Research Article

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Mesoporous silica nanoparticles functionalized with folic acid for targeted release Cis-Pt to glioblastoma cells

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Abstract: This work reports the preparation, characterization, and a drug release study of mesoporous silica nanoparticles (MNPSiO$_2$) functionalized with folic acid (FA) and loaded with Cis-Pt as a targeted release system to kill glioblastoma cancer cells. The MNPSiO$_2$ were synthesized by the Stöber method using hexadecyltrimethylammonium bromide as the templating agent, which was finally removed by calcination at 550$^\circ$C. The folic acid was chemically anchored to the silica nanoparticles surface by a carbodiimide reaction. Several physicochemical techniques were used for the MNPSiO$_2$ characterization, and a triplicate in vitro Cis-Pt release test was carried out. The release Cis-Pt experimental values were fitted to different theoretical models to find the Cis-Pt release mechanism. The cytotoxicity evaluation of the MNPSiO$_2$ was performed using LN 18 cells (human GBM cells). Homogeneous and well-defined nanoparticles with well-distributed and homogeneous porosity were obtained. The spectroscopic results show the proper functionalization of the mesoporous nanoparticles; besides, MNPSiO$_2$ showed high surface area and large pore size. High correlation coefficients were obtained. Though the best fitted was the Korsmeyer-Peppas kinetic model, the Higuchi model adjusted better to the results obtained for our system. The MNPSiO$_2$-FA were highly biocompatible, and they increased the cytotoxic effect of Cis-Pt loaded in them.

Keywords: Silica nanoparticles, drug release vehicle, brain cancer cells, cell uptake

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1 Introduction

Over the last few decades, mesoporous silica nanoparticles (MNPSiO$_2$) have been a focus of interest due to their morphological features such as high surface area (over 700 m$^2$/g), large pore volume (higher than 1cc/g), adjustable pore size (1.6-10 nm), superior surface properties, functionalization surfaces (internal and external), modifiable morphology (particle shape and size), and biocompatibility [1–3]. Thereby, MNPSiO$_2$ have significant applications in many research fields such as catalysis, adsorption, separation, sensing, and drug delivery [4, 5]. Mesoporous materials are usually prepared using self-assembled surfactant molecules as templates around which the silica precursors condense [4]. Then, the removal of the template carried out through heat treatment leads to a porous material. Size, morphology, pore size, and pore structure of MNPSiO$_2$ can be designed through the manipulation of the reaction parameters such as temperature, pH, surfactant concentration, and silica precursors, among others [6, 7].

There are mainly four methods to prepare MNPSiO$_2$, namely template-directed method, sol-gel method, microwave-assisted technique, and chemical etching technique [1, 8–11]. The sol-gel procedure is widely used to develop inorganic oxide networks from the hydrolysis and condensation of inorganic precursors (metal salts or metal alkoxides) [1]. The Stöber method, which is based
on sol-gel chemistry, is used to prepare uniform colloidal silica spheres [4, 6, 12, 13]. The Stöber procedure involves the hydrolysis and condensation of tetraethyl orthosilicate (TEOS) in alcohols used as solvents (ethanol, methanol, etc.) in the presence of water and a base-catalyst (ammonia, ammonium hydroxide, etc.) at room temperature. It is suggested that the uniform growth of the particles is based on the base-catalyzed reaction, which causes a rapid formation of nuclei due to the union of the constituent solutes in a kinetically controlled way. The Stöber method is the most common procedure for the synthesis of silica nanoparticles because it allows control of particle size and pore size distribution. MNPSiO$_2$ can be synthesized with different structural features, including particle sizes, shapes, surface areas, nanoscale pore sizes, pore volumes, and surface-modifying groups on their internal or external surfaces. Because of their exceptional textural and their biocompatibility properties, MNPSiO$_2$ have potential biomedical applications in drug delivery for cancer treatment, infectious treatment, and different bone diseases [1, 3, 5, 14–18].

The surface of MNPSiO$_2$ can be modified with cell-specific moieties such as organic molecules, peptides, and antibodies to achieve cell type or tissue specificity [3, 14–16, 19]. Specifically, the functionalization of the surface of MNPSiO$_2$ with ligands allows to interact selectively with specific cellular receptors overexpressed in tumor cells [20]. Some of the targeting ligands that can be linked to the MNPSiO$_2$ surface are transferrin, epidermal growth factor, folic acid, concanavalin A, and vascular endothelial growth factor, among others [20–22].

The connection of nanotechnology and medicine, specifically in drug delivery for drug targeting, is an essential subject in the biomedical knowledge [23, 24]. This is particularly true for cancer therapies, where the loss of the antitumor drug before it reaches targets results in adverse side effects and limits the drug’s effectiveness due to the unspecific drug action on healthy tissue. The functionalization of MNPSiO$_2$ with folic acid gives the nanoparticles the ability to release drugs selectively through their link to receptors on the cell surface [19].

Folate receptors are glycosylphosphatidylinositol proteins, which are expressed in many human cancer cells, where the level of folate receptors expression is related to the stage of cancer [25]. Folate receptors have a high affinity to the FA and its derivatives and mediate the delivery of the 5-methyltetrahydrofolate as physiological folate to the cell. However, non-cancerous cells possess a deficient level of folate receptors, while cancer cells bind to the FA group. Folate receptacles are overexpressed in different types of human cancer cells, such as breast, colorectal, ovarian, brain, epithelial, and lung cancer. As its expression is limited in healthy cells, it could be used as a biomarker for cancer [25, 26].

On the other hand, Glioblastoma (GB) is the most common and most lethal primary brain tumor in adult patients with an average patient survival of 12-15 months. The incidence is in the range of 2-3 cases per 100,000 per year [27] — current treatments include maximal safe resection, followed by radiotherapy with concomitant and adjuvant temozolomide. Surgical resection is not curative for glioblastoma, and even after gross total resection of the apparent tumor followed by radiotherapy with concurrent and adjuvant chemotherapy, tumor progression occurs [28, 29]. Cisplatin (Cis-Pt) is used as a first-line chemotherapy drug used against various cancers, including glioblastomas, metastatic melanomas, peritoneal, and pleural mesotheliomas [30]. The antitumor properties of Cis-Pt are attributed to the kinetics of its chloride ligand displacement reactions. Cis-Pt interacts with guanine and adenine N7 atoms located in the DNA major groove, leading to DNA bending and interfering with its replication and transcription, as well as other nuclear functions, thus avoiding cancer cell proliferation and tumor growth. However, the intravenous Cis-Pt administration can lead to nephrotoxicity, bone marrow toxicity, intractable vomiting, peripheral neuropathy, deafness, seizures, and blindness. Although Cis-Pt is toxic, it is used successfully in the clinical field.

In a previous work [31] we reported the synthesis of silica-based nanoparticles that served as vehicles for the release of Cis-Pt. In continuation of that previous work, in this present study we are reporting the functionalization of MNPSiO$_2$ with folic acid in order to release Cis-Pt molecules in a targeted way. The standard treatment for GBM consists of surgical removal of as much of the tumor as possible. However, the removal of the tumor is incomplete. Our proposal consists of placing the MNPSiO$_2$—FA/Cis-Pt systems in the cavity where the cancerous tumor was removed, so that the Cis-Pt reaches specifically the remaining cancer cells and eliminates them. Thus, the side effects are reduced, and crossing the blood-brain barrier is avoided, which hinders drugs reaching the brain.
2 Experimental

2.1 Sample preparation

Chemical substances

Sodium hydroxide (NaOH) (SIGMA-ALDRICH), Hexadecyltrimethylammonium bromide (CTAB) (SIGMA), Tetraethyl orthosilicate (TEOS) (ALDRICH, 98%), Dimethyl sulfoxide (DMSO) (SIGMA), Folic acid (AF) (SIGMA, 97%), diclohexylcarbodiimide (DCC) (SIGMA-ALDRICH, 99%), (3-aminopropyl)triethoxysilane (APTES) (SIGMA-ALDRICH), Toluene (SIGMA-ALDRICH), N-hydroxysuccinimide (N-HSA) (ALDRICH, 99%), Cis-Pt (ALDRICH, 99.9%).

MNPSiO$_2$

To prepare this sample we followed the procedure reported by [31]. Brief: 7 mmol of NaOH and 2.74 mmol of CTAB were mixed in 26.6 mol of deionized water, then temperature was raised to 70°C. Subsequently, 0.022 mol of Tetraethyl orthosilicate (TEOS) was slowly added. Once the addition of TEOS was completed, the final mixture was left with moderate agitation for 2h at 70°C. The supernatant was removed from the precipitate, and it was washed with a water/methanol (50:50 v/v) mixture. The sample was dried at 60°C for 24 hours. Finally, the sample was heated at 550°C for 4h in a conventional muffle.

MNPSiO$_2$-FA

A molar ratio of FA:MNPSiO$_2$ of 0.26:1.0 was used, and for the functionalization we followed the procedure reported in [32]. 0.126 mol of DMSO, 0.66mmol of FA, 0.76 mmol of N-HSA, 0.72 mmol of DCC, and 0.84 mmol of APTES were mixed. The entire mixture was kept under stirring for 6h. After this time, 12 ml of toluene and 150mg of MNPSiO$_2$ were mixed. After 6h, the nanoparticles in toluene were added to the mixture containing folic acid. The final mixture was left under stirring at room temperature for 72 h. Finally, the mixture was filtered and the solid was washed with toluene, DMSO, water, and acetone. The resulting powder was dried at 60°C by 12 h.

MNPSiO$_2$-FA/Cis-Pt

A solution of 10 mg of Cis-Pt in 20 ml of water (10% Cis-Pt by weight of MNPSiO$_2$-FA) was added to 100 mg of MNPSiO$_2$-FA. The solution was stirred at room temperature for 24 h, and the water was removed and left to dry at 20°C for three days. The weight percentage of Cis-Pt in MNPSiO$_2$-FA was 1:10.

2.2 Characterization

Scanning Electron Microscopy (SEM)

The SEM images of the samples were obtained using a Quanta 3D FEG Microscope.

Atomic Force Microscopy (AFM)

The AFM-images were acquired in a transmission electron microscope JEM2100 (VEECO) with filament LaB6. This apparatus is an opto-mechanical microscope that allows obtaining topographic images.

High-Resolution Transmission Electron Microscopy (HR-TEM)

A high-resolution transmission electron microscopy analysis was performed on a JEM 2100 microscope with voltages from 80 to 200 kV filament LaB6. For application, the samples were previously suspended in an Isopropanol solution and taken to a sonicator to increase the dispersion. Then samples were placed on a copper plate and introduced into the equipment for reading.

Fourier Transform Infrared spectroscopy (FTIR)

In this technique the KBr method was used, 5mg of the solid samples were mixed and ground with 95 mg of potassium bromide (KBr). The powder was then pressed into a translucent tablet, which was placed in an Affinity-1 Shimadzu spectrophotometer for measurement tasks. The range of analysis was 4000 to 400 cm$^{-1}$ wavenumbers.
X-Ray Diffraction (XRD)

The samples were characterized by X-ray diffraction (XRD) in Siemens D-500 equipment. The signal strength was measured by scanning in steps in the range of 4 and 80 degrees with a step of 0.03° and a measurement time of 2 s per point.

N₂ adsorption-desorption

The specific surface area, average pore diameter, and pore volume were determined from the N₂ adsorption–desorption isotherms. The samples were previously thermally treated at 250°C with a vacuum during 12 h. The N₂ gas adsorption-desorption was carried out at 77.3 K using the BelsorpII equipment. The specific surface areas were determined using the Brunauer–Emmet-Teller (BET) method, while the average pore size and pore volume were determined from the desorption isotherms using the Barret-Joyner-Halenda method.

2.3 Cis-Pt release test

Simulated cerebrospinal fluid (SCF)

SCF was used as a release medium, and it was prepared according to the information reported by [33].

Calibration curve

To build the calibration curve an initial solution of 10 mg Cis-Pt in 100 ml SCF was made. From this solution, 0.5, 1, 2, 4, and 6 ml aliquots were taken and calibrated to 25 ml each with SCF. Subsequently, a 3 ml aliquot was taken from each solution, and its absorbencies were measured in UV-Vis. From the results, a graph of Cis-Pt concentration versus maximum absorbance at 203 nm was made. Linear regression was performed on the resulting curve, and the molar extinction coefficient was determined.

Drug release test

The profile of the in-situ release kinetics of Cis-Pt was performed according to the procedure reported by [31], using artificial cerebrospinal fluid as the medium and evaluated by UV-Vis by measuring absorbance at 203 nm, which corresponded to Cis-Pt signal. The MNPSiO₂-F/A/Cis-Pt powder was slightly pressed to form three small cylinders (5-8 mg) which were individually added to 25 mL of SCF (pH = 7.3) maintaining the release medium with moderate stirring at 25°C for 8 h. At predetermined times, an aliquot of 3 mL was removed from the release medium for its measurement at 203 nm. After being measured, the aliquot was returned to the release medium. The Cis-Pt release procedure was made by triplicate.

2.4 Cytotoxicity evaluation

Cell Culture

LN 18 cells (human GBM cell line) [34] were obtained from American Type Culture Collection (ATCC# CRL-2610), and these were maintained in DMEM medium supplemented with 5% fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin at 37°C in a humidified 5% CO₂ atmosphere. The GBM cells were harvested by trypsinization and plated at 1×10⁵ cell/well in 96-well or 1×10⁵ in 35 mm culture plates. The procedure of cell culture was made according to the one described in [35].

Light microscopy

The morphological changes in LN18 cells by the presence of MNPSiO₂, MNPSiO₂-F/A, MNPSiO₂-F/A/Cis-Pt, and Cis-Pt (100 µg/mL) or by the vehicle (DMSO 1%) in the culture medium were recorded after 48 h, using a light microscope (CKX41, Olympus) at a magnification of 10×.

Tetrazolium reduction assay

After 24h cell growth, the medium was removed and replaced by 100µL fresh medium in the presence of MNPSiO₂, MNPSiO₂-F/A, MNPSiO₂-F/A/Cis-Pt, and Cis-Pt at different concentrations (10-1000 µg/mL) by 48 h. Later, the medium was removed and washed three times with PBS; and incubated with 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution (5mg/mL) at 37°C for 3 h to allow viable cells to convert tetrazolium salts into formazan crystals. The supernatant was removed, and the crystals were dissolved in 100 µL of isopropyl alcohol (acidified 0.04 HCl). Finally, the absorbance was read at 570 nm using a multi-well plate reader (Synergy HT, Biotek). The half inhibitory concentration IC₅₀ values were determined from in vitro dose-response curve. The data were analyzed using GraphPad Prism 8.0 and expressed as mean ± standard deviation. The cytotoxicity was determined using cell viability
= (OD of the experiment samples/OD of the control) × 100.

MTT assay was based in published protocol [36] but with some modifications.

3 Results and discussion

3.1 Scanning Electron Microscopy and Atomic Force Microscopy

Figure 1 reports the images obtained by atomic force microscopy (a) and scanning electron microscopy (b) of the MNPSiO₂ sample. Both figures show the well-defined and homogeneous spherical obtained nanoparticles with a size of approximately 100 nm.

Figure 1: (a) AFM-Image, and (b) SEM-Image of the MNPSiO₂ sample took at × 5,000 of magnification with a voltage of 5.0 kV. The bar is equivalent to 1 µm.

Figure 2: HR-TEM photographs of (a-d) MNPSiO₂, (e) MNPSiO₂-FA, and (f) MNPSiO₂-FA/Cis-Pt samples.
3.2 High-Resolution Transmission Electron Microscopy

Figure 2 corresponds to the HR-TEM images of MNPSiO₂, MNPSiO₂-FA, and MNPSiO₂-FA/Cis-Pt samples. The TEM images show features of silica mesoporous nanoparticles, consisting of highly ordered one-dimensional channels in hexagonally packed meso-structures. Nanoparticles smaller than 100 nm in size in the MNPSiO₂ sample were found (Figure 2a-c). The porosity confirms that the mesoporous structure of MCM-41 could also be obtained (Figure 2b). The pores had sizes of 3-5 nm (Figure 2c-d). The morphological structure of MNPSiO₂ did not change when it was functionalized with folic acid, and when Cis-Pt was loaded (Figures 2e and Figure 2f, respectively).

3.3 FTIR spectroscopy

Figure 3 shows the FT-IR spectra of the MNPSiO₂, MNPSiO₂-FA, and MNPSiO₂-FA/Cis-Pt samples. All samples present the characteristic vibrational modes bands of SiO₂, in agreement with the previous literature [31]. The distinctive bands of the MNPSiO₂ are observed in the 1020-1110 cm⁻¹ interval (Figure 3c). The band at 1089 cm⁻¹ belongs to the asymmetric stretching vibration of Si-O-Si bonds. The small bands found at 966 cm⁻¹ are related to bending vibrations from Si-O-H bonds. The band at 802 cm⁻¹ is associated with Si-O bending vibrations. Besides, another band was observed at 3323 cm⁻¹, which corresponds to hydroxyl groups from water molecules adsorbed on the MNPSiO₂ surface.

The MNPSiO₂-FA spectrum shows important bands that allow the identification of the folic acid linked to the surface of MNPSiO₂ (Figure 3b). The derived band from this spectrum agrees with our recently reported literature [32]. In brief, the band located at 3319 cm⁻¹ corresponds to the N-H stretching vibration from the pteridine ring. The peak at 2937 cm⁻¹ is attributed to vibrational C-H of CH₂ groups from the pteridine ring and glutamic acid group present in the FA. The bands at 1690 and 1608 cm⁻¹ belong to C=O and C=C vibrations in the FA, while the peak at 1526 cm⁻¹ confirmed the formation of an N-MNPSiO₂-FA bond during the functionalization of MNPSiO₂ with the folate [37].

The MNPSiO₂-FA/Cis-Pt spectrum shows the bands related to MNPSiO₂ and Folic acid attached to the silica nanoparticles. However, a stretching vibration of the N-H group, from Cis-Pt at 3305 cm⁻¹, is seen (Figure 3c), while the signals at 1700-1320 cm⁻¹ associated with H-N-H asymmetric and symmetric bending vibrations from Cis-Pt overlap with the signs of folic acid and silica.

3.4 X-Ray diffraction

Figure 4 displays the X-Ray diffraction partners of the MNPSiO₂, MNPSiO₂-FA/Cis-Pt, and Cis-Pt, samples. The MNPSiO₂ diffractogram exhibits the characteristic broad X-ray diffraction curve of the amorphous morphology of SiO₂. In addition to the broad silica peak at 10-30 degrees observed in the MNPSiO₂-FA/Cis-Pt diffractogram, other
peaks were seen at $2\theta = 7$, 14, 16, and 173 degrees, corresponding to the folic acid, which agrees with the data previously reported by [38]. In contrast, the Cis-Pt diffraction pattern contains several thin peaks due to the platinum atom in Cis-Pt. The XRD peaks that corresponded to Cis-Pt did not appear when Cis-Pt was loaded on MNPSiO$_2$-FA nanoparticles. This result agrees with our previously reported work [31], suggesting that Cis-Pt molecules are dispersed totally into MNPSiO$_2$-FA or that the detection levels of the equipment are not enough to detect the low amount of platinum.

3.5 N$_2$ adsorption-desorption isotherm

The Nitrogen adsorption-desorption isotherm of the MNPSiO$_2$ sample shows a characteristic type IV isotherm (Figure 5), indicating a uniform mesopore architecture. No hysteresis loop is observed in the sample. The surface area and pore volume values were calculated to be as high as about 1011 m$^2$/g and 1.10 cc/g, respectively. Moreover, the sample has an average pore size of about 4.35 nm, a value consistent with the observed by HR-TEM and pore size distribution (insert in Figure 5), which indicate the existence of uniform mesopores in the MNPSiO$_2$ sample.

![Figure 5: Nitrogen adsorption-desorption isotherms of the MNPSiO$_2$ sample. The insert corresponds to its pore size distribution.](image)

3.6 Cis-Pt releases tests

Figure 6a reports the calibration curve obtained by plotting five different known increasing concentrations of Cis-Pt versus the absorbance at 203 nm obtained from the UV spectra shown in the insert. Simulated cerebrospinal fluid (SCF) was the aqueous medium to get solutions with different Cis-Pt concentrations. The Lambert-Beer law was used to determine the molar extinction coefficient of Cis-Pt, as shown in Figure 6a.

The in vitro Cis-Pt release test was made by triplicate using SCF as the release medium, and Cis-Pt concentrations were obtained from UV-Vis spectroscopy by measuring absorbance at 203 nm (Cis-Pt signal) at each predetermined time.

The cumulative release of Cis-Pt from the MNPSiO$_2$ sample was calculated according to the following equation [39].

\[
\text{Cumulative Release (\%)} = \left( \frac{M_t}{M_a} \right) \times 100 \quad (1)
\]

where $M_t$ is the Cis-Pt mass released from the MNPSiO$_2$-FA samples at time $t$, and $M_a$ is the estimated Cis-Pt mass loaded in the MNPSiO$_2$-FA samples.

The experimental Cis-Pt release profiles were obtained plotting the cumulative release (\%) values vs. time, as seen in Figure 6b. The insert in Figure 6b shows the spectra taken as a function of time during the release test, where the features Cis-Pt signal can be observed. Figure 6c shows the average Cis-Pt release profile calculated from the experimental results of Figure 6b. A sustained Cis-Pt release as a function of time can be observed. 60% of the Cis-Pt was released approximately after 7h. The primary release mechanism from the mesoporous silica nanoparticles is mainly through a diffusion process, which depends on the porosity properties [31]. In this case, there is no initial release burst due to the large BET surface area, well ordered pores, uniform pore diameter, and high total pore volume of the pure silica nanoparticles, as confirmed by the N$_2$ adsorption-desorption isotherm analysis. A large surface area was suitable to a high Cis-Pt dispersion, while a large pore volume allowed the Cis-Pt to be well housed mostly inside the pores and almost nothing on the nanoparticles’ outer surface. Therefore, the high pores’ homogeneity in the nanoparticles (see Figure 2) allows a Cis-Pt diffusion in a sustained way. Besides, the pore size distribution (insert in Figure 5) shows a narrow pore size distribution, which corroborates what was observed by TEM images. The silica nanoparticles have well-ordered pores that allow a homogeneous suitable diffusion of Cis-Pt from these pores and not from the nanoparticles’ external surface, as it usually happens with other mesoporous materials, that exhibit an initial drug burst.
3.6.1 Theoretical models applied to the experimental Cis-Pt release profiles

To explain the theoretical Cis-Pt release mechanism from the MNPSiO₂, the experimental release values were fitted to various kinetic mathematical models: Zero-order, First-order, Higuchi, Korsmeyer -Peppas, and Hixson-Crowell [40–42].

(1) Zero-order model
Zero-order kinetics defines a process of constant drug release from a drug delivery system, and the drug level remains constant throughout the delivery process. Drug release from the dosage form can be represented by the (2) and (3) equations.

\[ C_t = C_0 - K_0 t \]  

\[ C_t = C_0 + K_0 t \]  

where \( C_t \) is the amount of drug released at time \( t \), \( C_0 \) is the initial concentration of drug at time \( t = 0 \), \( K_0 \) is the zero-order rate constant.

(2) First-order model
The first-order model is suitable for porous drug carriers with insolubility in water. The release of drug which follows first-order kinetics is represented by the (4) equation.

\[ \frac{DC}{dt} = -K_1 C \]  

where, \( K_1 \) is the first-order rate constant, expressed in time⁻¹ or per hour following linear kinetics. After rearranging and integrating the equation, the following (5) equation remains.

\[ \log C = \log C_0 - K_1 t/2.303 \]  

(3) Higuchi model
The Higuchi model concentrates on the drug release from a matrix. The release of a drug from a drug delivery system involves both dissolution and diffusion. Several mathematical equation models describe drug dissolution and release from a drug delivery system. Higuchi equation is a prominent kinetic equation, which evidences drug dissolution studies that are recognized as an important element in drug delivery systems development. Today the Higuchi equation is considered one of the most widely used and the most
well-known controlled-release equation. The classical basic Higuchi equation is represented by (6).

\[ Q = A \sqrt{D (2C_0 - CS) CST} \quad (6) \]

where \( Q \) is the cumulative amount of drug released in time per unit area, \( C_0 \) is the initial drug concentration, \( C_S \) is the drug solubility in the matrix, and \( D \) is the diffusion coefficient of the drug molecule in the matrix.

The simplified Higuchi equation can be represented in the following form (7).

\[ Q = K_H \times t^{1/2} \quad (7) \]

where \( K_H \) is the Higuchi dissolution constant.

(4) Korsmeyer-Peppas model

To describe the drug release from a polymeric system, Korsmeyer and Peppas use a simple relationship represented by the (8) equation:

\[ \frac{Mt}{M_\infty} = K_{kp}t^n \quad (8) \]

where \( \frac{Mt}{M_\infty} \) is a fraction of drug released at time \( t \).

\[ \log(\frac{Mt}{M_\infty}) = \log K_{kp} + n \log t \quad (9) \]

where \( M_t \) is the amount of drug released in time \( t \), \( M_\infty \) is the amount of drug released after time \( \infty \), \( n \) is the diffusional exponent or drug release exponent, and \( K_{kp} \) is the Korsmeyer release rate constant.

In Korsmeyer-Peppas model ‘\( n \)’ is the diffusion exponent that explains the drug release mechanism. According to this model, drug release takes place by Fickian diffusion if it is \( n < 0.43 \), by Anomalous (non-Fickian) diffusion if it is \( 0.43 < n < 0.89 \), by case-II transport if it is \( n = 0.89 \) and by Supercase-II transport, if it is \( n > 0.89 \).

(5) Hixson-Crowell model

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. Therefore, particles of regular area are proportional to the cube root of its volume. Crowell established a relationship between drug release and time, which can be represented by the (10) equation.

\[ W_0^{1/3} - W_t^{1/3} = K_{HC}t \quad (10) \]

where \( W_0 \) is the initial amount of drug in the pharmaceutical dosage form (Amount of drug remaining at time 0); \( W_t \) is the remaining amount of drug in the pharmaceutical dosage form at time \( t \); \( K_{HC} \) is the Hixson-Crowell constant describing surface volume relation.

Table 1: Correlation Coefficients (\( R^2 \)) for linear relationship from different mathematical models of MNPSiO\(_2\)-FA/Cis-Pt sample.

| Mathematical model       | \( R^2 \)     |
|--------------------------|---------------|
| Zero-order               | 0.93357       |
| First-order              | 0.9773        |
| Higuchi                  | 0.98927       |
| Korsmeyer-Peppas         | 1.0, n=1.0    |
| Hixson-Crowell           | 0.78096       |

Figure 7 shows the fitted experimental values to the different theoretical models, and table 1 summarizes the correlation coefficient (\( R^2 \)) obtained for each model as well as the \( n \) value. The correlation coefficient \( R^2 \) is the criteria commonly used to select the model that most reliably describes the general release drug mechanism. In a reliable prediction model, the \( R^2 \) value should be as close to 1 as possible. According to the results in Table 1, the First-order, Higuchi, and Korsmeyer-Peppas models showed an \( R^2 \) greater than 0.95, while the \( R^2 \) values for the zero-order and Hixson-Crowell model were 0.933 and 0.780, respectively. Therefore, the Cis-Pt release mechanism may follow the rules of any of the three models. The first-order model explains the drug release from the matrix where the drug release rate is concentration-dependent [43]. The Higuchi model describes the release of the drug from the insoluble matrix as a square root of time-dependent process based on Fickian diffusion. The Korsmeyer model derives a simple mathematical relationship that describes the drug release from a polymeric system [43]. As shown in Table 1, the value of the release exponent (\( n \)) was 1.0, indicating the presence of a Super Case II, where drug transport type is controlled mainly by the swelling of the drug-loaded systems, and this pattern regularly exhibited a longer period of increased swelling than the period of relaxing [44]. However, this model cannot be used for silica, since this one is not a polymeric matrix that can suffer swelling processes. In our particular case, the Higuchi model effectively describes the Cis-Pt release mechanism from MNPSiO\(_2\).

Some postulates of the Higuchi model are [45]:

1. The initial drug concentration in the matrix must be higher than its solubility in the medium.
2. The mathematical analysis is based on one-dimensional diffusion.
3. The drug is considered in a molecularly dispersed state with particles much smaller in diameter than the matrix thickness.
4. The dissolution of the matrix carrier is negligible.
5. The drug diffusibility is constant.
Figure 7: Mathematical Models fitted to experimental kinetic release of Cis-Pt. SQRT refers to square root, and CBR to cube root.
These postulates are consistent with the release profile obtained from our Cis-Pt/MNPSiO₂ system. Nanoparticles containing Cis-Pt are placed in contact with the liquid medium; the fluid enters through the particle pores; it reaches the Cis-Pt and slowly dissolves it, thus allowing the drug to disperse in the fluid phase [45].

### 3.7 Cytotoxic effects of MNPs over cell viability

The effects of MNPSiO₂, MNPSiO₂-FA, MNPSiO₂-FA/Cis-Pt, and Cis-Pt on the GBM cancer cells are shown in Figure 8; LN18 cells morphology can be observed (Figure 8a). The presence of MNPSiO₂-FA/Cis-Pt and Cis-Pt (100 µg/mL, 48 h) visibly affected cell morphology. With MNPSiO₂-FA/Cis-Pt the cell morphology changed slightly, and some cells began to die. Besides, the cell number decreased, and Cis-Pt induced cells to lose their structure and present round shapes. Cytotoxic effects of mesoporous nanoparticles on cell viability was evaluated (Figure 8b). MNPSiO₂ showed cytotoxicity only at higher concentrations (100µg/mL), but when the nanoparticles were coupled to folic acid (MNPSiO₂-FA), the effect was similar to control cells in all concentrations probed. The MNPSiO₂-FA/Cis-Pt and Cis-Pt affected the cells in a dose-dependent manner, and the magnitude of effects varied depending on the doses. This was better observed in a log graph (Figure 8c). The IC₅₀ values were lower for Cis-Pt exposure than to MNPSiO₂-FA/Cis-Pt, 78.6 µg/mL, and 149 µg/mL, respectively.

### 4 Conclusions

MNPSiO₂ with well-ordered pores and homogeneous morphology were successfully synthesized and functionalized with folic acid. The SEM, AFM, and TEM results show uniform spherical nanoparticles with sizes less than 100 nm. The infrared spectroscopic results revealed the successful functionalization of the MNPSiO₂ with folic acid. Large sur-

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**Figure 8**: Cytotoxic effects of MNPs over cell viability: (a) LM photographs of LN18 cells. (b) Dose-response of mesoporous nanoparticles, (c) Dose-dependent curves of Cis-Pt (squares), and MNPSiO₂-FA/Cis-Pt (circles).
face areas (1011 m²/g), mesopores (4.35 nm), and high pore volume (1.10 cc/g) to MNPSiO₂ were found. These properties were influential in obtaining a sustained Cis-Pt release profile over time. The drug was released in a homogeneous way from the silica pores, without presenting an initial burst release. When the experimental release results were fitted to the mathematical model, high correlation coefficients (r²) were obtained. The best fitted model was the Korsmeyer-Peppas kinetic model (R² = 1.0), indicating that a super case-II transport controls the Cis-Pt release mechanism. However, the model that best fitted our system was Higuchi’s. The theoretical release mechanism of Cis-Pt consists of the initial leaching of the drug into the fluid medium that enters the matrix phase through the pores. Then, the Cis-Pt slowly dissolves into the fluid phase and is released by diffusion through the solvent-filled pores. The MNPSiO₂ functionalized with folic acid were highly bio-compatible. MNPSiO₂-FA loaded with cis-Pt increased the cytotoxic effect of the drug, thus MNPSiO₂-FA/Cis-Pt may have an essential role in GBM cancer treatment, but further in vivo studies using animal models will be necessary for elucidating the mechanism of their action in cancer cells.

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Authors Contribution: Emma Ortiz-Islas developed the project, was in charge of supervising the activities and writing the article.

Anahí Sosa-Arróniz executed the experimental part on the synthesis of the nanoparticles as well as the obtaining of the infrared spectra and the adsorption-desorption of nitrogen.

Ma Elena Manriquez-Ramirez obtained and interpreted the high-resolution transmission electronic micrographs, atomic force images, and electron scanning figures.

C. Ekaterina Rodriguez-Perez developed and supervised the in vitro biological tests using glioblastoma cells.

Francisco Tzompantzi supervised and analyzed the infrared and X-ray diffraction spectra.

Juan Manuel Padilla supervised the activities of student Anahi Sosa and helped write the article.

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