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作品名稱　Synthesis of functionalized mesoporous silica nanoparticles as drug delivery carriers for therapeutic agents

得獎獎項　三等獎

盧森堡國際科學博覽會

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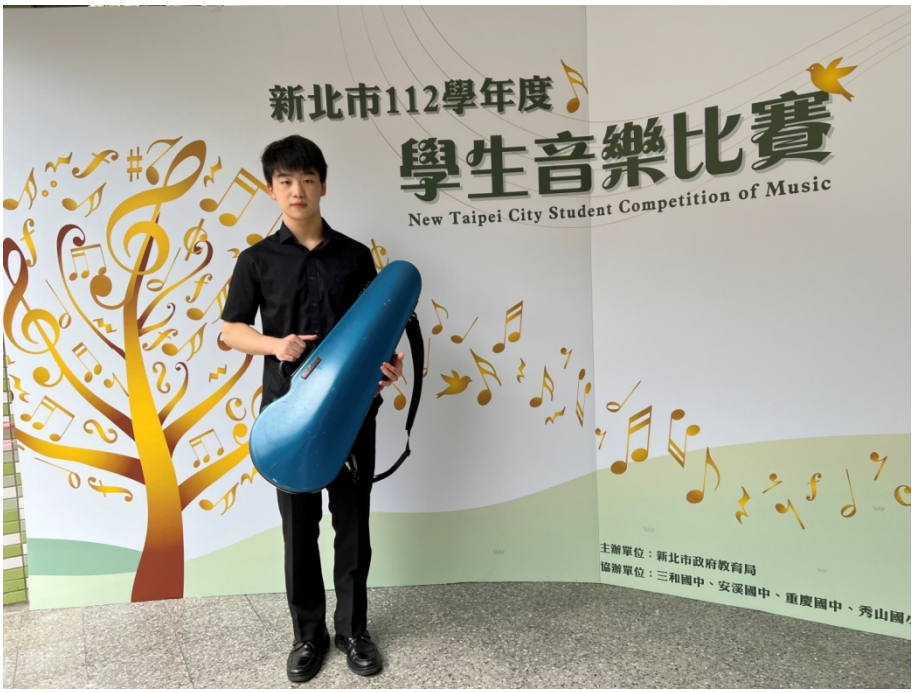
關鍵詞　mesoporous silica nanoparticles、slow release、drug delivery

作者簡介



Hi, I am Sean Lee, a junior at Kang Chiao Internationl School in New Taipei, Taiwan. In this science fair, I dive deep into the world of organic chemistry and its applications, and found chemistry truely fascinating. Apart from spending time in labs, I play the viola and fence in my spare time. I have won multiple music awards, including national champion and first prize in International European Music Competition, and I am a member of my school's chamber piano quintet. Apart from music, I enjoy fencing as well, and I rank second in my city. I am also on the school's robotics team and reached the semifinals in an international VEX robotics event. I'm also an active achiever, participating in many school activities and tutoring kids in the school library. With all those experiences and interest combined, I hope to study chemistry more in the future while maintaining a balanced and enthusiastic life.

摘要

奈米藥物在臨床治療引入了重大創新。多孔性二氧化矽奈米粒子 (mesoporous silica nanoparticle) 具有生物相容性和高孔隙度的特性，被運用在奈米藥物傳遞和標靶治療。奈米粒子經過表面修飾可提高藥物載荷效能以達到有效的治療。

本研究建立一項有效的奈米藥物傳遞載體的開發系統，目標在合成多孔性二氧化矽奈米粒子，使用幾丁聚糖對表面進行修飾，以提升地塞米松藥物載荷和穩定的藥物釋放。應用 X-射線繞射和傅立葉轉換紅外光譜來分析奈米粒子產物和表面官能基。應用分光光譜儀進行測定藥物的載荷和釋放速率。幾丁聚糖修飾包覆的多孔性二氧化矽奈米粒子具有 53.7%的藥物載荷率，比較未修飾的粒子只有 27.9%。藥物釋放實驗，幾丁聚糖包覆的二氧化矽奈米粒子可以延緩藥物釋放，在 5 天釋放 19.7%的地塞米松。本次研究說明幾丁聚糖包覆多孔性二氧化矽奈米粒子是有潛力的奈米藥物載體。

Abstract

Nanomedicine has introduced significant innovations in clinical practice. Mesoporous silica nanoparticles (MSN) with adaptable surface modifications allow accommodation of various therapeutic agents for targeted drug delivery. Dexamethasone is an anti-inflammatory drug which may be used in lowering mortality rates in severe COVID-19 cases.

A simple and smart drug controlled delivery system is developed in this work. This study aims to synthesize functionalized MSNs to achieve prospective loading efficiency of dexamethasone and controlled drug release with constant rate. Synthesis of MSN is verified with X-ray diffractometer. Grafted functional groups with amine and chitosan on MSN are characterized by Fourier transform infrared spectroscopy. The amounts of dexamethasone loading and cumulative release are quantified by ultraviolet–visible spectrophotometer. The pure MSNs present 27.9% dexamethasone loading. The chitosan capped MSNs promote dexamethasone loading efficiency to 53.7%. The release of dexamethasone in the unmodified MSNs reveals early and fast, which shows 50% in 12 hours and 90% after 5 days. The release pattern of dexamethasone in chitosan capped MSNs is constant and delayed, which reveals 19.7% after 5 days. Taken together, this research provides the potential of chitosan capped MSNs as an efficient nanoparticle-based triggered drug delivery system.

1. Introduction

1.1. Background

Nanomedicine has brought about promising advancements in clinical practice to solve unmet medical needs. Nanoparticles serve as carriers for small molecular medicine such as dexamethasone, enhancing their ability to penetrate cell membrane. Within the extensive spectrum of nanoparticles, mesoporous silica nanoparticles (MSN) stand out as a distinct class of inorganic nanoparticles possessing a diverse range of functional properties. This is due to their favorable characteristics, such as good biocompatibility, high porosity, extensive surface area, substantial pore volume, and the ease of their surface modification to accommodate organic and inorganic therapeutic agents for targeting drug delivery. Depending on the porous size and shape, MSN can carry drugs such as protein, amino acids, nucleic acids, antibodies, or even other nanoparticles, efficiently delivering and releasing the drug at regular intervals. The pore size and shape of MSN can be altered by adding chemicals during the synthesis stage. MSNs are classified by their 3D cylindrical form, porous size, and porous shape. There are different types of MSNs, such as hexagonal MCM-41 with round pores, cubic MCM-48 with spherical pores, or laminar MCM-50. Besides, modified shapes of MSNs significantly impact the behavior of the delivery system. Modification of the MSN surface using various components, such as polymers, through direct immobilization or functionalization of the mesostructured surfaces offers the gate-keeping of the drug molecules by ensuring their safety from premature release.

Dexamethasone (DEX) is a drug aimed to treat asthma inflammation, multiple allergies, cardiovascular disorders, and rheumatology. When used in synchronization with other anticancer treatments, it can even alleviate the side effects of chemotherapy, such as nausea and vomiting. Furthermore, steroidal dexamethasone is a recent advancement that significantly reduces the mortality rate among severe COVID-19 cases and helps combat the pandemic.

1.2. Motivation

Often, patients have to take high doses of medicine at regular intervals to attain the ideal therapy effect. However, this treatment involves certain undesired side effects, and it is demanding for certain patients to follow the medicine intake prescriptions every time. For example, patients with dementia will likely forget the prescription schedule and amount, resulting in potential serious consequences and even hindering the curing process. There are multiple drug carriers that improve the safety and efficiency of drug dosing. Due to its biocompatibility, low toxicity and stability, MSN is an excellent candidate for drug loading and release. Furthermore, with advanced surface modification by adding functional groups, MSN can achieve stable controlled drug release, promoting more efficient and safer drug treatment.

1.3. Purpose

The following are three primary objectives of this research:

1. Define the properties and characteristics of MSNs with different types of surface modification.
2. Compare the loading efficiency of DEX in functionalized MSNs
3. Adjust the release profile of DEX in functionalized MSNs at slow and constant rate

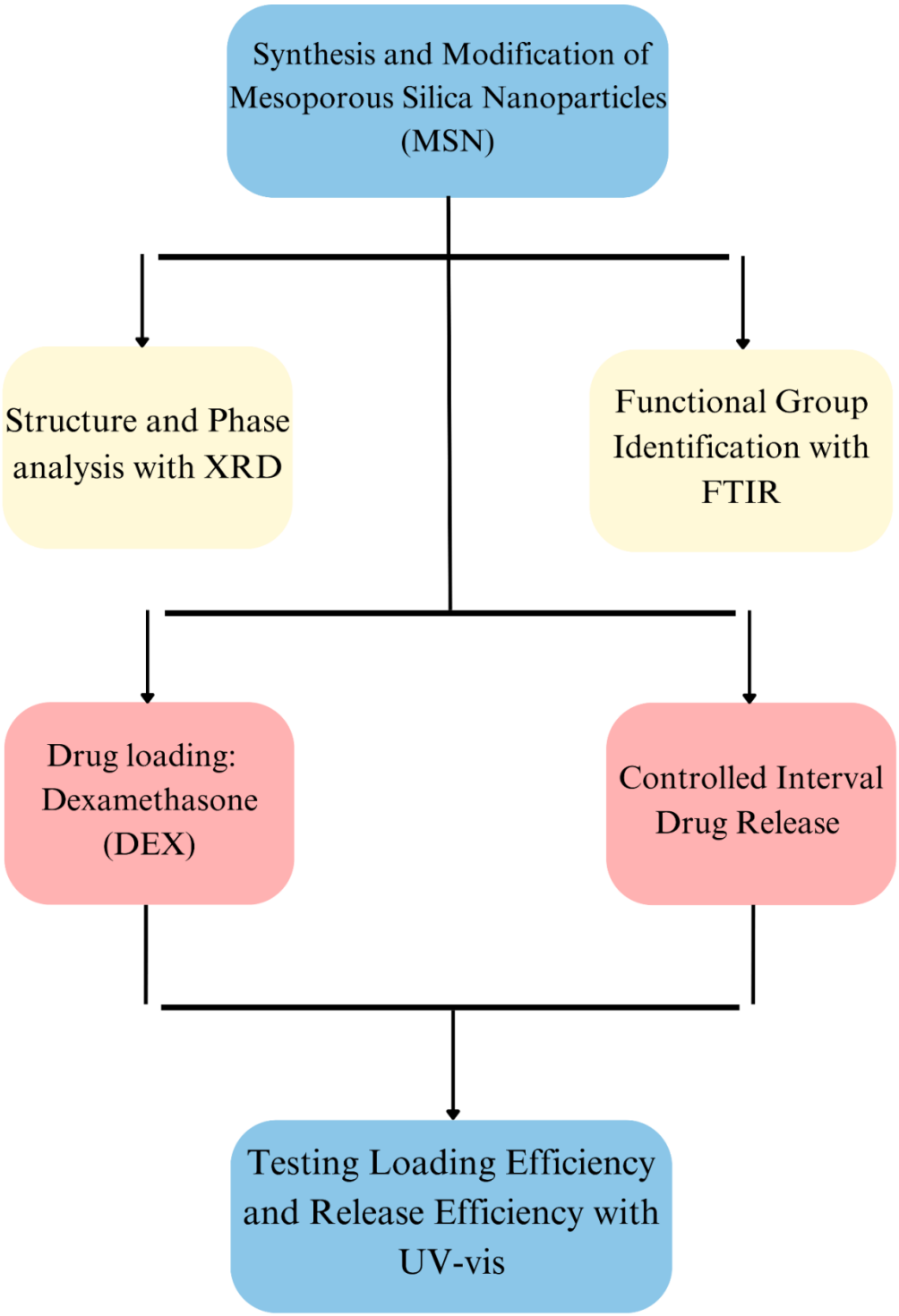
1.4. Review of Literatures

Article Title	Author(s)	Summary
Targeted delivery of curcumin in breast cancer cells via hyaluronic acid modified mesoporous silica nanoparticle to enhance anticancer efficiency	Shatadal Ghosh, Sayanta Dutta, Abhijit Sarkar , Mousumi Kundu, Parames C Sil. *	Targeted delivery of curcumin in cancer cells was achieved by conjugating hyaluronic acid (HA) on the surface of MSN. Synthesis of HA tagged MSNs loaded with curcumin was verified by several characterization techniques like TEM, FESEM and DLS. Drug loading content and release behavior were calculated. Cell viability assay showed that MSN-HA-C was almost nontoxic to normal cells.

Nanoarchitectured Structure and Surface Biofunctionality of Mesoporous Silica Nanoparticles	Ranjith Kumar Kankala, Ya-Hui Han, Jongbeom Na, Chia-Hung Lee, Ziqi Sun, Shi-Bin Wang, Tatsuo Kimura, Yong Sik Ok, Yusuke Yamauchi,* Ai-Zheng Chen,* and Kevin C.-W. Wu*	A review paper about MSN, including MSN developing history, MSN classification, and application. MSNs are synthesized based on the surfactant-templating approach, which predominantly utilizes surfactant molecules. Advanced fabrication of MSNs includes surface engineering, framework modification, tuning the mesopores and nanoarchitecting morphology. This paper discusses the biofunctionality of MSN and its possible application in medical fields.
Dexamethasone-Loaded Radially Mesoporous Silica Nanoparticles for Sustained Anti-Inflammatory Effects in Rheumatoid Arthritis	Sang Jun Kim, Youngbo Choi, Khee Tae Min, Surin Hong*	RMSNs with protonated amine functionality are proposed to be a dexamethasone carrier that could achieve a sustained effect. UV visible spectroscopy machine was used to quantify the amount of dexamethasone loaded at around 250nm. The maximum loading efficiency into the RMSNs was attained at about 76 wt%
Mesoporous Silica Nanoparticles for Drug Delivery	Miguel Manzano, María Vallet-Regí. *	A review progress report. MSNs are conventionally produced through the hydrolytic sol–gel process. Different porous morphologies and textures for MSNs have been reported. Safety and biodegradation of MSN are discussed. MSN as smart drug delivery systems includes release on-demand and transporting to the right place.
A pH-responsive drug delivery system based on chitosan coated mesoporous silica nanoparticles	Amirali Popat, Jian Liu, Gao Qing (Max) Lua, Shi Zhang Qiao*	Report a core–shell pH-responsive drug-carrier based on chitosan-coated MSNs. TEM and Small angle XRD for exam of MSN as MCM-41, (2D hexagonal). Chitosan capped onto phosphonate functionalized MSNs were prepared for loading of ibuprofen drug. Cumulative release of drug was calculated in 50 hours.

2. Materials and Methods

2.1. Flow chart of experiment process



2.2. Reagents and apparatus

Table 1. Laboratory Chemicals

Dexamethasone (DEX, Sigma-Aldrich)	Tetraethyl orthosilicate (TEOS, Tokyo Chemical Industry)	Cetyltrimethylammonium bromide (CTAB, Tokyo Chemical Industry)	3-Aminopropyl triethoxysilane (APTES, Tokyo Chemical Industry)
Phosphate buffer saline (PBS)	Ethanol	Glacial Acetic	Glutaraldehyde
NaOH	HCl	Chitosan (CHIT)	

Table 2. Laboratory Instruments

Pipet	Syringe	Centrifuge machine (Model Himact-18r)	Pellets
Magnetic stir bars & stirrer	Oil bath shaker	Cold water tank & Graham condenser	Ultrasonic processor
Ultrasonic shaker	Vortex mixer	Vacuum incubator	Calcination oven
Microbalance (±0.000001 g)	Three neck flask	Laboratory freezer	Vacuum pump
Fourier-transform infrared spectroscopy machine (FTIR)			
X ray diffractometer (XRD)			
Ultraviolet visible spectroscopy machine (UV-Vis, Model JASCO V-670 UV-Vis spectrophotometer)			

2.3. Preparation of mesoporous silica nanoparticles (MSN)

The experimental apparatus was setup and shown in Figure 1. CTAB (1g) was dissolved in a solution containing deionized water (480 mL) and 2N NaOH (3 mL). The mixed solution was heated to 80 ℃ and stirred for 2 hrs vigorously. After that, 98% TEOS (5 mL) was rapidly added to the above solution, and the mixture was maintained at 80 ℃ for 2 hrs until a white precipitate formed. After that, the products were obtained through centrifugation at 15,000 rpm for 10 min and rinsed with deionized water and 75% ethanol. Then the sample was vacuum-dried (60℃, 24 hrs) to produce the as-synthesized MSN. Finally, the as-synthesized MSN was calcined at 550 ℃ for 4 hrs to remove the surfactant template (CTAB), then the MSN sample was finally synthesized. The process of MSN preparation was illustrated in Figure 2.

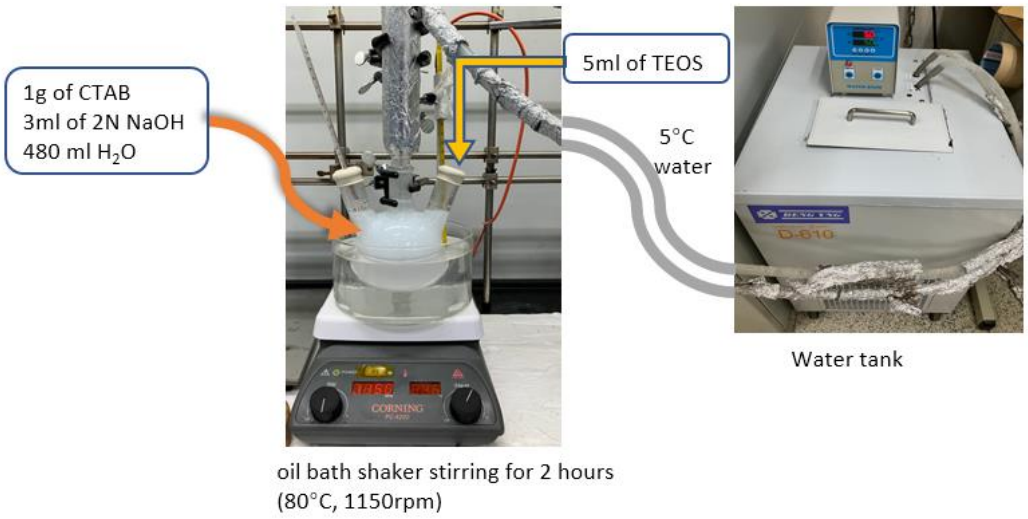


Figure 1. Experimental apparatus for MSN synthesis

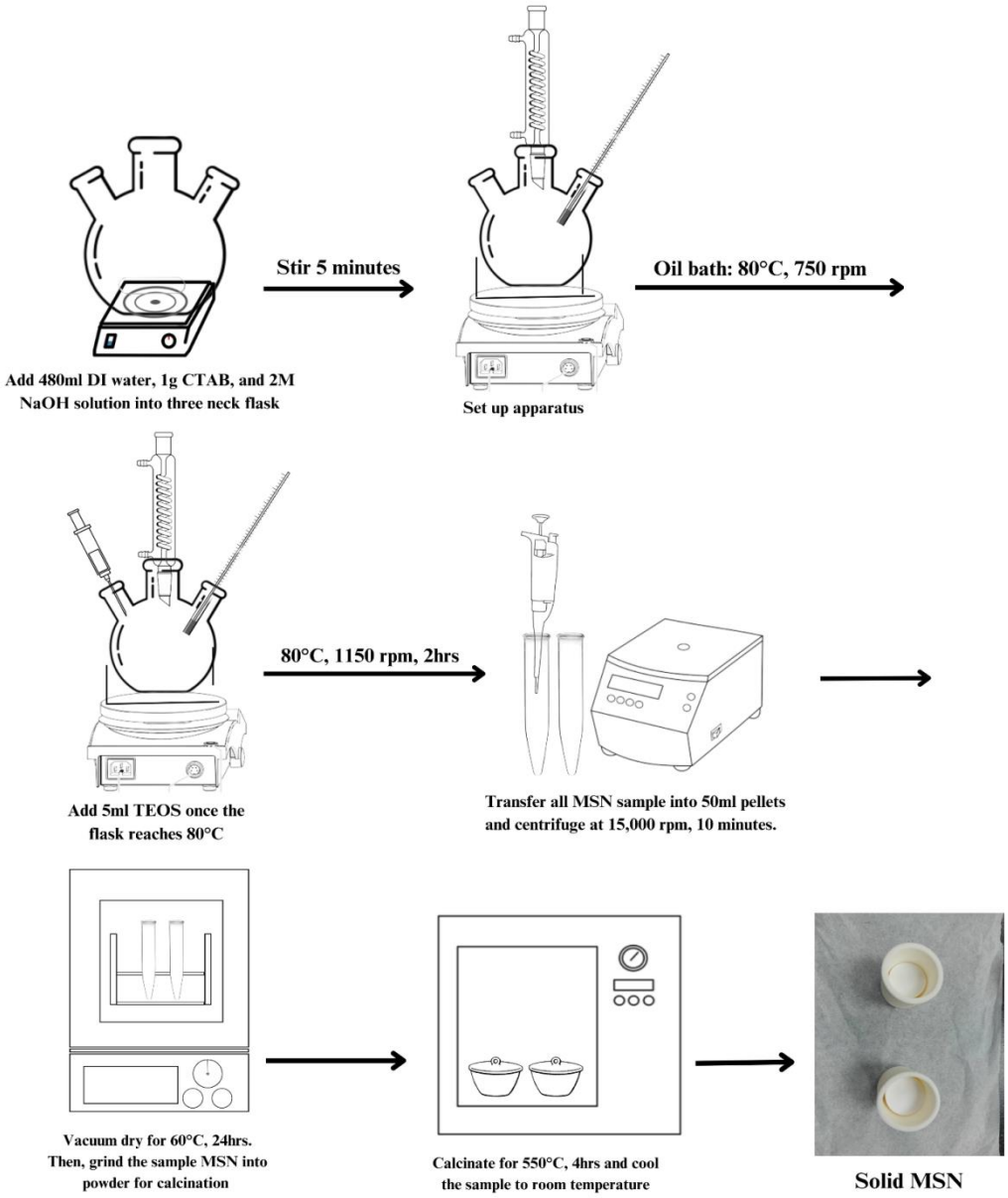


Figure 2. Schematic illustration showing the preparation of MSN

2.4. Preparation of Amine functionalized MSN (MSN-NH_2)

Namely, the MSN dried overnight (500mg) was suspended in 15 ml of deionized water, 7.5ml of glacial acetic, and 30ml of 75% ethanol. The particle suspension was stirred for 10 minutes. Next, 0.5 ml of 98% APTES was added dropwise into the suspended solution of MSN and stirred for 24 hrs. The MSN solution was centrifuged at 15,000 rpm, 10 minutes and washed twice with ethanol and deionized water. Subsequently, the sample was dried at 60°C for 24 hrs. The fabricated MSN-NH_2 obtained after drying was 410mg.

2.5. Synthesis of Chitosan capped MSN (MSN-NH_2-CHIT)

At first, MSN-NH_2 (410mg) was suspended in 40ml of deionized water and 0.4ml of glutaraldehyde and stirred (4°C, 1 hr). Then, the mixture was transferred into pellets and centrifuged (12,000 rpm, 25°C) for 10 minutes and washed with ethanol and deionized water. Finally, the precipitate was vacuum-dried (60°C, 24hrs) and grinded into powder. Chitosan (50mg) was added to 30 ml of 0.1M HCl under constant stirring at room temperature for 4 hrs to obtain chitosan solution. The prepared MSN powder (404mg) was dispersed in the chitosan solution and stirred (25°C, 24 hrs). The prepared MSN-NH_2-CHIT was collected by centrifugation (15,000 rpm) for 10 min and was washed by deionized water and ethanol for two times followed by vacuum-dried overnight at 25°C. The fabricated MSN-NH_2-CHIT obtained was 395mg.

2.6. Characterization of Fabricated MSN

XRD

The crystalline and surface structure of nanomaterial MSN were characterized using X-ray diffractometer (XRD) with 2-theta from 0 to 10 degrees

FTIR

Fourier transform infrared (FTIR) spectroscopy was used to confirm the successful synthesis of MSN-CTAB, MSN, MSN-NH_2, and MSN-NH_2-CHIT. The frequency range of FTIR spectral scanning was between 4000 and 500 cm^{-1}.

2.7. Dexamethasone Loading in MSN-DEX, MSN-NH_2-DEX, MSN-NH_2-CHIT

Dexamethasone loading in MSN (MSN-DEX) was done. Dexamethasone (20mg) was added to 20 ml phosphate buffer saline (PBS) at pH 7.4. MSN (100mg) was dissolved in the DEX-PBS solution. Then, the solution was stirred (25 °C, 2 hrs) using a magnetic stirrer. Then the sample was centrifuged (12,000 rpm at 25 °C). The supernatant was collected for analysis of loading efficiency. Further washing was conducted twice by adding PBS to the pellet and centrifuged (12,000 rpm at 25 °C) for 5 min. Then, the supernatant was collected and added to the previous collected one for loading efficiency analysis.

MSN-NH_2-DEX and MSN-NH_2-CHIT-DEX were both prepared as described above.

2.8. Analysis of Dexamethasone

UV-Vis Spectrometer

The absorbance of dexamethasone in solution was measured using ultraviolet–visible spectrophotometer (UV-Vis) with the scanning wavelength range between 200 and 400 nm. The optical densities of the dexamethasone concentrations in different solutions were measured at 254 nm.

2.9. Loading efficiency

To calculate the amount of dexamethasone encapsulated in MSNs, the prepared MSN-DEX, MSN-NH_2-DEX, and MSN-NH_2-CHIT-DEX solutions were centrifuged and washed with ethanol and water. The supernatants were carefully collected and the cal densities were measured at 254 nm using UV–Vis spectrophotometer. The concentration of free-DEX in the supernatant was then calculated. Finally, dexamethasone loading efficiency was calculated using the following formula.

Dexamethasone loading efficiency =

$$\frac{total\ amount\ of\ DEX\ -\ amount\ of\ free\ DEX\ in\ supernatant}{total\ amount\ of\ DEX} \times 100$$

2.10. Release profile

All dexamethasone loaded MSN samples were vacuum-dried (60°C, 24hrs). Three pellets of 30ml PBS (pH 7.4), each with 100mg of MSN-DEX, MSN-NH_2-DEX, and MSN-NH_2-CHIT-DEX respectively, are put into water bath shaker and then shaken (100 rpm at 25 °C) for 5 days under dark conditions. At scheduled time intervals (0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120 h), 4 ml of the PBS solution was withdrawn from each release media, and then the dexamethasone concentration was quantified spectrophotometrically at 254 nm. To maintain a constant volume, the withdrawn amount was returned, after each measurement, to the release media to keep a constant cumulative release percentage. After which, the cumulative release percentage was calculated using the following formula.

Cumulative release of dexamethasone (%) =

$$\frac{total\ amount\ of\ DEX\ released}{total\ amount\ of\ DEX} \times 100$$

3. Results and Discussion

3.1. Synthesis of the prepared MSN nanocomposites

The synthesis of MSNs was schematically illustrated in Figure 3. MSNs were prepared by a condensation method with TEOS as silica source. CTAB as the organic surfactant template would self-assembly into hexagonal micelles in alkaline aqueous solution. These micelles formed templates that helped build up the mesoporous framework. The hexagonal prism with micelles was formed and white precipitate after centrifugation was collected (Figure 4). Then the surfactant CTAB was oxidized and disappeared after calcination (Figure 5). The fabricated MSNs were functionalized with incorporation of amine groups on the external and internal surfaces. The aminated MSNs were capped with chitosan on the surface. The small molecular therapeutic agent, dexamethasone, was loaded on functionalized MSNs.

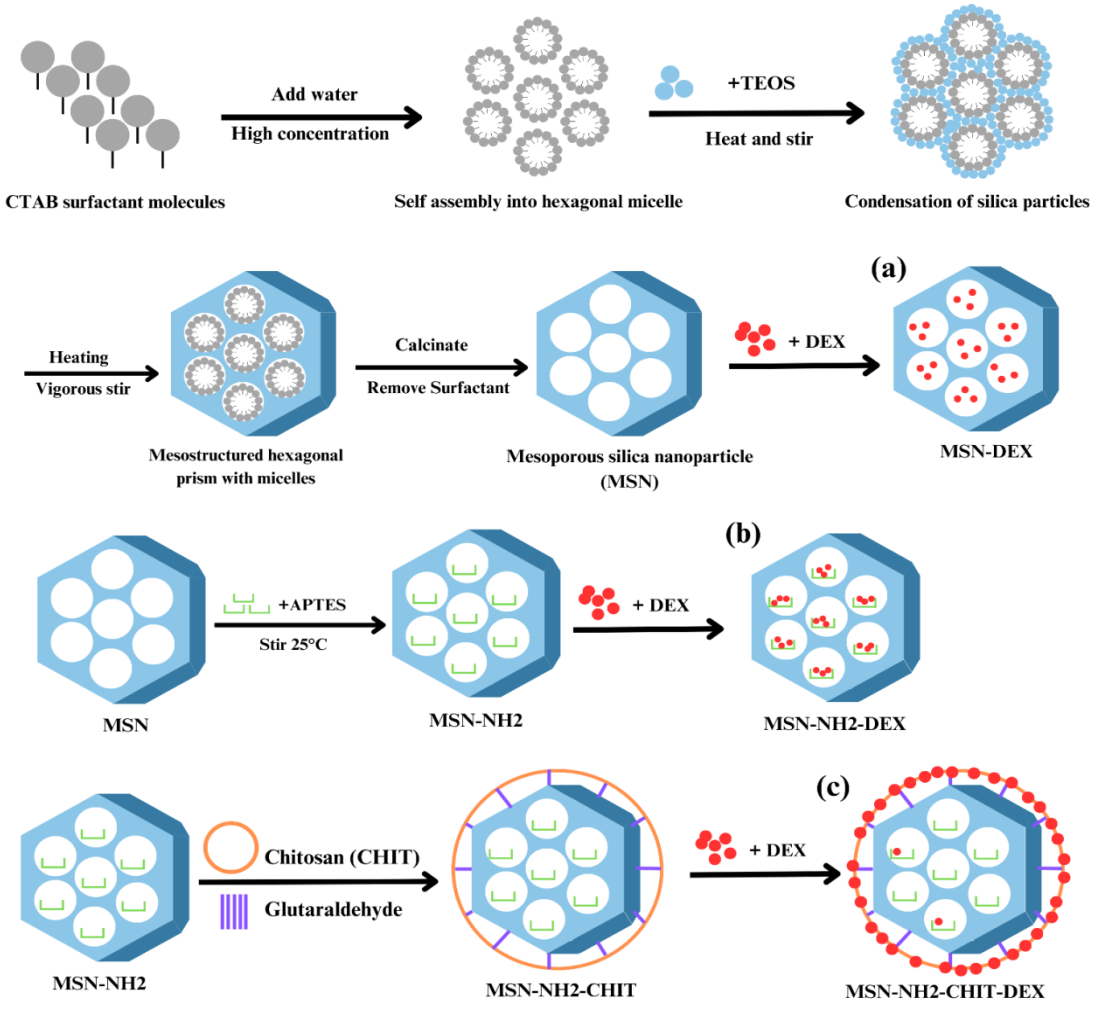


Figure 3. Schematic illustration showing synthesis and modification of MSN.
(a) MSN-DEX. (b) MSN-NH_2-DEX. (c) MSN-NH_2-CHIT-DEX



Figure 4. MSN precipitate after centrifugation.

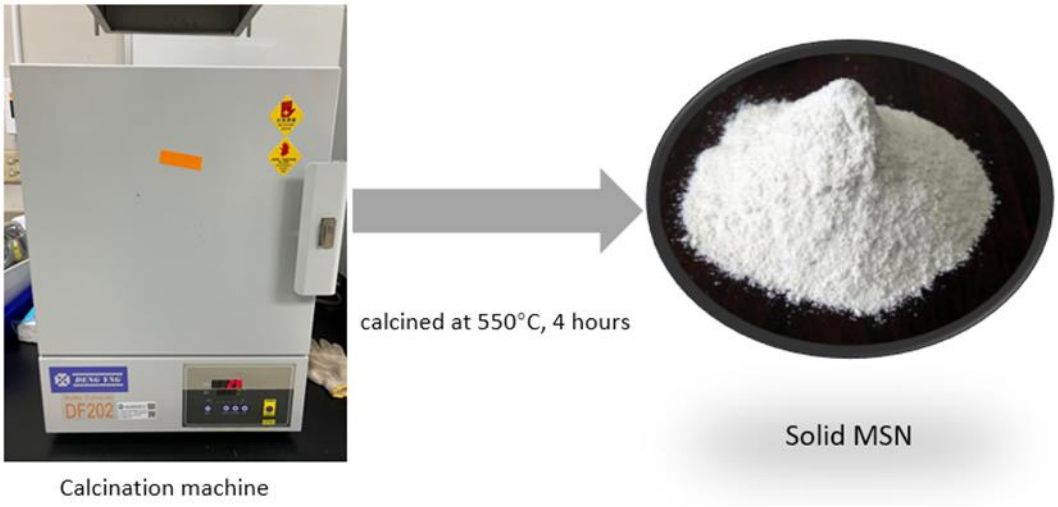


Figure 5. Calcination of MSN

Cetryl-trimethylammonium bromide (CTAB) was a quaternary ammonium salt with a carbon chain, hydrophobic tail and a hydrophilic head composed of complex ammonium ion (Figure 6). When exposed in water, CTAB could self arrange. The nonpolar, hydrophobic tail groups then clustered at the center to minimize contact areas with water, while the ionic, hydrophilic head groups were gathered on the outside, forming a small vesicle. At a high concentration, the surfactant micelles self assembled into hexagonal arrays, known as the hexagonal close-packed structure. The surfactant molecules preferred hexagon shapes due to the efficiency of hexagon, enabling maximum surface exposure with the least surface area, maximizing the

contact areas between the ionic head groups while preventing hydrophobic tail groups from contacting water. NaOH was added in water to facilitate the self assembly of CTAB. The attraction force between -OH ions and the ammonium cation heads formed stronger affinity between surfactant molecules.

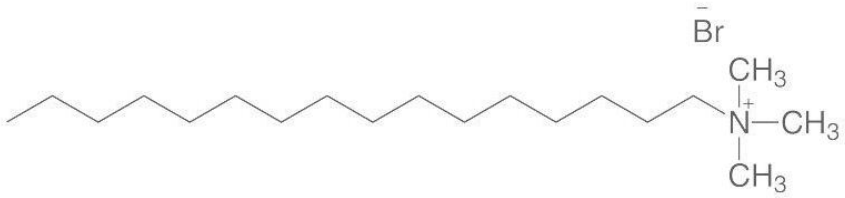


Figure 6. Chemical structure of CTAB.

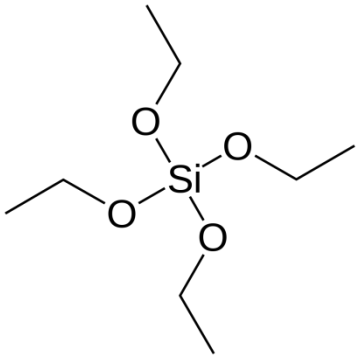


Figure 7. Chemical Structure of TEOS

Tetraethyl orthosilicate (TEOS) was an organic silica compound (Figure 7), with silica in the center surrounded by oxygen and carbon. At high concentration and vigorous stir, the tetrahedral structure and micro-size of TEOS allowed the compound to effectively disperse and fill the spaces between the surfactant micelles. After heating at 80°C, the silica particles condensed into hexagonal prisms surrounding the surfactant templates.

MSN was functionalized with 3-aminopropyl triethoxysilane (APTES) to incorporate aminopropyl groups on the surface (MSN-NH_2). The Si-O group of APTES was bonded to the Si group on the MSN with covalent bonds (Figure 8).

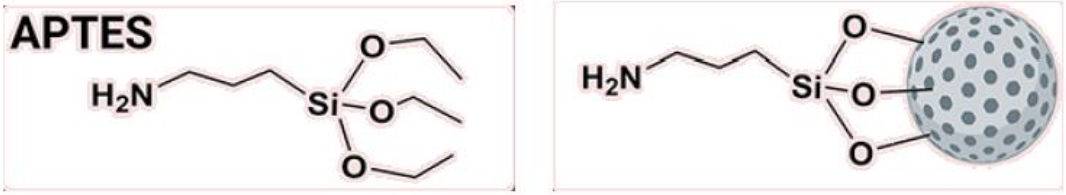


Figure 8. APTES bonded to MSN

This was followed by modification with chitosan on the MSN surface via crosslinking with glutaraldehyde. Chitosan was a chitin-derived natural polysaccharide, which could be applied as a pharmaceutical material due to its low toxicity, good biocompatibility and antimicrobial activities. Glutaraldehyde was widely used as a crosslink agent for primary amines, forming a characteristic imine bond (N=C) between aldehyde carbon and primary amine (Figure 9). The process of chitosan modified on the surface of MSN was known as chitosan encapsulation. The amine groups on chitosan were horizontally bonded to the carbonyl groups of glutaraldehyde, forming a cap. Ultimately, chitosan polymers would be horizontally bonded to glutaraldehyde molecules on the MSN surface, forming a spherical cap on the surface of MSN.

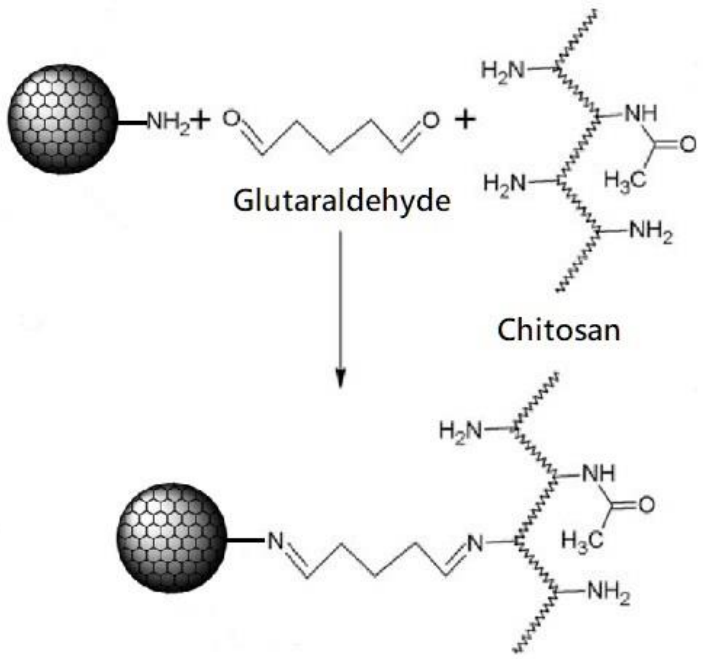


Figure 9. Crosslinking between glutaraldehyde and chitosan

3.2. Characterization of the prepared nanoparticles

3.2.1. XRD pattern of calcined MSN

The XRD pattern of the mesoporous silica nanoparticles was presented in Figure 10. The findings revealed that a broad diffraction peak was emerged at 2θ =2.4°, 4.0°, and 4.6° in the calcined MSN sample, representing the reflections of the ordered two-dimensional hexagonal mesoporous structure. Three well-resolved diffraction peaks, assigned as (100), (110) and (200) planes, respectively, were clearly observed in the XRD pattern of MSN sample. The two theta positions corresponded to the spacing between the crystals or atoms, determined by the angle of diffraction from the incident x-ray sent into the sample. The height of the peaks showed a greater intensity, representing the amount of molecules within that spacing. In this work, the ordered mesoporous structure of the silica nanoparticles confirmed by small angle XRD measurement was consistent with the characteristic diffraction pattern of MCM-41 type MSN, representing the reflections of the ordered two-dimensional hexagonal mesoporous structure. The MCM-41 type MSN was 2-4 nm in pore size and 0.6-1.2 nm in wall thicknesses. Silica described as mesoporous because of their pore diameters ranging between 2 nm < d < 50 nm.

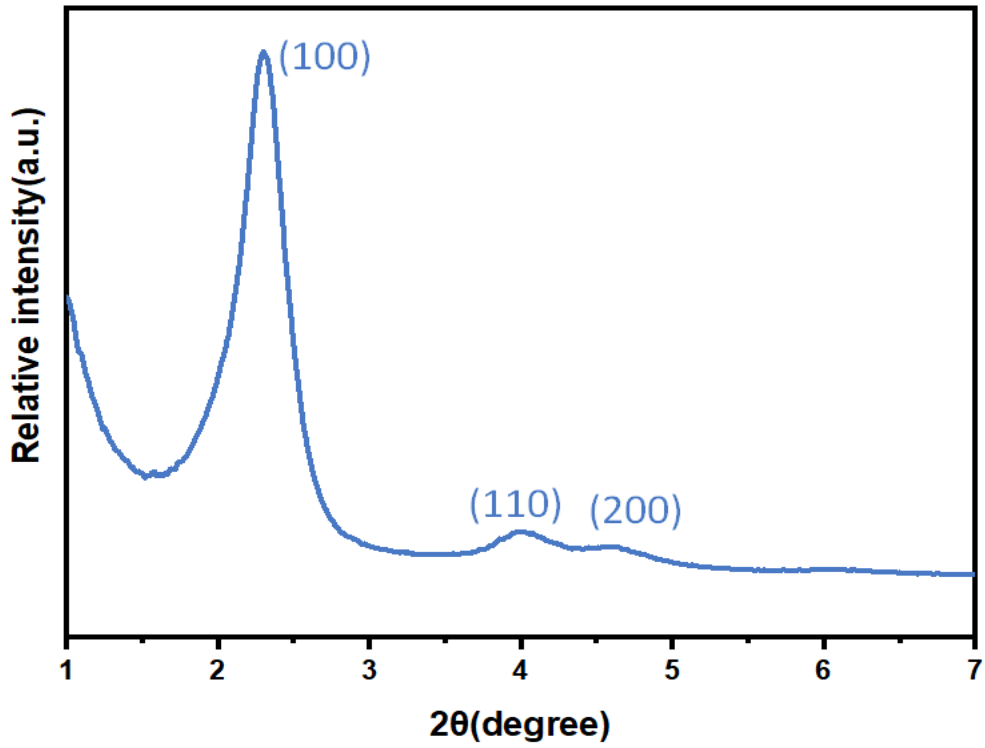


Figure 10. XRD pattern of calcined MSN

3.2.2. Fourier transform infrared (FTIR) spectra of MSNs

Fourier transform infrared spectroscopy (FTIR) was a powerful tool to investigate interface interactions in hybrid organic-inorganic materials. It could be used to assess the molecular structure of surface grafted functionalizing groups.

The FTIR spectra of the MSN-based nanostructures (MSN-CTAB, MSN, MSN-NH2 and MSN-NH2-CHIT) were shown in Figure 11. All four groups of MSN showed distinctive peaks at 800 cm^{-1}, which corresponded to the bending vibrations of Si–O bond. The peaks at 1084 $cm^{-1}$and 1750 cm^{-1} suggested the stretching and bending vibrations of Si–O–Si bond. The strong absorbance peak at 3468 cm^{-1} was assigned to stretching and bending modes of the silanol (Si–OH) groups on the surface of the MSNs due bonds formed by TEOS. These results indicated successful synthesis of the silica-based materials.

Complete removal of the surfactant CTAB from the MSNs was confirmed by the disappearance of the wide absorption C–H peak at 2810 cm^{-1} in MSN and MSN-NH_2. For MSN-NH_2, the corresponding fingerprints of aminopropyl functional groups in the low frequency region were the band at 1554 cm^{-1} due to the –NH2 bending mode. The successful addition of chitosan was confirmed by the disappearance of the N–H peak at 1554 cm^{-1}, as the amino group on APTES had formed an amine bond with glutaraldehyde, which was then bonded to chitosan. Furthermore, in the case of MSN-NH_2-CHIT, only the band of alkyl groups was noted by the weak C–H stretching vibrations at 2980 cm^{-1}. Other peaks such as NH, OH and CO related to chitosan were overlapped with the band of silanols after the surface modification. The frequencies of each functional group were listed in Table 3.

Table 3. Frequencies of different functional group

Functional group	Wavenumber (cm^{-1})	Functional group	Wavenumber (cm^{-1})
Si-O	800 cm^{-1}	-NH_2	1554 cm^{-1}
Si-O-Si	1084-1230 cm^{-1}	-CH_2	2810 cm^{-1}
Si-O-Si	1750 cm^{-1}	Si-OH	3468 cm^{-1}

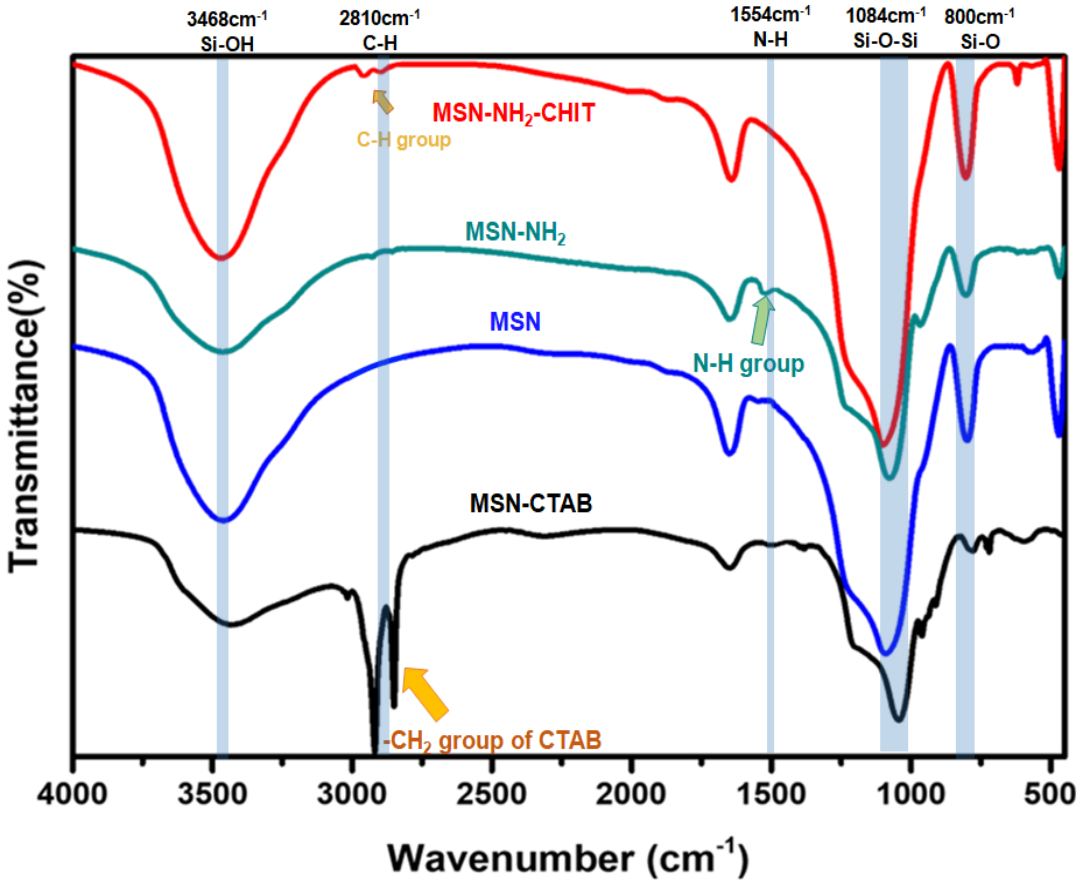


Figure 11. FTIR spectra of KBr pellets of MSN-CTAB, MSN, MSN-NH_2, MSN-NH_2-CHIT

3.3. Dexamethasone loading

UV-Vis spectroscopy was a powerful tool for analyzing specific chemical compounds by quantifying ultraviolet beam pathways, with each unique compound matching with a specific wavelength. According to Beer-Lambert Law, each molecule had a unique molar absorptivity, producing different absorbance.

The amount of 20 mg of dexamethasone was loading to the 100 mg of MSN, MSN-NH_2, MSN-NH_2-CHIT in 20ml PBS solution respectively. DEX was prepared as blank control with 20 mg of dexamethasone in 20 ml PBS solution (pH=7.4). The concentration of DEX in the supernatant after centrifuge was quantified by UV-Vis spectrometer (Figure 12). The wavelength of DEX was set at 254 nm, following the Beer–Lambert law for DEX concentrations of 2.72 µg/ml. A peak at 254 nm was present for all MSN-DEX, MSN-NH_2-DEX, MSN-NH_2-CHIT-DEX, indicating a successful loading of DEX in all MSN samples (Figure 12).

The concentrations of DEX in the supernatant of the MSN-DEX, MSN-NH_2-DEX, MSN-NH_2-CHIT-DEX solutions were provided in Table 4. The concentration of DEX loaded presented graphically, with MSN-NH_2-CHIT-DEX the highest loading efficiency at 53.7%, followed by MSN-NH_2-DEX 34.9%, and MSN-DEX 27.9% (Figure 13).

The results demonstrated a promising effect of the modification on MSN surface which showed higher loading efficiency than non-modified MSN. The speculation below was in accordance with experimental results for the DEX loading efficiency. In order to achieve high loading efficiency for DEX, it is necessary to induce specific and strong interactions between the surface of the silica carrier and DEX molecules. Functionalizing the surface of the MSNs with an amine group could enhance the interaction with DEX molecules by forming stronger intermolecular forces. Chitosan layer was capped on MSN-NH_2 to provide higher affinity with DEX molecules.

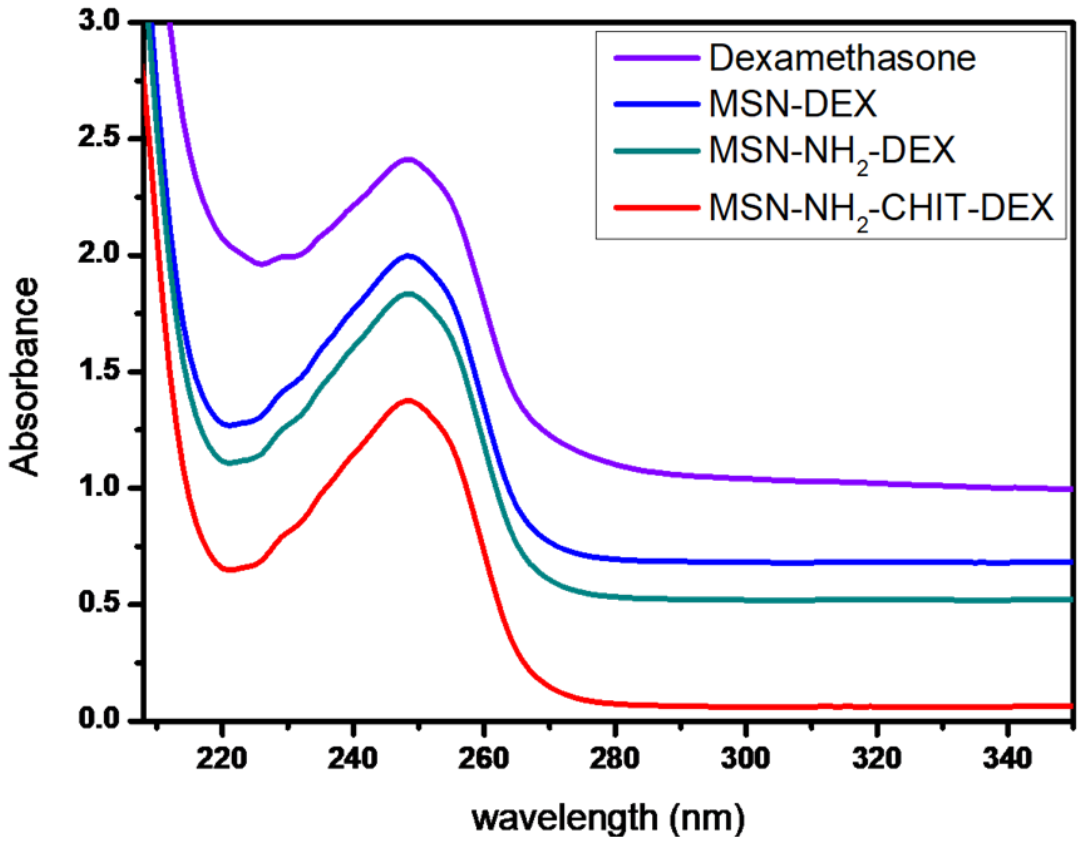


Figure 12. UV-Vis spectra of DEX, MSN-DEX, MSN-NH_2-DEX, MSN-NH_2-CHIT-DEX

Table 4. Amount of DEX in centrifuged supernatant from MSN-DEX, MSN-NH2-DEX, MSN-NH2-CHIT-DEX

	DEX amount in supernatant (μg/ml)
DEX	2.72
MSN-DEX	1.96
MSN-NH2-DEX	1.77
MSN-NH2-CHIT-DEX	1.26

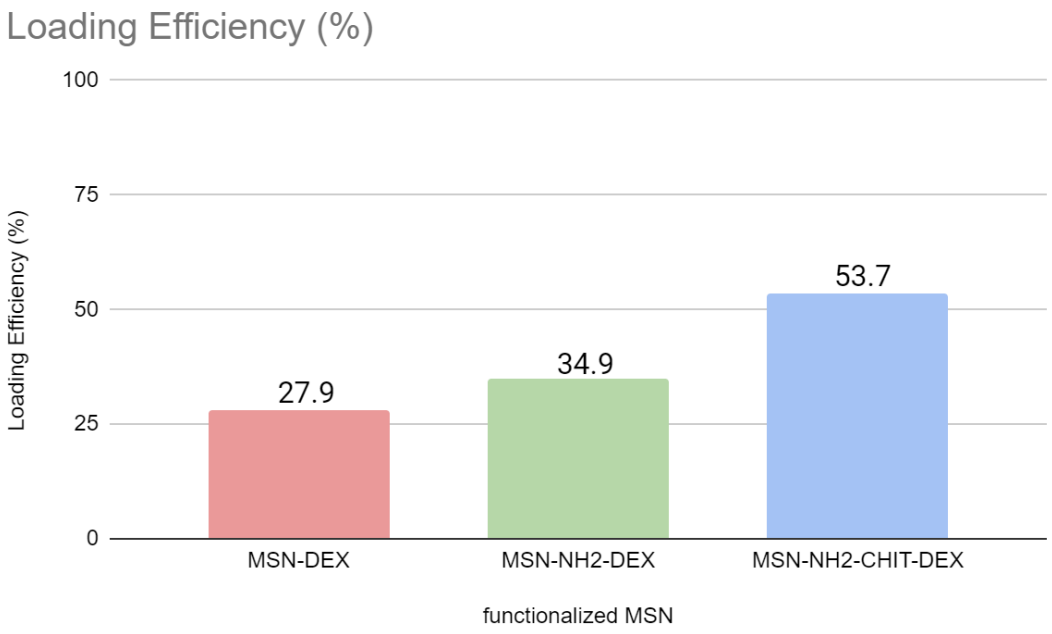


Figure 13. Determined loading efficiency of DEX in MSN-DEX, MSN-NH_2-DEX, MSN-NH_2-CHIT-DEX

3.4. Dexamethasone releasing

The dexamethasone-loaded MSN was kept in PBS buffer at 25°C up to different time intervals. The release kinetics of dexamethasone from MSN-DEX, MSN-NH2-DEX, MSN-NH_2-CHIT-DEX in PBS buffer at uniform pH 7.4 were shown in Figure 14. The graphic pattern showed that the release of dexamethasone in MSN-DEX and MSN-NH_2-DEX already reached around 50% at 12th hour and then around 80% at 48th hour, revealing a quite similar release profile. The initial bursting release, noted as "premature release", was shown from 0 to 48 hrs. Then it showed a ceasing trend of DEX release as indicated by the flat plateau from 48 to 120 hrs, indicating that both carriers reached a release terminal. On the other hand, MSN-NH_2-CHIT-DEX only released around 5% at 12th hour, reaching merely 10% at 48th hour. The release was slow and sustained throughout the time intervals, increasing at a constant rate in 120 hrs.

The amounts of released dexamethasone after 5 days were listed on Table 5. The determined cumulative release (%) was shown in Figure 15. The cumulative release after 120 hrs followed a decreasing pattern with more fabrication on the MSN surface, with MSN-DEX releasing the most and MSN-NH_2-CHIT-DEX the least. The cumulative release of dexamethasone from dispersed MSN-DEX, MSN-NH_2-DEX, and MSN-NH_2-CHIT-DEX reached 90.7%, 87.3% and 19.3% respectively. These results indicated a high release rate of MSN-DEX and MSN-NH2-DEX, as both carriers reached their release terminal at high 90.7% and 87.3%, which were close to 100% fully released. In MSN-DEX and MSN-NH2-DEX, the DEX molecules were loaded in the pores via weak intermolecular forces.It consisted on diffusion process of incorporated dexamethasone from the mesoporous channels toward the PBS media. This diffusion process was triggered immediately after the contact of the nanocarriers with the media. As a result, the DEX molecules easily diffused out of the pores, resulting in large amount premature release in short period of time. It could be attributed to the lack of control and gatekeeping for the DEX molecules.

In order to build the sustained drug delivery systems with slow and constant release rate, chitosan was capped around MSNs as controller for dexamethasone release. MSN-NH2-CHIT-DEX provided an alternative loading mechanism than attaching to dexamethasone to the porous surface. Chitosan encapsulation formed a cap on the outside of MSN, grabbing free DEX molecules and forming firm covalent bonds. Few DEX molecules that were not bonded to chitosan entered the MSN pores, in which it

was hard for the molecules to escape due to the spherical capping of chitosan on the outside. The slow release of MSN-NH_2-CHIT-DEX showed a linear progression, proving a constant and sustained dexamethasone release.Overall, these results suggested that the chitosan capped MSN could be a suitable drug carrier for sustained therapeutic effects.

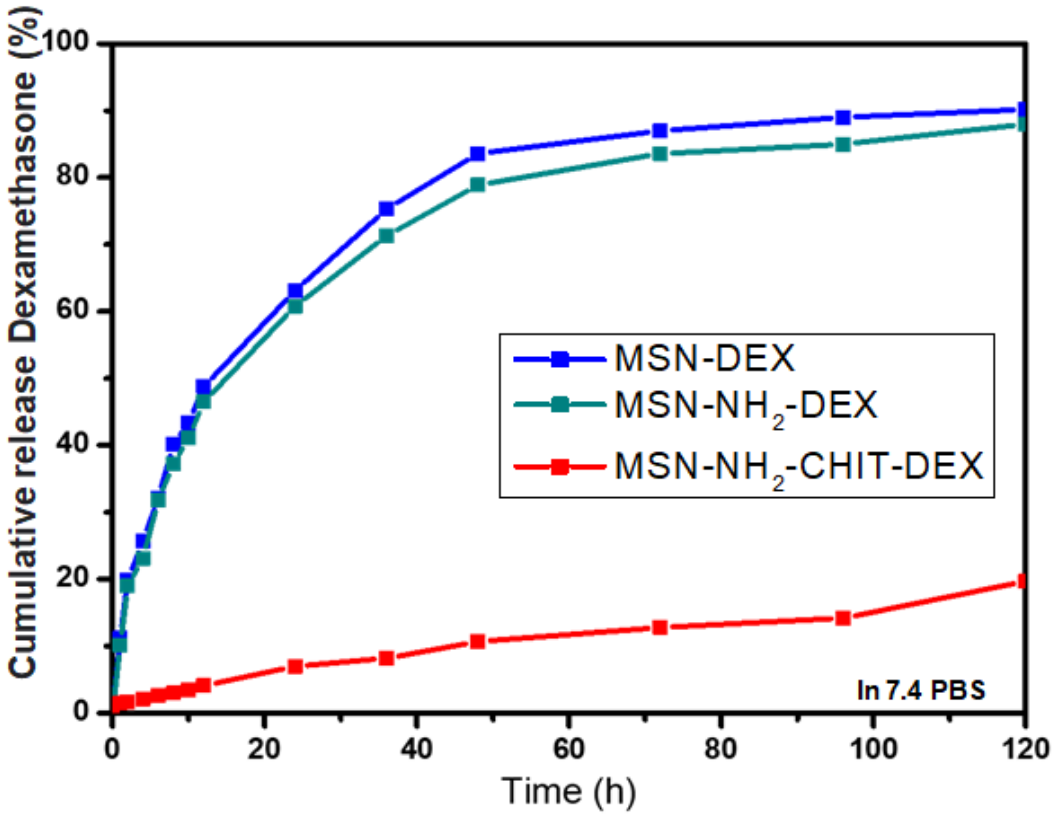


Figure 14. Kinetic release profile of DEX from MSN-DEX, MSN-NH_2-DEX, MSN-NH_2-CHIT-DEX

Table 5. Amounts of DEX released from MSN-DEX, MSN-NH_2-DEX, MSN-NH_2-CHIT-DEX

	DEX released after 5 days (μg/ml)
MSN-DEX	0.69
MSN-NH2-DEX	0.83
MSN-NH2-CHIT-DEX	0.287

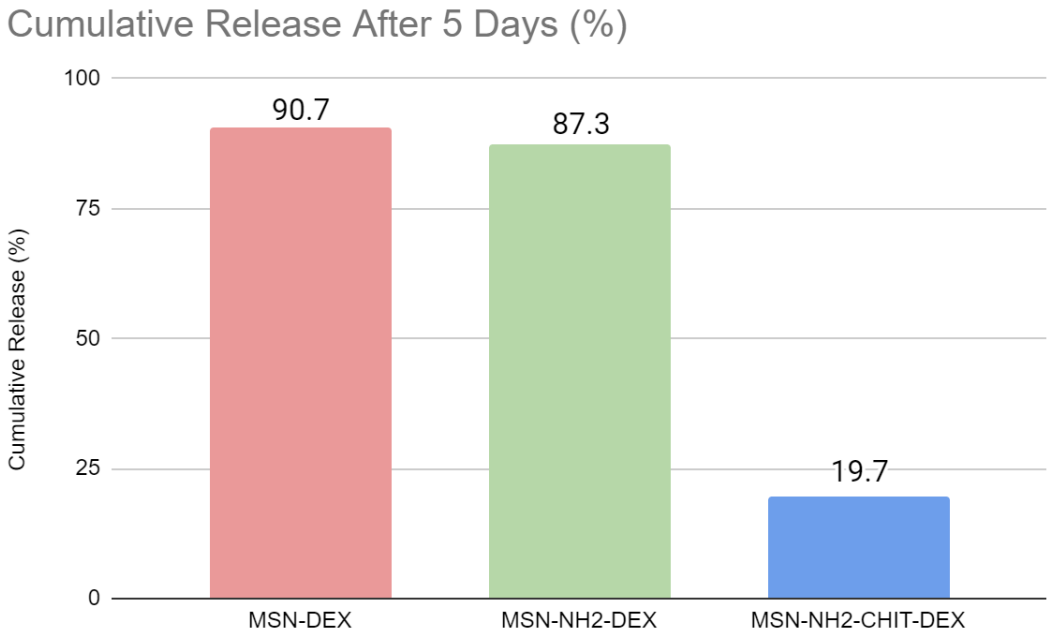


Figure 15. Cumulative release profile of MSN-DEX, MSN-NH_2-DEX, MSN-NH_2-CHIT-DEX after 5 days.

4. Conclusions

In this study, we achieved successful functionalization of MSNs with chitosan through two-step conjugations of amination and crosslinking. We also confirmed the fabricated MSN as MCM-41 type with hexagonal mesoporous structure. In addition, we identified organic functional group of grafted MSN with FTIR. Furthermore, we illustrated the fabrication process in Figure 3 that demonstrated our current understanding of how to synthesize hexagonal MSN and how to modify the surface of MSN, followed with dexamethasone drug loading. The concept was clearly demonstrated and might be used as reference for future studies.

We built a model for controlled drug delivery systems with mesoporous silica nanocarriers. Our findings displayed that chitosan functionalized MSN was a promising carrier for medicine, providing protection from its premature release under undesirable conditions. The anti-inflammatory drug, dexamethasone, was applied in this study to load and release at 25°C in pH 7.4 PBS solution. MSN-NH_2-CHIT-DEX that featured high loading efficiency of 53.7% and sustained slow release of 19.7% after 120 hrs was proposed as a desired drug carrier. Accordingly, this drug delivery system would represent a smart and stable nanocarrier for controlled drug release in vitro and might be suitable to apply in the mammalian internal environment.

5. Future Work and Application

For future work, we would like to measure the amount of dexamethasone release from MSN-NH_2-CHIT-DEX in longer periods to gain a long term profile. Besides, we would like to test if pH value could adjust the drug loading and release as referred from the review literatures. According to results, we could propose to investigate for drug effect in cell culture assay and for biosafety evaluations in animal study.

As for the drug loading, we would like to test different kinds of COVID-19 medicine, such as methotrexate and curcumin. Dexamethasone, methotrexate and curcumin had the similar and nanoscaled molecular sizes. These drugs might be loaded on designed MSNs with controlled release at target tissues and cells to slow the progression of COVID-19 to life-threatening stages.

6. Bibliography

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【評語】030013

合成多孔性二氧化矽奈米粒子用於奈米藥物傳遞載體的開發，並使用幾丁聚糖對表面進行修飾，以提升地塞米松藥物載荷以及穩定的藥物釋放。整體實驗清晰有條理，需對 MSN-NH2-CHIT 作更長時間的藥物釋放觀察以確認其體內代謝狀況，並加入以 CHIT 作為藥物傳遞載體的控制組。