI, coating of MSNs
13 with low molecular weight PEI polymers has been used to increase delivery of paclitaxel into
14 cancer cells²⁹. For CDDP delivery, the introduction of low molecular weight PEI
15 ($M_w = 800$ g/mol) into CDDP-encapsulated liposomes enhanced cellular uptake and
16 extended cellular retention, thus facilitating DNA exposure to cisplatin³⁰.

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32 Regarding CDDP delivery using MSNs, Gu *et al.* used the properties of carboxylic groups to
33 coordinate with platinum and form a complex by replacing the chloride ion ligands^{31,32}. Later,
34 Lin *et al.* incorporated CDDP-carboxylate complexes with a pH-sensitive hydrazone bond to
35 tether CDDP complexes to the internal surfaces of MSNs, leading to the release of CDDP-
36 carboxylate complexes under endosomal or lysosomal pHs³³. The effect of amine and
37 carboxyl functionalization of MCM-41 microparticles on the release of cisplatin were also
38 studied by Arian *et al.*³⁴. Later, Vivero *et al.* reported the use of folic acid-conjugated MSNs
39 for the delivery of the cisplatin(IV) prodrug³⁵. More recently, Huang *et al.* developed MSNs
40 based copolymer composites where cisplatin release was controlled through coordination
41 interaction between carboxyl groups of (poly(MPC-co-IA)) and CDDP³⁶. Depending on the
42 chemical properties of the materials, CDDP loading generally varied from 0.9 to 26.5 wt%.
43 While dimethylsulfoxide (DMSO) is often used as a solubilizer during the loading process, it
44 has been reported that it inhibited cisplatin cytotoxicity and its ability to initiate cell death³⁷.
45 Consequently, the use of DMSO during the loading step must be avoided at all cost. High
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3 adsorption of CDDP has been also observed on mercapto-silylated silica surfaces^{38,39}.
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5 Although these studies have reported efficient loading and prolonged delivery of cisplatin,
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7 the effect of MSNs surface modification using stabilizing or transfection agents, such as
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9 PEG or PEI, on the loading and release of CDDP, has not been investigated. Moreover, very
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11 limited attention has been directed to the spectroscopic properties and spatial distribution of
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13 CDDP once loaded into MSNs.
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16 In the present work, we report the surface functionalization of MSNs with PEG or PEI using
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18 a two-step post-synthesis grafting approach (Fig. 1(a-b)). A low molecular weight (1.8 kDa)
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20 branched PEI was used because of its low toxicity. Given a strong affinity of sulfur for
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22 platinum, thiol-functionalized MSNs were synthesized as a reference using a co-
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24 condensation approach (Fig. 1(c)). The nanomaterials were thoroughly characterized by an
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26 array of techniques including transmission electron microscopy (TEM), dynamic light
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28 scattering (DLS), ²⁹Si magic angle spinning nuclear magnetic resonance (²⁹Si MAS NMR),
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30 Raman and Fourier-transform infrared (FTIR) spectroscopies, thermogravimetric analysis
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32 (TGA), elemental analysis (EA), X-ray diffraction (XRD) as well as nitrogen sorption
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34 measurements. To compare the loading capacity of the functionalized nanomaterials, CDDP
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36 was loaded either by adsorption or impregnation of an aqueous solution of CDDP without
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38 the use of dimethylsulfoxide (DMSO). CDDP loading was evaluated by scanning electron
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40 microscopy (SEM), scanning transmission electron microscopy (STEM) - high-angle annular
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42 dark field (HAADF) and Raman spectroscopy measurements. Additionally, preliminary
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44 release studies were performed at 37°C in 1 mM NaCl. This study sought to identify an
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46 appropriate surface-functionalized MSNs delivery system for further biomedical applications.
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2. Materials and Methods

2.1 Materials

Tetraethyl orthosilicate (TEOS, Aldrich), cisplatin (CDDP, Aldrich), *n*-cetyltrimethylammonium bromide (C₁₆TAB, Aldrich), standard solution of sodium hydroxide (2 M NaOH, Aldrich), ammonium nitrate (NH₄NO₃, Fisher Chemicals, ≥ 99.0 %), hydrochloric acid (HCl, Fisher Chemicals, 35-38 %), ethanol (C₂H₅OH, Fisher Chemicals, 95 %), (3-mercaptopropyl)triethoxysilane (MPTES, Fluorochem), (3-triethoxysilyl)propylsuccinic anhydride (TESP, Fluorochem), poly(ethylene glycol) monomethyl ether amine (mPEG₁₀₀₀₀-NH₂, M_w = 11,153 Da, Iris-Biotech), polyethylenimine (PEI₁₈₀₀, MW = 1800 g.mol⁻¹, 50 % (w/w) in water, Aldrich), N-hydroxysuccinimide (NHS, Aldrich, 98 %), (1-ethyl-3-[3-dimethylamniopropyl])carbodiimide hydrochloride (EDC, Aldrich), cisplatin (CDDP, Aldrich, ≥ 99.9 %), orthophenylenediamine (OPDA, Aldrich, ≥ 98.0 %), dimethylformamide (DMF, Aldrich, HPLC grade), dichloromethane (DCM, Aldrich, HPLC grade), diethyl ether (DE, Aldrich, HPLC grade) and RPMI 1640 medium (Biowhittaker) were used without further purification. Chemical modification of MSNs was performed in dry toluene (homemade distillation). Ultrapure water (18 MΩ.cm) was used for all synthesis and purification steps (pure UHQ, Elga, France).

2.2 Synthesis of pure MSNs

Bare mesoporous silica nanoparticles (MSNs) were prepared according to our previous procedure^{40,41}. Briefly, 416.6 mg of CTAB (1.14 mmol) were dissolved in 200 mL of water in a 500 mL four-neck round bottom flask. The solution was purged thrice with a N₂ flow (1 L.min⁻¹) and a 2 M NaOH standard solution protected from ambient air (1.45 mL, 2.90 mmol) was added under the same N₂ flow. After stirring for 2 h at 70°C under a N₂ flow of 150 mL.min⁻¹, 2.01 mL of TEOS were added at 1 mL.min⁻¹ under a N₂ flow of 1 L.min⁻¹. The molar composition of the solution was 1 TEOS: 0.32 NaOH: 0.127 C₁₆TAB: 1235 H₂O. After 2 h of stirring, the pH of the colloidal suspension was adjusted to 7.5 by adding 1 M HCl solution. The mixture was stirred for another 24 h under static N₂ atmosphere. The sample

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3 was separated by centrifugation (25 000g, 20 min, 20°C). The solid cake was redispersed in
4 ultrapure water using an ultrasonic cleaner for 1 min. The centrifugation-redispersion
5 procedure was repeated until the conductivity of the supernatant was lower than 10 μ S/cm.
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7 Water was then slowly removed by rotary evaporation at 80°C for 1.5 h. The sample was
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9 heated at 90°C under vacuum for 1 h to ensure the complete removal of the solvent. The
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11 template was extracted by using the chemical route proposed by Lang *et al.* in order to
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13 prevent irreversible aggregation of nanoparticles observed with calcination . Typically, 600
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15 mg of MSNs were refluxed in a $\text{NH}_4\text{NO}_3/\text{EtOH}$ (2 g/100 mL) mixture for 1 h. This procedure
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17 was repeated four times and centrifugation (12 000g, 10 min, 20°C) was used to wash the
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19 particles between each step. At the end of this process, MSNs were then extensively rinsed
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21 with ethanol. After complete removal of the solvent under vacuum, the powder was stored in
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23 a desiccator. In these storage conditions, structural properties of the MSNs are preserved at
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25 least 8 months. These mesoporous nanoparticles were referred to as MSNs.
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30 **2.3 Grafting of carboxylic groups at the surface of MSNs by silanization (MSNs-** 31 **COOH)**

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33 Mesoporous silica nanoparticles were first synthesized according to the procedure described
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35 for pure MSNs until the centrifugation-redispersion step. Then, the nanoparticles were
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37 dispersed in ethanol. In order to remove adsorbed surfactants at the surface of the
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39 nanoparticles, a Soxhlet extraction has been performed in ethanol for 24 h (Fig. 1(a)). The
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41 resulting nanoparticles were then collected by centrifugation (12 000g, 10 min, 20°C),
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43 washed, redispersed with deionized water and finally freeze-dried. Grafting of carboxylic
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45 groups on the surface of MSNs was performed according to a reported procedure published
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47 for mesoporous materials^{43,44}. Briefly, 150 mg of lyophilized MSNs were degassed under
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49 vacuum for 12 h and suspended in 15 mL of dry toluene. Then, 34 μ L of TESP (120 μ mol)
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51 were added dropwise to the mixture. After 24 h of stirring under static N_2 atmosphere, the
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53 obtained solid was washed 3 times with a mixture of diethyl ether and dichloromethane
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55 (1:1, v/v) and finally dried at 60°C for 12 h in air. The template was then extracted by using
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57 the method described for pure MSNs. After complete removal of the solvent under vacuum,
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the powder was stored in a desiccator. These nanoparticles were referred to as MSNs-COOH.

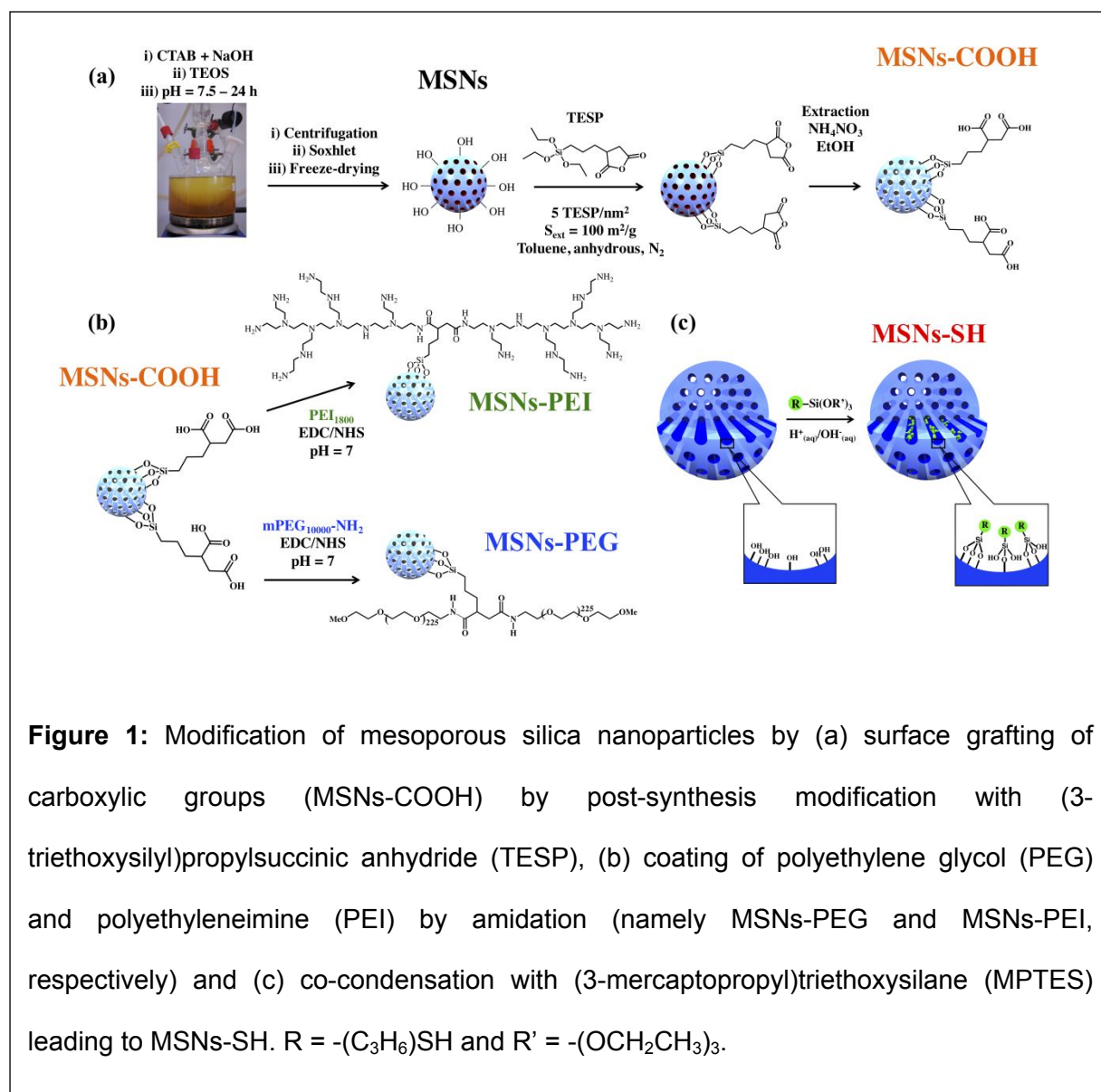


Figure 1: Modification of mesoporous silica nanoparticles by (a) surface grafting of carboxylic groups (MSNs-COOH) by post-synthesis modification with (3-triethoxysilyl)propylsuccinic anhydride (TESP), (b) coating of polyethylene glycol (PEG) and polyethyleneimine (PEI) by amidation (namely MSNs-PEG and MSNs-PEI, respectively) and (c) co-condensation with (3-mercaptopropyl)triethoxysilane (MPTES) leading to MSNs-SH. R = -(C₃H₆)SH and R' = -(OCH₂CH₃)₃.

2.4 Coating of polymers by amidation (MSNs-PEI and MSNs-PEG)

PEI₁₈₀₀ or mPEG₁₀₀₀₀-NH₂ were grafted to MSNs-COOH in the presence of EDC and NHS (EDC:COOH = NHS:COOH = 2:1) (Fig. 1(b)). The molar composition was 1 COOH: 5 NH₂: 10 EDC: 10 NHS. In a typical procedure, 100 mg of NHS were dissolved in DMSO before adding 5 mL of ultrapure water, EDC (160 mg dissolved in 5 mL of ultrapure water) and 10 mL of MSNs-COOH suspension (150 mg in 10 mL of water, pH = 7). To this, PEI₁₈₀₀ (450 mg of a solution at 50 % in weight) or mPEG₁₀₀₀₀-NH₂ (4.8 mg) dissolved in 5 mL of

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3 water were subsequently added to the reaction mixture and let stirred for 24 h at 20°C. The
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5 resulting nanoparticles were then intensively washed with ultrapure water. After complete
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7 removal of the solvent under vacuum, powders were stored in a desiccator. The
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9 nanoparticles were referred to as MSNs-PEI and MSNs-PEG.

11 **2.5 Synthesis of mercapto-functionalized mesoporous silica nanoparticles (MSNs- 12 SH)**

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15 The synthesis of MSNs-SH was achieved by a co-condensation procedure using the same
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17 protocol as described above for pristine MSNs. The mixture containing the
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19 organoalkoxysilane precursor (MPTES) and TEOS was added to the CTAB solution under
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21 N₂ flow of 1 L.min⁻¹ (Fig. 1(c)). The amount of organoalkoxysilane was 10 mol% of the total
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23 amount of silane. The pH aging process, product isolation and surfactant removal were
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25 carried out by the method described above except that at the end of the template removal
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27 process, a supplementary step was processed by redispersing the pellet in a solution of 10
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29 mL concentrated hydrochloric acid in 100 mL of ethanol. After refluxing for 1 h, the MSNs-
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31 SH were separated by centrifugation (12 000g, 10 min) and extensively washed with
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33 ethanol. After complete removal of the solvent under vacuum, the powder was stored in a
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35 desiccator. These nanoparticles were referred to as MSNs-SH.

37 **2.6 Physico-chemical characterizations of the nanomaterials**

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40 MSNs samples were characterized by nitrogen physisorption analysis at 77 K using a
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42 Micromeritics ASAP 2020 setup. Samples were degassed prior analysis at 363 K. The
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44 specific surface area (S_{BET}) of the materials was calculated using BET method in the relative
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46 pressure range between 0.06 and 0.25. The pore-size distribution (D_p) and pore volume (V_m)
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48 were determined from the desorption branches of the isotherms using the BJH-method at
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50 $P/P_0 \leq 0.75$ in order to remove the contribution of interparticle mesoporosity. The structural
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52 properties of the samples were characterized by low angle powder X-Ray Diffraction (XRD)
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54 using a Bruker D8 Advance diffractometer equipped with Vantec linear PSD, using CuK α
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56 radiation (35 kV, 35 mA). The diffraction patterns were recorded over the range 0.5°-10°
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58 (2θ), with a 0.017° step and a step time of 3 s. The hydrodynamic diameter of the particles
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3 was measured by Dynamic Light Scattering (DLS) at 173° angle using a Malvern Nano ZS
4 instrument (Malvern Instruments, UK) equipped with a 4 mW He-Ne laser ($\lambda = 633$ nm). The
5 mean particle size, D_H , was obtained from a fit of a single exponential to the correlation
6 function with the corresponding polydispersity index (PDI). Each measurement was carried
7 out in triplicate. The plotted data are mean values of the three independent measurements.
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9 Zeta potential measurements were performed on a zeta-Nanosizer (Malvern Instruments,
10 UK) supported by a DTS Nano V6.20 software. The nanomaterials were dispersed in 10^{-2} M
11 NaCl aqueous solutions at around 1 mg/mL. The pH of the solution was adjusted from 3 to
12 11 by addition of HCl or NaOH solutions. The decomposition of organic species was studied
13 by thermogravimetric analysis (TA instruments, Discovery, USA). The heating rate was
14 2 °C.min⁻¹ from 25°C to 800°C under air flow (25 mL.min⁻¹). Sample weight was around 5-
15 10 mg. Precision of the instrument is 0.1 wt%. The elemental analysis of the materials was
16 obtained from a Thermo Scientific Flash EA 1112 HT. The morphology and the
17 mesostructure of the prepared materials were characterized using a Transmission Electron
18 Microscope (TEM, JEM2100 LaB₆ cathode, JEOL, Japan) operating at 200 kV and fitted with
19 a high tilt pole piece achieving a point-to-point resolution of 0.25 nm. A drop of the diluted
20 suspension was placed on a 300-mesh carbon film coated copper grid. After solvent
21 evaporation (ethanol), the size of 100 particles was measured to determine the intensity-
22 weighed mean diameter of the particles, d_{TEM} . FTIR characterizations of dried powders were
23 performed at room temperature using a Bruker Equinox 55. Dried powders were disposed
24 between 2 pellets of KBr. Solid-state nuclear magnetic resonance (NMR) experiments were
25 performed on an ASX 400 spectrometer (Bruker) operating at a Larmor frequency of 79.49
26 MHz. The samples were placed in a 7.5-mm MAS probe and spun at 10 kHz. To obtain
27 quantitative spectra for all samples, single-pulse excitation with continuous decoupling was
28 used for ²⁹Si MAS NMR, with a 5 μ s $\pi/6$ pulse duration and a recycle delay of 60 s. ²⁹Si CP
29 MAS NMR spectra were used to fix chemical shifts and were acquired with a 5 s recycle
30 delay, 2 μ s $\pi/6$ (1H) pulse duration and 3 ms contact time. It was checked that no relaxation
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3 effect could affect relative line intensities in ^{29}Si experiments; a recycle delay of 240 s did not
4 lead to any change in the relative ratio of the individual components as obtained from
5 spectral decomposition. Solid-state NMR spectra were reconstructed using Dmfit software⁴⁵.
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7 The Raman spectra of MSNs-SH were recorded using a Renishaw InVia setup with a 532-
8 nm incident laser wavelength. Conversely, loaded-MSNs were characterized as follows.
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10 STEM-HAADF micrographs were taken on a JEOL JEM 2100F instrument equipped with a
11 FEG type cathode operating at 200 kV. Scanning electron microscopy (SEM) was performed
12 using a JEOL JSM 7600F instrument. Samples for SEM were prepared by dispersing the
13 powders on the conductive carbon tape. For Raman spectroscopy of the loaded samples, a
14 785-nm incident laser wavelength was used instead to avoid photoluminescence.
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24 **2.7 Loading of cisplatin by adsorption or impregnation**

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26 To study the adsorption capacity of the nanomaterials, CDDP (20 mg) was completely
27 dissolved in 20 mL of water (1 mg/mL) and aged for 6 h in order to get its first hydrolysis
28 product $[\text{Pt}(\text{NH}_3)_2\text{Cl}(\text{OH}_2)]^{+46}$. Then, the prepared MSNs-SH, MSNs-PEI, MSNs-PEG and
29 pure MSNs (100 mg) were added to the CDDP solution (100 mg of MSNs/20 mg CDDP) and
30 sonicated. After stirring for 18 h in the dark at 20°C, the resulting suspensions were
31 centrifuged (25 000g, 15 min, 20°C). Supernatants were then collected and the CDDP
32 content was determined by derivatization with *o*-phenylenediamine (OPDA) as previously
33 described with slight modifications⁴⁷. In a typical procedure, 100 μL of a CDDP solution
34 (standards or supernatants) were mixed with 30 μL NaCl solution (5 M in water) and 870 μL
35 ultrapure water. The solution was kept in the dark for 5 days at 20°C. Then, 1 mL of OPDA
36 solution (2.15 mg/mL in DMF) was added and the mixture was heated for 30 min at 90°C,
37 allowed to cool down, and then the volume was completed to 10 mL with a mixture of DMF
38 and water (7:3, v/v). The maximum absorbance of the product was then recorded at 705 nm
39 (UV-2550, Shimadzu, Japan). For loading cisplatin by impregnation, the same protocol used
40 to load CDDP by adsorption was applied, except that the resulting suspensions were dried in
41 the dark at 40°C for 24 h after the adsorption step. Then, the resulting powders were
42 collected and dispersed in ethanol. After 15 min of settling, the supernatants were collected
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3 and centrifuged (25 000g, 15 min, 20°C). Finally, the loaded nanomaterials were dried under
4 vacuum and the CDDP content was measured by inductively coupled plasma atomic
5 emission spectroscopy (ICP-AES) performed on an Analytik Jena AG contra 700 instrument.
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9 **3. Results and discussion**

10 **3.1 Overall description of the functionalization methods**

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12 Non-functionalized pure-silica MCM-like materials (pristine MSNs) were synthesized
13 according to our previous procedure leading to stable colloidal suspensions of nanoparticles
14 with a diameter of 160 nm and with a high structural stability^{40,41}. As described in Fig. 1(a),
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16 Pristine MSNs were then coated by the reaction between MSNs free hydroxyl groups and (3-
17 triethoxysilyl)propylsuccinic anhydride, to afford carboxyl-functionalized MSNs (MSNs-
18 COOH). This step was performed in dry toluene after eliminating the template at the surface
19 of the nanoparticles using a Soxhlet extraction. After freeze-drying the nanoparticles without
20 altering the structure, the nanohybrids (MSNs-COOH) were coupled with amino-terminated-
21 PEG chains (PEG-NH₂) or low molecular weight branched PEI by EDC coupling, to afford
22 MSNs-PEG or MSNs-PEI^{48,49} (Fig. 1(b)). A co-condensation approach was also employed
23 to introduce mercaptopropylsilane into the silica networks^{50,51} (Fig. 1(c)).
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37 **3.2 Physico-chemical properties of PEG or PEI-functionalized mesoporous** 38 **nanomaterials**

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40 TEM analysis showed that the surface-functionalized nanoparticles were flattened spheres,
41 a morphology similar to that of pristine MCM-41 nanoparticles, proving that the surface
42 modification did not affect the morphology (Fig. 2(a-d and Fig. S1(a-d)). TEM images also
43 did not reveal the presence of bonds between particles (Fig. 2(a-d)) and DLS showed
44 narrow particle size distributions (Fig. 2(e)). The size of functionalized MSNs was very
45 similar to that of pristine MSNs ($d_{\text{TEM}} \sim 150$ nm and $D_{\text{H}} \sim 200$ nm), indicating that no
46 aggregates were formed during the modification procedures (Table 1). The nanoparticles
47 were rather monodispersed in size as demonstrated by PDI and c_v lower than 0.23 and 0.20,
48 respectively (Table 1). In addition, MSNs-PEI and MSNs-PEG were colloidally stable for
49 several days in an aqueous solution containing 1 mM NaCl, pH = 7 (Fig. S2).
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X-Ray diffraction (XRD) patterns further confirmed the typical structure of MCM-41 materials (Fig. S3). Well-defined (100) peaks, along with the (110) and (200) peaks, characteristic of the 2D hexagonal mesoporous structure were observed, indicating that the surface modification procedure, including the Soxhlet and freeze-drying steps, did not alter either the MCM-41-like structure or the lattice parameter a_0 (Table 1). The honeycomb-like mesoporous structure was also confirmed by TEM (Fig. 2).

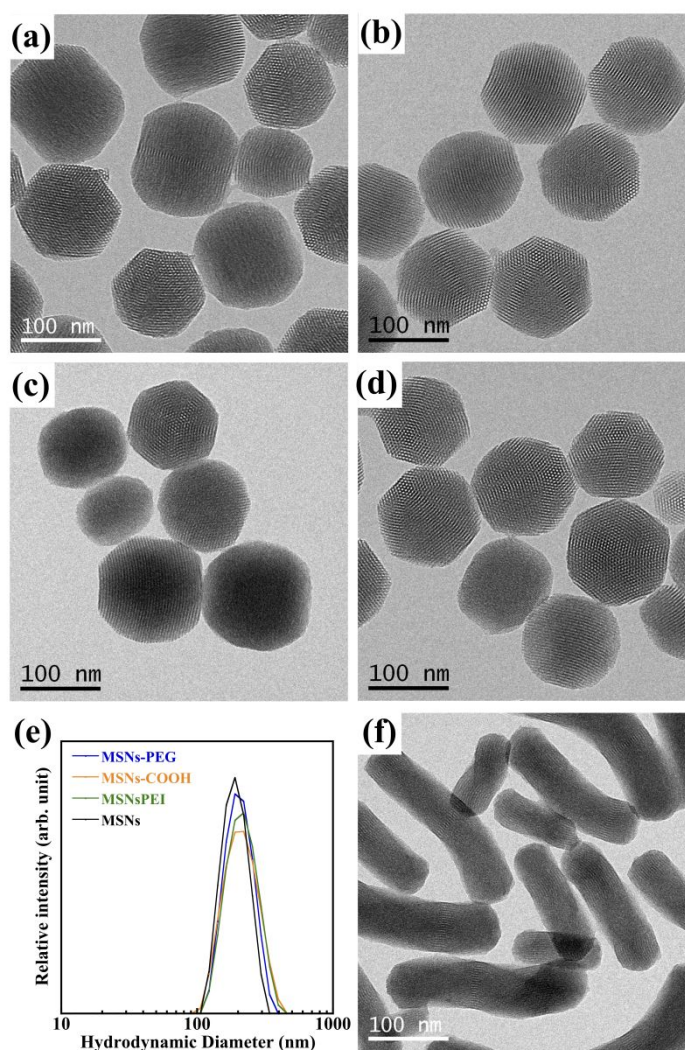


Figure 2: TEM images of (a) pure MSNs (MSNs), (b) MSNs-COOH, (c) MSNs-PEI, (d) MSNs-PEG, (e) the corresponding size distributions obtained by DLS and (f) TEM image of MSNs-SH.

Table 1: Properties of the functionalized mesoporous silica nanoparticles.

Sample	D_H^a (nm) (PDI^b)	d_{TEM}^c (nm) (c_v^d)	a_0^e (nm)	S_{BET}^f (m^2/g)	V_m^g (cm^3/g)	D_p^h (nm)	T_{TGA}^i (molec/nm^2) (mmol/g)	T_{EA}^j (molec/nm^2) (mmol/g)
MSNs	221 (0.23)	130 (0.20)	4.85	1062	0.96	2.8	-	-
MSNs-COOH	203 (0.10)	142 (0.14)	4.70	904	0.75	2.6	1.21COOH 0.56	1.48COOH 0.69
MSNs-PEG	197 (0.05)	139 (0.15)	4.74	455	0.31	2.2	0.10PEG 0.02	0.09PEG 0.02
MSNs-PEI	224 (0.22)	144 (0.17)	4.68	580	0.39	2.1	0.50PEI 0.08	0.74PEI 0.12
MSNs-SH	-	70(L)/327(I) (0.21(L)/0.36(I))	4.17	1225	0.75	2.3	0.67SH 1.36	0.61SH 1.20

^a D_H : mean size of the extracted nanoparticles. [NaCl] = 10^{-3} M. Data are means of three independent measurements.

^bPDI: polydispersity index (width of the distribution) from the Cumulants analysis.

^c d_{TEM} : mean TEM diameter calculated by measuring 100 particles ($\Sigma d_i^3 / \Sigma d_i^2$). (L) and (I) represent the width and the length of the particles, respectively.

^d c_v : relative standard deviation, $c_v = \sigma / d_{TEM}$ where σ is the standard deviation of the distribution.

^e a_0 : lattice parameter ($a_0 = 2d_{100} / \sqrt{3}$) where d_{100} represents the spacing of (100) for the extracted samples.

^f S_{BET} : BET specific surface area calculated in the relative pressure range between 0.06 and 0.25.

^g V_m : pore volume calculated at $P/P_0 \leq 0.75$ in order to remove the contribution interparticle mesoporosity.

^h D_p : BJH pore diameter calculated from desorption branch.

ⁱ T_{TGA} : Determined by TGA by subtracting the weight loss determined for pristine MSNs (for MSNs-SH and MSNs-COOH) or MSNs-COOH (for MSNs-PEG and MSNs-PEI).

^j T_{EA} : Determined by EA. Relative elemental contents of carbon, sulfur and nitrogen were used respectively for MSNs-PEG/MSNs-COOH, MSN-SH and MSNs-PEI. Specific surface area of 560 m^2/g and 100 m^2/g were considered for MSNs-COOH and MSNs-PEG/PEI, respectively. Subtraction of carbon contents with the corresponding materials were used to determine the grafting densities of MSNs-COOH, MSNs-PEG and MSNs-PEI.

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3 The textural properties of pristine and functionalized-MSNs were evaluated by nitrogen
4 adsorption-desorption technique. BET (Brunauer-Emmet-Teller) and BJH (Barrett-Joyner-
5 Halenda) methods were used to calculate the surface area (Table 1) and plot the pore size
6 distribution (Fig. S3). Type IV isotherms were observed for all samples with a sharp capillary
7 condensation step between 0.2 – 0.4 p/p_0 (Fig. S3). A hysteresis loop at high relative
8 pressure (~ 0.9) was observed and attributed to inter-particle porosity. After addition of
9 carboxylic silane, the sorption properties of the nanomaterials did not change significantly
10 since the specific surface area (S_{BET}) slightly decreased from 1062 m^2/g to 904 m^2/g , the
11 pore volume (V_m) from 0.96 to 0.75 cm^3/g and the pore diameter (D_p) from 2.8 to 2.6 nm
12 (Table 1). However, after polymer grafting, N_2 adsorption isotherms showed a significant
13 decrease of the capillary condensation step at 0.2 – 0.4 p/p_0 which also shifted to lower
14 pressure. Consistently, S_{BET} , V_m and D_p drastically decreased (Table 1). It shows that part of
15 the pores is not accessible anymore or some pores have been partially obstructed,
16 suggesting that the grafting has occurred both at the external surface of the MSNs and
17 slightly inside the pores. Sorption studies performed on pristine MSNs after Soxhlet
18 extraction corroborate this latest point. Indeed, the high specific surface area after Soxhlet
19 extraction ($S_{\text{BET}} = 560 \text{ m}^2/\text{g}$) indicates that the condensation of the carboxylic silane was
20 partly possible at the entrance of the pores, allowing subsequently the grafting of the
21 polymer inside the channels of MSNs.

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43 ζ -Potential measurements indicated an Isoelectric point (IEP) at 3.8 for pure-MSNs, which is
44 in good agreement with previous reports^{52,53} (Fig. 3(a)). ζ -potential of MSNs measured at
45 pH7 shifted from positive (+30 mV) to negative (-30 mV) after the Soxhlet extraction (not
46 shown), thus proving the surface of the MSNs was free of surfactants. ζ -potential of MSNs-
47 COOH was negative along the range of pH, confirming the presence of acid groups onto the
48 surface of MSNs. For MSNs-PEI, the IEP shifted at pH 9.6 reflecting the presence of basic
49 groups onto the surface of MSNs. This value is in good agreement with the literature^{24,54}.

Neutral polymer like PEG was grafted at the surface of the MSNs as potentials were very close to 0 mV along the range of pH (Fig. 3(a)).

Grafting densities were determined using TGA and EA. Thermogravimetric analyses of the materials are presented in Fig. 3(b) (started at 130°C to compensate for different water losses) and Fig. S4 (full analyses with derivatives). As described for pristine MSNs (Fig. S4), the weight losses between 25-150°C and 150-400°C were respectively attributed to physisorbed and chemisorbed water (8 and 4.2 %, respectively) and corresponds to 3.7

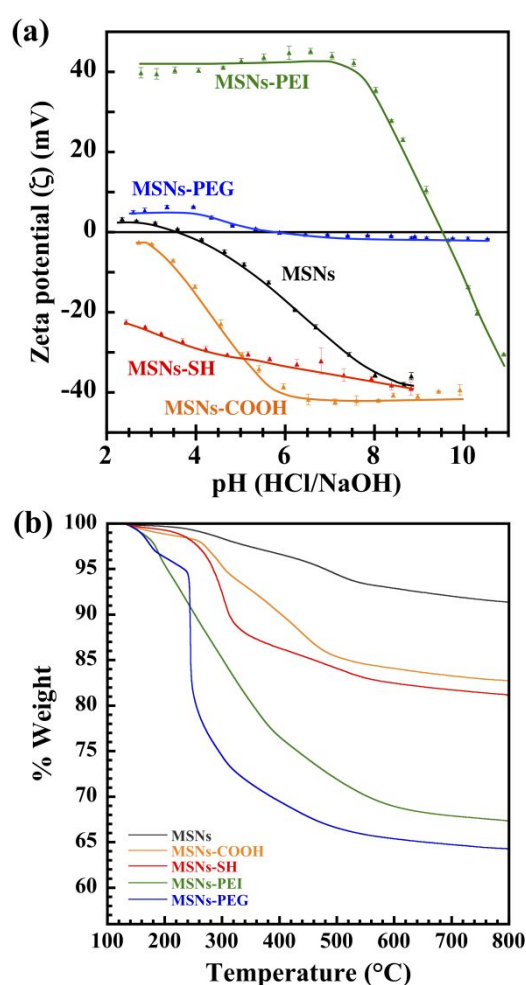


Figure 3: Properties of the functionalized mesoporous nanoparticles obtained by (a) zeta potential measurements and (b) thermogravimetric analysis (starting at 130°C to compensate for different water losses). $[\text{NaCl}] = 10^{-2} \text{ mol.L}^{-1}$ for zeta potential measurements.

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3 molecules of H₂O per nm², in good agreement with the literature⁵⁵. The weight loss after
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5 400°C was attributed to the condensation of silanol groups which can occur until 1100°C⁵⁶.
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7 For functionalized-MSNs, the weight losses between 25-150°C due to physisorbed water
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9 were lower than that of pristine MSNs. The weight losses due to the presence of the
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11 functional groups generally occurred between 200°C and 600°C (Fig. S4). For MSNs-
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13 COOH, the weight loss observed before 200°C was attributed to the formation of the
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15 succinic anhydride⁵⁷. For MSNs-COOH, the grafting density was calculated by subtracting
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17 the weight loss determined for pristine MSNs at 200°C. For MSNs-PEG and MSNs-PEI,
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19 such subtraction was performed using MSNs-COOH at 70°C. Grafting densities obtained by
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21 TGA and EA were in very good agreement and varied from 0.02 to 0.69 mmol/g or from 0.09
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23 to 1.48 molec/nm² depending on the functional group (Table 1). Compared with the number
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25 of PEI/nm², the number of PEG/nm² was very low (only 6 % of carboxylic groups were used)
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27 and is attributed to the greater steric hindrance and coiling and consequently less effective
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29 surface binding of the PEG-NH₂ to the carboxylic surface³⁵. FTIR spectroscopy was used to
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31 confirm the presence of the functional groups as well as the amide formation (Fig. 4(a)). For
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33 all samples, FTIR showed typical bands of the silica framework⁵⁸ between 1300 cm⁻¹ and
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35 500 cm⁻¹ (Fig. 4(a)): $\nu_{as}(\text{O-Si-O})$ at 1078 cm⁻¹, $\nu_s(\text{O-Si-O})$ at 807 cm⁻¹, $\nu_{as}(\text{Si-O-Si})$ at
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37 1221 cm⁻¹ and $\nu(\text{Si-OH})$ at 970 cm⁻¹. PEI vibrations observed at 2938 cm⁻¹ (C-H asymmetric
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39 stretching) and 2846 cm⁻¹ (C-H symmetric stretching) were identified on both PEI and MSNs-
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41 PEI spectra while missing for pure MSNs spectrum (Fig. 4(a)), highlighted by double stars)⁵⁹.
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43 Similarly, PEG vibrations were identified on PEG and MSNs-PEG spectra while missing from
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45 pure MSNs spectrum: C-H stretching modes of the methyl and methylene groups of the
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47 polymer chain between 2980-2880 cm⁻¹ (three stars), CH₂ wagging at 1340 cm⁻¹ (four stars)
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49 and CH₂ scissoring at 1465 cm⁻¹ (five stars)²³. For all spectra, the bending mode vibration of
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51 water⁶⁰ is observed at 1628 cm⁻¹. In addition, C=O stretching vibration at 1719 cm⁻¹ (single
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53 star) was only observed for MSNs-COOH and MSNs-PEG and confirmed our TGA/EA
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55 results showing that a low number of carboxylic groups was used for the conjugation with
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PEG. Disappearance of this vibration band for MSNs-PEI highlighted that more carboxylic groups was used for the conjugation with PEI. For both MSNs-PEG and MSNs-PEI, the band observed at 1700 cm^{-1} could be characteristic of the free remaining carboxylic groups. Finally, vibrations around 1550 cm^{-1} (1558 and 1569 cm^{-1} for MSNs-PEI and MSNs-PEG, respectively), assigned as Amide II (N-H bending and C-N stretching), were observed for MSNs-PEG and MSNs-PEI, thus proving successful amide bond formation^{61,62}. Solid state ^{29}Si MAS NMR provided information about the silicon environment and the degree of functionalization. Cross-polarized ^{29}Si MAS NMR (qualitative) was first used to settle chemical shifts (Fig. 4(b)), allowing to determine peak areas obtained using a single pulse

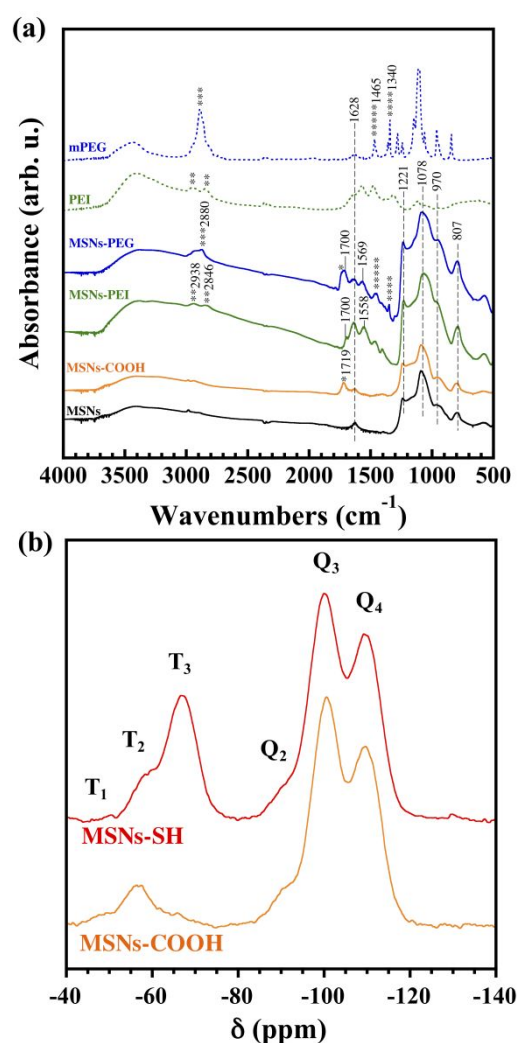


Figure 4: Spectroscopic characterizations of the mesoporous silica nanoparticles by (a) FT-IR performed under air and (b) Cross-polarized ^{29}Si MAS NMR.

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3 experiment (Fig. S5). For MSNs-COOH, typical resonances of Q^{2-4} ($Q^n = (\text{SiO})_n(\text{OH})_{4-n}$) for
4 the siloxane moieties were observed at -91 (Q^2), -100 (Q^3) and -110 (Q^4) ppm. As expected,
5 the grafting process resulted in a decrease in the intensity of the Q^3 resonance and a
6 concomitant increase in the intensity of the Q^4 resonance (Fig. S5). Consequently, the ratio
7 of the relative peak areas of the deconvoluted peaks, Q^4/Q^3 , increased from 1.6 to 1.9 with
8 the functionalization. Upon grafting the surface with carboxylic groups, the ^{29}Si MAS NMR
9 spectrum showed three additional resonances corresponding to the incorporated
10 organosilanes ($T^m = \text{RSi}(\text{OSi})_m(\text{OH})_{3-m}$, $m=1-3$) observed at -48 (T^1), -58 (T^2) and -67
11 (T^3) ppm.
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24 3.3 Physico-chemical properties of the thiol-functionalized MSNs

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26 By introduction of thiol groups in mesoporous silica using a co-condensation approach,
27 anisotropic nanoparticles were formed, as observed by TEM (Fig. 2(f) and Fig. S1(f)). The
28 diameter of the rods was highly uniform with a size of 70 nm ($c_v = 0.21$) while the length was
29 327 nm ($c_v = 0.36$) (Table 1). Due to the anisotropic shape of the nanoparticles, DLS was
30 inappropriate. The pore system appeared to be well-ordered at a local scale with parallel
31 pores aligned to the long axis of the rods (Fig. 2(f)). Such morphology has been already
32 observed by several authors^{50,63,64}. Sadasivan *et al.* proposed that repulsive interactions
33 between the negatively charged cylinder walls and soluble deprotonated thiol-containing
34 silica surfactant micelles, along with steric hindrance due to disulfide linkages would favor
35 the particle growth along the channel direction to produce nanofibers with a hexagonal
36 cross-section⁶³. The diffraction pattern and sorption isotherm were also typical of ordered
37 MCM-41 type materials (Fig. S3). Compared to pristine MSNs, a reduction in the pore
38 diameter D_p and lattice parameter a_0 was observed (Table 1), while the specific surface area
39 was higher (1225 m^2/g). As previously observed in the literature^{65,66}, ζ -potential
40 measurements exhibited negative potentials for MSNs-SH over the range of pH (Fig. 3).
41 Grafting densities determined by TGA and EA were in very good agreement and showed
42 that the nanoparticles contained 1.2 mmol/g or 0.6 SH/ nm^2 (Table 1).
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3 Raman spectroscopy is much more sensitive to thiols groups than FTIR spectroscopy due to
4 the small dipolar moment⁶⁷. For this reason, the spectroscopic properties of MSNs-SH were
5 determined by Raman instead of FTIR spectroscopy (Fig. S6). First of all, Raman bands of
6 silica were observed for both MSNs-SH and MSNs : 486 cm^{-1} (vibration of tetra
7 cyclosiloxane rings), 800 cm^{-1} (symmetric stretching modes of SiO_4 units (Si-O-Si)) and
8 978 cm^{-1} (vibration associated with the Si-OH stretching mode of the surface hydroxyls)⁵⁰. In
9 addition, as described in Fig. S6(a), the main peaks of the MPTES precursor were identified
10 within the MSNs-SH spectrum : 651 cm^{-1} (Si-C stretching), 1304 cm^{-1} (out of plane CH_2
11 bending) and 1452 cm^{-1} (CH_2 and CH_3 in plane bending). The Raman band at 509 cm^{-1}
12 assigned to the stretching of the di-sulfur bond demonstrated the dimerization of some thiol
13 groups. This band was also observed in the precursor but with a smaller intensity. The peak
14 at 2581 cm^{-1} assigned to the stretching of the S-H bond were identified in the spectrum of
15 MSNs-SH (Fig. S6(b)). Compared to pristine MSNs, the increase of the peaks between 2800
16 and 3000 cm^{-1} (CH stretching region) observed for the MSNs-SH spectrum was obvious and
17 demonstrated that thiol groups were potentially grafted to the silica (Fig. S6(b)).

18
19 Additional spectroscopic evidence for the anchoring of the thiol groups was obtained from
20 ²⁹Si CP MAS NMR (Fig. 4(b) and Fig. S5). Resonances of Q^{2-4} were observed at -91 (Q^2), -
21 100 (Q^3) and -110 (Q^4) ppm. The ratio of the relative peak areas of the deconvoluted peaks,
22 Q^4/Q^3 , was lower than that of pristine MSNs and was 1.4, which suggests a lower
23 polycondensation degree of the silica network. Unlike MSNs-COOH, MSNs-SH showed only
24 T^2 (-58 ppm) and T^3 (-67 ppm) resonances with relative peak areas of 3 and 12 %, respectively.
25 For MSNs-COOH, the relative peak areas for T^1 , T^2 and T^3 were 0.4, 2.8 and
26 0.2 %, respectively, which suggests that mercaptosiloxane moieties were more tightly
27 incorporated to the silica surface compared to carboxysilane moieties.

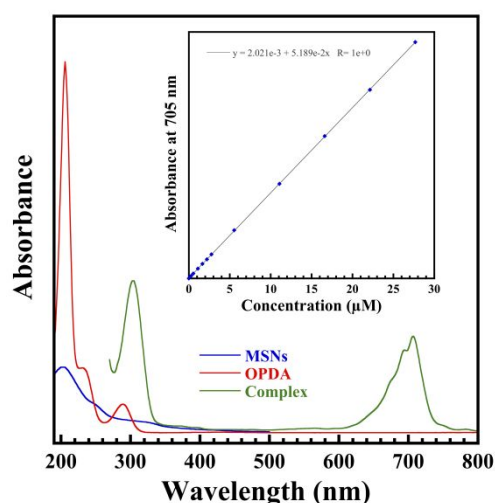
3.4 Quantification of cisplatin using OPDA derivatization

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29 The relative adsorption capacity of cisplatin in pristine or functionalized MSNs was studied,
30 using a sensitive and simple method allowing the quantification of cisplatin within
31 supernatants. As cisplatin has a low absorbance in the UV region, no fluorochrome and over

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3 time its UV profile drifts due to hydrolysis⁴⁶, quantification methods involving derivatization
4 were developed⁶⁸. For instance, Golla *et al.* were first to develop a method based on the
5 derivatization of CDDP using *o*-phenylenediamine (OPDA)⁶⁹, giving rise after only 30 min at
6 90°C to a complex that strongly absorbs at 705 nm. With slight modifications, this method
7 was used. First, its specificity was evaluated by comparing UV spectra of OPDA, MSNs and
8 cisplatin-OPDA (Fig. 5). UV spectrum of the complex showed a strong absorbance peak at
9 705 nm with no interference. Linearity was studied in the concentration range of 0.55 μM to
10 27.67 μM by visual inspection of a calibration curve (inset of Fig. 5) and by calculating the
11 regression equation and the correlation coefficient (R^2) using the method of least squares.
12 The correlation coefficient was 0.999, which indicates a good linearity since a regression
13 coefficient $R^2 > 0.998$ is generally considered as evidence of acceptable fit of the data to the
14 regression line⁷⁰.
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29 3.5 Loading of cisplatin by adsorption or impregnation

30 Although CDDP is soluble in DMF (20 mg/mL) or DMAC (18 mg/mL), the use of such
31 solvents during the loading step is to be avoided due to their high toxicity. CDDP is highly
32 soluble in DMSO (350 mg/mL), which is usually well tolerated with no observable toxic
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Figure 5: UV-Visible spectra of MSNs, *o*-phenylenediamine (OPDA) and complex formed by reacting cisplatin with OPDA at 90°C for 30 min in a mixture of DMF:water (1:1, v/v).

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3 effects to cells up to 1 % (v/v). However, the use of DMSO to dissolve CDDP must be
4 prohibited as it has been reported to inhibit its cytotoxicity and its ability to initiate cell
5 death³⁷. In the literature, cisplatin loading is generally performed by stirring the
6 nanomaterials at 37°C in an aqueous solution (PBS or water) of CDDP up to 48 h
7 (adsorption). Herein, our nanomaterials were loaded either by adsorption or impregnation.
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9 The adsorption study was performed by stirring the nanomaterials at 20°C for 18 h in a 6 h
10 aged solution of CDDP prepared in water at 1 mg/mL. Under these conditions, the most
11 abundant species was its first hydrolysis product $[\text{Pt}(\text{NH}_3)_2\text{Cl}(\text{H}_2\text{O})]^+$ according to cisplatin
12 hydrolysis reactions⁷¹. Results were expressed as the mass percentage of CDDP compared
13 to the total mass of sample (Table 2). The final results for the adsorbed CDDP determined
14 by UV-Vis spectroscopy showed the strongest affinity within the following sequence MSNs-
15 SH (16.09 wt%) > MSNs-PEG (3.32 wt%) > MSNs-PEI (0.99 wt%) > MSNs (0.36 wt%).
16 Compared to the other nanoparticles, MSNs-SH showed the highest amount of adsorbed
17 cisplatin, close to the maximal adsorption value of 16.67 wt%. This result is consistent with
18 the literature showing that the formation of complexes with Pt ions and biological thiols such
19 as GSH, metallothioneine and other protein thiols defend the cell against CDDP by limiting
20 the amount of drug available for binding to DNA⁷². Several authors also demonstrated the
21 formation of a strong covalent Pt – S bond after adsorption of CDDP onto mercapto-silylated
22 silica surfaces^{38,39}. Compared to pristine MSNs, MSNs-PEG showed greater adsorption
23 capacity, probably reflecting the high electronegativity of the oxygen atoms in the PEG
24 chains. Contrastingly, no significant difference was also observed between pristine MSNs
25 and MSNs-PEI even if the first hydrolysis product of CDPP ($[\text{Pt}(\text{NH}_3)_2\text{Cl}(\text{H}_2\text{O})]^+$) is highly
26 reactive to nucleophiles such as amines⁷³. This was confirmed by using ICP-AES as a good
27 correlation was observed with the adsorption results determined by UV-Vis spectroscopy
28 (Table 2). At the loading pH, the fact that the amino groups are protonated could limited the
29 amount of adsorbed CDDP. Compared to the literature, our CDDP loadings obtained by
30 adsorption are lower. For instance, under the same loading conditions, Arian *et al.* reported
31 a higher CDDP loading with amino-functionalized MCM-41 (6.1 wt%) materials, despite a
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low CDDP loading was obtained with carboxy-functionalized MCM-41 (1.5 wt%)³⁴. In their study, the number of complexing sites, which is linked to the number of amino groups introduced in the materials, was considerably higher than that of MSNs-PEI and could explain why the drug loading was higher. While using DMSO during the loading step, different authors also reported high CDDP loading up to 20 wt% using carboxylic groups to coordinate with platinum groups^{31,32}. Vivero *et al.* also reported a CDDP loading of 12.5 wt% with pristine MSNs, but still, DMSO was used to load CDDP⁷⁴. Lately, Sun *et al.* described a drug loading capacity of 10.1 wt% by using a nanosized porous/hollow PEI functionalized Gd₂O₃/Fe₃O₄ composite. More recently, Huang *et al.* developed MSNs based copolymer composites and reported an incredibly high drug loading of 26.5 wt% through the use of coordination between carboxyl groups of (poly(MPC-co-IA)) and CDDP³⁶. In order to increase the drug loading capacity of our nanocarriers, CDDP was loaded by impregnation. The nanomaterials were stirred at 20°C in an aqueous solution of CDDP for 18 h. Then, the suspensions were dried at 40°C for 24 h. The aim was to promote preferential attachment of CDDP within the pores of the MSNs by slowly evaporating the solvent. After washing the nanomaterials, high CDDP loadings were obtained within the following sequence: MSNs-SH (16.07 wt%) > MSNs-PEI (11.30 wt%) > MSNs-PEG (10.07 wt%) > MSNs (3.91 wt%)

Table 2: Amount of adsorbed and impregnated CDDP determined for pristine and functionalized-MSNs. (Pt/Si) distributions were determined by SEM using EDS.

Sample	Adsorbed CDDP ^a (%)	Adsorbed CDDP ^b (%)	Impregnated CDDP ^b (%)	% (Pt/Si) _{local}	% (Pt/Si) _{global}	Ratio (local/global)
CDDP@MSNs	0.36 ± 0.89	0.82 ± 0.04	3.91 ± 0.20	2.32	7.15	0.32
CDDP@MSNs-PEG	3.32 ± 0.57	2.90 ± 0.15	10.07 ± 0.50	2.83	4.80	0.59
CDDP@MSNs-PEI	0.99 ± 0.38	0.88 ± 0.04	11.30 ± 0.57	6.48	8.01	0.81
CDDP@MSNs-SH	16.09 ± 0.29	16.09 ± 0.80	16.07 ± 0.80	16.57	16.95	0.98

^aDetermined by UV-Vis spectroscopy.

^bDetermined by ICP-AES.

(Table 2). However, due to the loading process employed, some CDDP crystals were expected to be observed outside the pores. Thus, CDDP distribution within the loaded nanomaterials was investigated.

3.6 Cisplatin distribution by SEM, TEM and STEM-HAADF

In the literature, very few studies paid attention to the spatial distribution of CDDP once loaded into MSNs. Herein, CDDP loaded MSNs were characterized by SEM using Backscattered-Electron (BSE) mode (Fig. 6). CDDP crystals (brighter area) with size ranging from few micrometers to 30 μm were observed for all samples, except for CDDP@MSNs-SH. EDS analysis (Fig. 6: arrow number 1) showed that it corresponded to partially hydrolyzed cisplatin as the Pt/Cl atomic ratio was found equal to 0.45 (Pt/Cl = 0.5 without hydrolysis). To estimate the proportion of CDDP remaining outside the nanomaterials, two

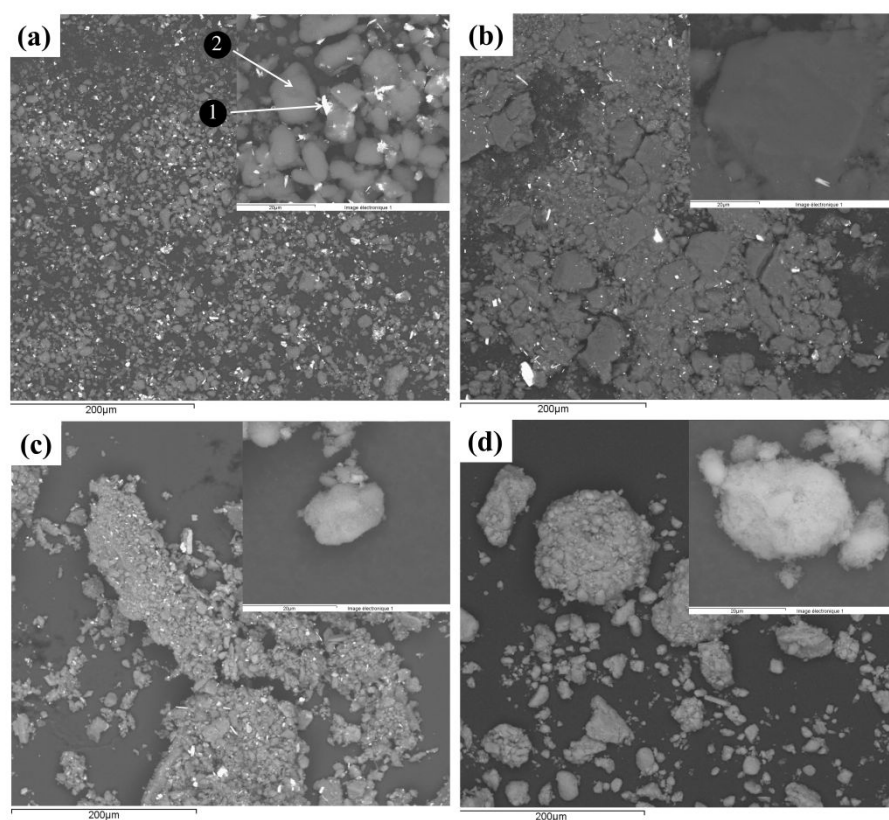


Figure 6: SEM images of (a) CDDP@MSNs, (b) CDDP@MSNs-PEG, (c) CDDP@MSNs-PEI and (d) CDDP@MSNs-SH.

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3 different Pt/Si atomic ratios were determined: $(\text{Pt/Si})_{\text{local}}$ and $(\text{Pt/Si})_{\text{global}}$. On the one hand,
4 $(\text{Pt/Si})_{\text{local}}$ was directly determined on a cluster of loaded-materials nanoparticles taking care
5 to avoid CDDP crystals, as depicted by the arrow number 2 (Fig. 6). On the other hand,
6 $(\text{Pt/Si})_{\text{global}}$ was determined on a large zone (around 0.25 mm²) covering both loaded-
7 materials and CDDP crystals. Then, the homogeneity of cisplatin distribution was evaluated
8 by calculating the following ratio: $(\text{Pt/Si})_{\text{local}}/(\text{Pt/Si})_{\text{global}}$ (Table 2). The ratio (local/global)
9 showed that the homogeneity decreases within the following sequence MSNs-SH (0.98) >
10 MSNs-PEI (0.81) > MSNs-PEG (0.59) > MSNs (0.32). This trend demonstrated that more
11 CDDP was loaded within the pores of the MSNs-SH, contrary to pristine MSNs. Cisplatin
12 distribution was also characterized at the nanometer scale. For CDDP@MSNs, STEM-
13 HAADF images showed nanocrystals of CDDP (Fig. 7). For the other samples, CDDP was
14 homogeneously distributed (Fig. 7). For CDDP@MSNs, TEM and STEM-HAADF images
15 obtained by tilting the sample along y-axis (0°, +20°, +40°), showed that the nanocrystals of
16 CDDP were aligned with the pores and evidenced that CDDP was encapsulated into the
17 pores (Fig. 8).

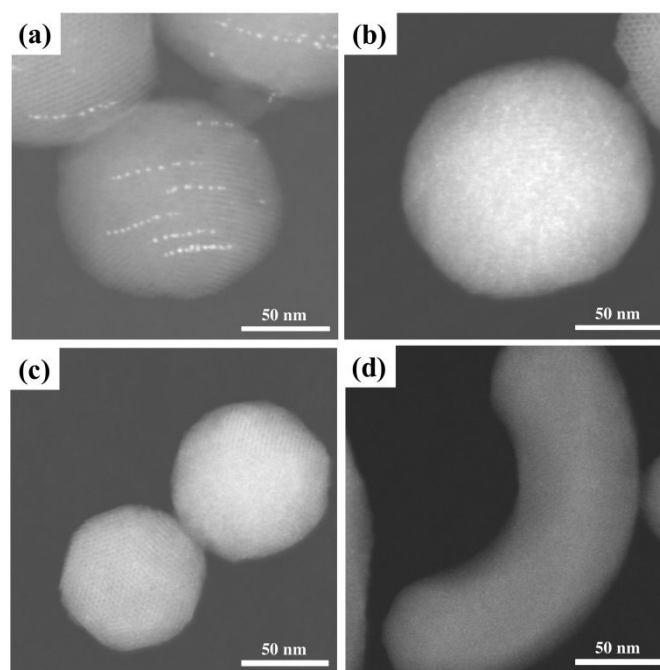


Figure 7: STEM-HAADF images of (a) CDDP@MSNs, (b) CDDP@MSNs-PEG, (c) CDDP@MSNs-PEI and (d) CDDP@MSNs-SH.

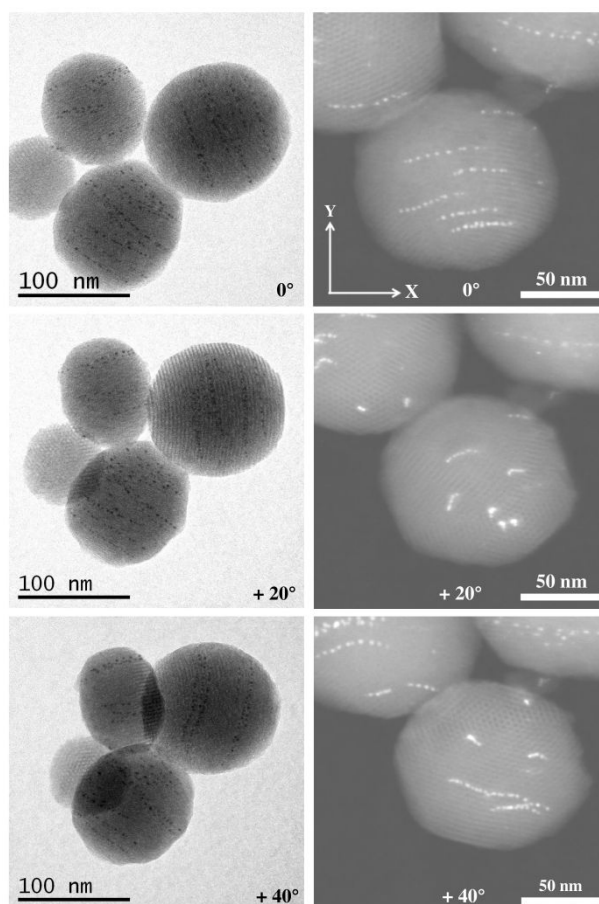


Figure 8. TEM (left) and STEM-HAADF (right) images of CDDP@MSNs after tilting the sample along y-axis.

3.7 Spectroscopic properties of cisplatin-loaded nanomaterials

The Raman spectrum of cisplatin (Fig. 9(a)) showed 4 vibration bands at 162, 255, 322 and 524 cm^{-1} that were respectively attributed to the skeletal in-plane deformation modes $\delta_{\text{Cl-Pt-Cl}}$, $\delta_{\text{N-Pt-N}}$ and the Pt-Cl ($\nu_{\text{Pt-Cl}}$) and Pt-N ($\nu_{\text{Pt-N}}$) stretching modes^{75,76}. The Raman bands of CDDP were observed for all spectra, except for CDDP@MSNs-SH (Fig. 9(d)) and CDDP@MSNs-PEI (Fig. 9(e)). This confirmed the presence of CDDP either in the pores or outside the particles. For CDDP@MSNs-SH, two broad vibration bands were observed at 310 and 486 cm^{-1} . The vibration band at 310 cm^{-1} corresponded to the Pt-S stretching mode ($\nu_{\text{Pt-S}}$), proving coordination of CDDP by thiol groups. The vibration band at 486 cm^{-1} attested the presence of silica (fourfold siloxane rings, $\nu(\text{SiO}_4)$, D1)^{77,78}. For CDDP@MSNs-PEI, two new

bands appeared at 410 cm^{-1} and 598 cm^{-1} . Further data obtained with MSNs functionalized with amino groups and loaded with CDDP showed a vibration band at 396 cm^{-1} (data not shown), suggest formation of a complex between the primary amines of the amino groups and cisplatin. The broader band observed in the case of CDDP@MSNs-PEI may result from the different nature of the amines (primary, secondary and tertiary). However, the literature is lacking in this domain and more work is required to determine the composition of the complexes associated with these bands.

3.8 Preliminary study of CDDP release

To determine which of the surface-functionalized MSNs was the most appropriate in terms of CDDP release, preliminary release kinetics were performed using the well-known dynamic dialysis method⁷⁹. Loaded MSNs were dispersed in 1 mM NaCl at a silica concentration of 4 mg/mL. Then, 2.5 mL of these suspensions were introduced in a dialysis bag (MWCO: 12 – 14 kDa). Platinum concentration outside the dialysis membrane was monitored over time by ICP-AES after the dialysis bag was immersed in 47.5 mL of RPMI 1640 culture medium

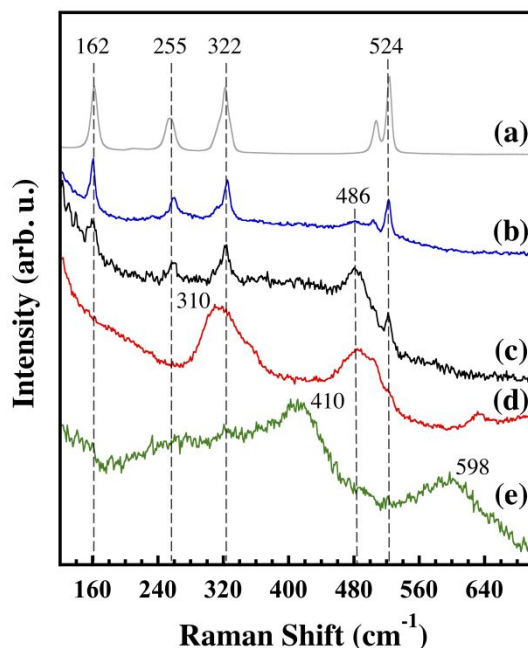


Figure 9: Raman spectra of (a) CDDP, (b) CDDP@MSNs-PEG, (c) CDDP@MSNs, (d) CDDP@MSNs-SH and (e) CDDP@MSNs-PEI.

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3 incubated at 37°C (Fig. S7). As expected, the observation of a burst release was closely
4 related to the homogeneity factor and followed this sequence after only 1 h of incubation:
5 MSNs (29.3 %) > MSNs-PEG (23.1 %) > MSNs-PEI (2.5%) > MSNs-SH (0 %). As the
6 homogeneity decreases, the burst release increases. This preliminary study also showed
7 that despite no burst release was observed with CDDP@MSNs-SH, only 1.4 % of platinum-
8 based species were released after 192 h of incubation, which confirm that platinum has a
9 strong affinity for sulfur. However, the release of CDDP from this material could be triggered
10 by the presence of biochemical thiols such as glutathione. Further studies are needed to
11 confirm this hypothesis. On the opposite, a sustained release was observed for
12 CDDP@MSNs, CDDP@MSNs-PEG and CDDP@MSNs-PEI with 53.8, 40.4 and 22.9 % of
13 platinum-based species being released after 72 h of incubation, time for which the release
14 process ended.

30 **4. Conclusion**

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32 In this study, we have successfully synthesized MCM-41 silica nanoparticles functionalized
33 either with (3-mercaptopropyl)triethoxysilane (MPTES) by co-condensation or with
34 polyethylene glycol or low molecular weight branched polyethyleneimine using a
35 postsynthesis grafting procedure. With a number of analytical tools, we have shown that the
36 thiol groups were incorporated into the pores of the nanoparticles. Moreover, surface-
37 modification with polymers allowed us to obtain monodispersed nanoparticles with a size of
38 about 160 nm, with high porosity and colloidal stability for several days in aqueous solutions,
39 which make these hybrid MSNs suitable for drug delivery applications.

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41 To the best of our knowledge, this is the first study to describe the spectroscopic properties
42 and spatial distribution of CDDP once loaded into MSNs. Depending on the functional group,
43 platinum-based species were either dispersed in the nanomaterials as nanocrystals or as
44 uniformly distributed species. Interestingly, spectral signature of CDDP was lost once loaded
45 into PEI or thiol-functionalized MSNs.

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3 Finally, PEI-functionalization showed the most promising results in terms of drug release
4 considering the absence of burst release and a sustained release over several days.
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6 Combined with the property of low molecular weight PEI to increase the delivery of
7 anticancer drugs into cancer cells while presenting a low cytotoxicity profile, this study
8 highlights the potential of PEI-functionalized MSNs for efficient and safe cancer therapy
9 using cisplatin.
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18 **Supporting Information**

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20 High magnification TEM images, colloidal stability of MSNs-PEI and MSNs-PEG, BET
21 isotherms, BJH pore distributions, XRD patterns and TGA measurements, single-pulse ²⁹Si
22 MAS NMR spectra of MSNs-SH, MSNs-COOH and pristine MSNs, Raman spectra of MSNs-
23 SH and CDDP release profiles.
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31
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36 spectroscopy.
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45 **Conflict of interests**

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47 The authors declare no conflict of interest.
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