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作品名稱　Feasible fabrication of chitosan capped mesoporous silica nanoparticles as a smart mucoadhesive drug delivery platform for dexamethasone

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關鍵詞　chitosan、mesoporous silica nanoparticle、dexamethasone

作者簡介

Hi, I am Sean Lee, a senior at Kang Chiao International School. My passion in science originated from a school science project in fourth grade on electrophoresis, and since then I have participated in a science fair every year, solving common problems like environment, food safety, health, and energies. Apart from science, I find joy in music and athletics. Having played the piano, violin, and viola, I channel my inner peace with music, as it calms and relaxes my brain during intense weeks of experiments. As a national-level fencer, I train a competitive mindset and physique, motivating me to strive for further research. I am also a societal caregiver. Joining youth rotary club since 8th grade, I volunteered for multiple special-needs organizations, such as autism foundation and wheelchair fencing association. From them, I was inspired to use science to help society.

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研究報告

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（編號由國立臺灣科學教育館統一填列）

摘要

中孔二氧化矽納米顆粒(MSN)由於其高孔隙率而適合成為藥物載體，可增加 ul 藥物的負載量。幾丁聚糖是一種帶正電的聚合物，用於修飾 MSN 表面，以達到強力的靜電吸附力，並進一步提高藥物負載能力，以及可持續併緩慢藥物釋放的控制。

MCM-41 和 MCM-48 型的 MSN，通過 CTAB 界面活性劑為模版，以溶膠–凝膠法制備。SBA-15 型的 MSN 由 P123 為模版製備。MCM-41 通過戊二醛的交聯進一步被幾丁聚糖包覆（MCM-41-CHIT）。 利用 X 射線繞射儀驗證了所有載體皆是中孔洞的六方密堆積晶體結構。利用傅里葉變換紅外光譜，鑒定了烷基、胺基、和二氧化矽官能團，證實了表面的幾丁聚糖。

MCM-41-CHIT 的地塞米松載藥量為 53.7%。MCM-41 有突發釋放的現象，在兩天內釋放出 80%。另一方面，MCM-41-CHIT 中的藥物釋放，表現出恆定的釋放，五天後僅釋放出 19.7%。

這項研究確定了 MCM-41-CHIT 是可應用在粘膜吸附藥物遞送系統，可做為好的候選藥物載體。

Abstract

Among the carriers, mesoporous silica nanoparticles (MSN) were promising for drug carrier due to its biocompatibility and high porosity, thereby increasing the loading of therapeutic agents. Chitosan, a biocompatible polymer with positive charge, was used to modify the MSN surface in order to achieve strong electrostatic mucoadhesion and further to improve drug loading capacity and sustainable release profile.

The MCM-41 type and MCM-48 type MSNs were prepared by a CTAB-templated sol-gel method. The SBA-15 type MSNs was prepared by P123 surfactant template. MCM-41 was further coated by chitosan via the crosslinking of glutaraldehyde.

With X-ray diffractometer, the hexagonal close-packed crystal structure of MSN was verified for MCM-41, MCM-48 and SBA-15. SEM demonstrated the particle morphology and distribution of MSNs.Utilizing Fourier transform infrared spectroscopy, vibration modes of alkyl, amine, hydroxyl, and silica function groups were identified to confirm the encapsulation of chitosan on MCM-41 (MCM-41-CHIT) surface.

The small dexamethasone was encapsulated in the chitosan capped MCM-41. The drug loading capacity of MCM-41-CHIT revealed 53.7%. The burst release of dexamethasone in the bare MSNs was noted, which showed 80% in 24 hours. On the other hand, drug release in MCM-41-CHIT showed constant and delayed release, which showed only 19.7% in 120 hours.

In summary, this research established chitosan capped MCM-41 as a potentially efficient candidate for the mucoadhesive drug delivery system.

1. Introduction

1.1. Background

COVID-19 was a virus-induced lung disease that caused millions of deaths globally. In the therapy trial study, the use of dexamethasone (DEX) daily for up to 10 days resulted in lower 28-day mortality among those severe hospitalized patients with intensive care. Dexamethasone was a corticosteroid, which worked on the immune system to help relieve swelling, redness, itching, and allergic reactions. Oral and intravenous dexamethasone therapy in the long term treatment course would cause systemic adverse effects. Direct drug delivery to the airway or inhalation therapy was commonly used to treat lung diseases. Inhalation therapy was accessible to deliver the therapeutic drug to the lung mucosa and, therefore, could significantly reduce the drug's dose and further reduce its side effects.

A long term release and high concentration of dexamethasone were required to cure the lung inflammatory disease. The control of release over a long period of time was important factor to be taken into account for improving maximum treatment benefits. Thus, to reduce the adverse effects of drug resulting from high dosage burst release, sustainable drug delivery system should be designed with the biocompatible nanomaterials.

For drug delivery purposes, the mechanism of mucoadhesion involved attachment of a drug carrier system to the mucous membrane of a tissue. The mucous membrane lining the respiratory system, including the nose, the larynx, the trachea and the lung, had great surface area and played a crucial role in protection of the inhaled air. Nanoparticle composites offered advantages in improving cell penetration and thus demonstrated a promising tool for treating lung diseases. As delivered to the airway via inhalation, these nanoparticles could remain or penetrate in the mucus layer through adhesion-mediated approaches.

Mesoporous silica nanoparticles (MSNs) with pore size 2–50 nm played an important role as a carrier in mucoadhesive delivery and control of drug release in pharmaceutical technology. The benefits of utilizing MSNs in medical applications were enhanced drug bioavailability and reduced unwanted toxicity due to their modifiable surface, suitable nano-size, tunable pore size and chemical stability.

Chitosan, a derivative obtained from the deacetylation of chitin, had distinctive biological properties and had been widely used for sustained release and targeted

preparations of drugs. Because the abundant amino groups of chitosan were protonated and positively charged, chitosan could effectively react with the negative charge of mucus gel. When chitosan-based drug carriers were inhaled into the respiratory tract, the strong electrostatic adhesion of chitosan to the mucous membrane occured. Chitosan with particular mucoadhesion ability could increases the retention time of the drug in the pulmonary mucosa, resulting in slow and cumulative release of the drug,

In this study, we proposed a simple and feasible method to fabricate a MSN and chitosan nano-composite for the construction of mucoadhesive drug delivery platform. Firstly, MSNs in three biodegradable nanoparticle forms were synthesized by the use of tetraethyl orthosilicate (TEOS). Secondly, MSNs were modified with APTES. Aminated MSNs were capped with chitosan mediated by glutaraldehyde as a cross-linker. Subsequently, the nanoparticles size, pore size, surface morphology and dexamethasone loading content were investigated. Cummulative drug release in physiological condition was monitored.

1.2. Purpose

Our work focused on the following key contributions:

1. Establish the feasible fabrication processes of MSNs
2. Adjust MSNs with different particle size and various pore size
3. Synthesize chitosan capped MSNs via the crosslinking of glutaraldehyde.
4. Define the characteristics of MSNs
5. Evaluate the loading capacity of dexamethasone in MSNs
6. Control the release profile of dexamethasone in MSNs

2. Materials and Methods

2.1 Reagents and apparatus

Table. Laboratory Chemicals

Dexamethasone sodium phosphate (DEX, ASTAR CHEMICAL)	Tetraethyl orthosilicate (TEOS, Tokyo Chemical Industry)	Cetyltrimethylammonium bromide (CTAB, Tokyo Chemical Industry)	3-Aminopropyl triethoxysilane (APTES, Tokyo Chemical Industry)
P123 (Sigma; 5800 g/mol, 1.11 g/mL)	Ammonium hydride (28%, SHOWA)	Chitosan (CHIT)	Phosphate buffer saline (PBS)
Glutaraldehyde	Glacial Acetic	NaOH	HCl

Table. Laboratory Instruments

Pipet	Syringe	Oil bath shaker	Pellets
Magnetic stir bars & stirrer	Centrifuge (Himact-18r)	Cold water tank & Graham condenser	Ultrasonic processor
Ultrasonic shaker	Vortex mixer	Vacuum incubator	Calcination oven
Microbalance	Three neck flask	Laboratory freezer	Vacuum pump
pH meter (sensION+)	Heating mantle & heat sensor		
Scanning electron microscope (SEM) (prototype: HITACHI S-4800)			
X ray diffractometer (XRD)			
Fourier-transform infrared spectroscopy machine (FTIR)			
BET Surface Analysis			
Ultraviolet visible spectroscopy machine (UV-Vis) (Model JASCO V-670)			

2.2 Fabrication process and modification

2.2.1 Preparation of mesoporous silica nanoparticles (MSN)

2.2.1.1 MCM-41 synthesis procedure:

1. Add 480mL DI water and 3.5mL 2N NaOH into three neck flask
2. Add 1g CTAB, stir until the solution is clear
3. Stir at 2 different condition: 80°C, 2 h and 60°C, 1 h
4. Add 5 ml 98% TEOS, stir. The solution was milk-white suspension
5. Centrifuge with 50 mL pellets (15,000 rpm, 10 min).
6. Wash twice with DI water and 75% ethanol
7. Vacuum dry the sample (60°C, 24 h)
8. pour out the solid sample and grind it into powder with mortar and pestle
9. Calcinate at 550°C, 4 h (+5°C/min)
10. The final mass was MCM-41(80) 0.60 g and MCM-41(60) 0.60 g.

The experimental apparatus was setup (Figure 1). The process of MSN preparation was illustrated (Figure 2). .

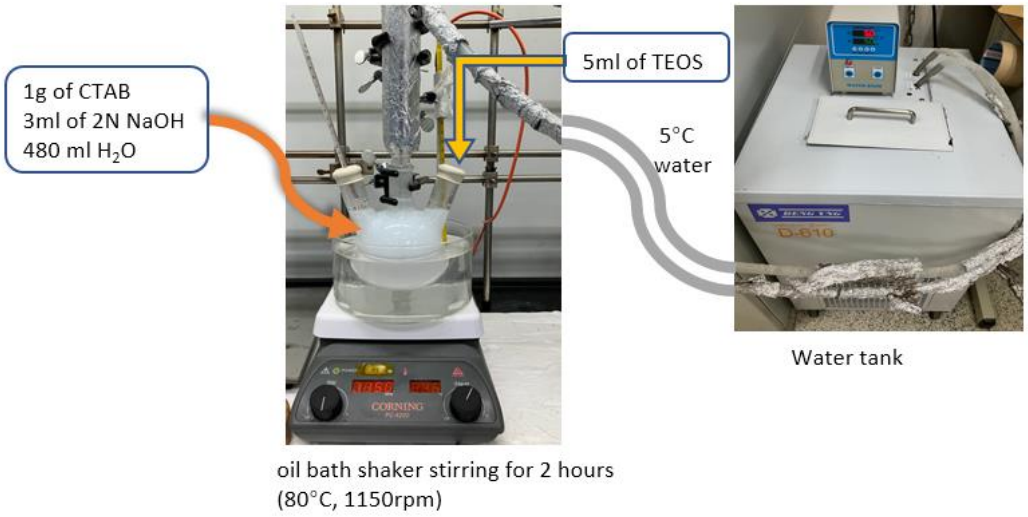


Figure 1. Experimental apparatus for MSN synthesis

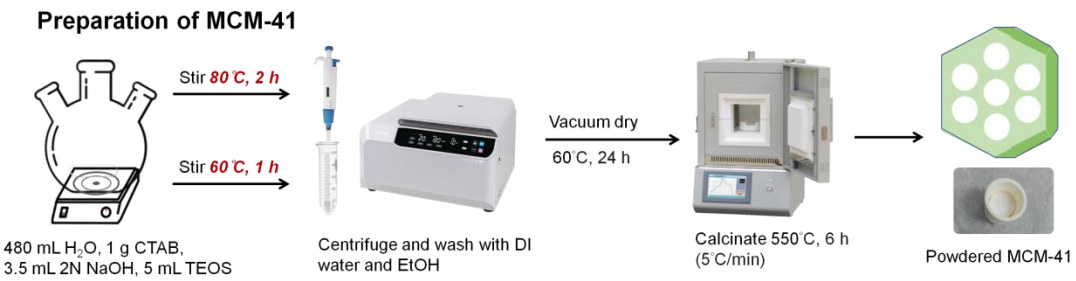


Figure 2. Schematic illustration showing the preparation of MCM-41

2.2.1.2 MCM-48 synthesis procedure

1. Add 240mL DI water and 100 mL ethanol into three neck flask
2. Add 5g CTAB, stir until the solution is clear
3. Add 24mL of ammonium hydride (pH = 11.19), stir (25°C, 5 min)
4. Add 7 ml TEOS, the solution was milk-white suspension
5. Stirred at 25°C in 2 different periods: 15 h and 32 h
6. Centrifuge with 50mL pellets (12,000 rpm, 15 min)
7. Wash twice with DI water and 95% ethanol
8. Vacuum dry the sample (25°C, 24 h)
9. Pour out the solid sample and grind it into powder with mortar and pestle
10. Transfer powder into heat crucible
11. Calcinate at 550°C, 6 h (+5°C/min)
12. The final mass was 0.657 g

2.2.1.3 SBA-15 synthesis procedure

1. Add 4g of P123 and 123 mL of HCl (pH = 3.00) into a two-neck bottle
2. Heat the mixture with oil bath (650 rpm, 40°C,)
3. When the solution is clear, add 8 mL of TEOS with a 10 mL syringe.
4. Keep stirring and heating with the oil bath (1150 rpm, 40°C, 24 h). The solution was milk-white after stirring
5. Put the flask into the heating mantle after stirring. Place a graham condenser in one neck. Cover the other neck with a rubber stopper
6. Stick a thermometer and heat sensor through the rubber stopper
7. Set the target temperature to 100°C
8. Heat for 2 different time period: 3 days and 7 days.
9. After heating, pour out the solid sample and grind it into powder with mortar and pestle
10. Transfer powder into heat crucible
11. Calcinate at 550°C, 6 h (+5°C/min)
12. The final mass SBA-15 (D3) was 0.743 g and SBA-15 (D7) was 1.24 g.

The process of MSN preparation was illustrated (Figure 2). .

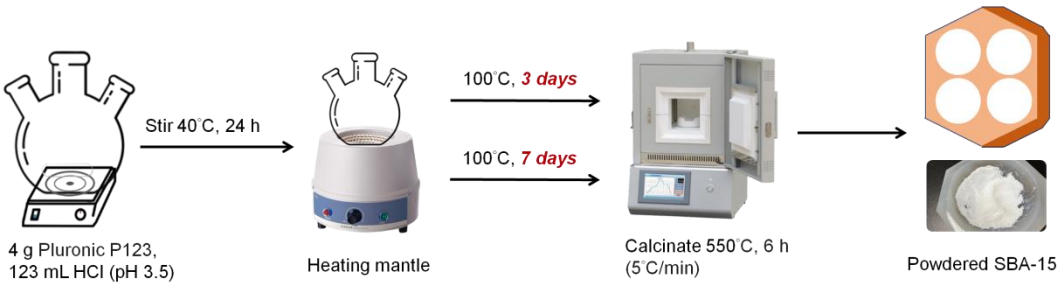


Figure 3. Schematic illustration showing the preparation of SBA-15

2.2.2 Functionalization of MSNs with amine groups of APTES

1. The MCM-41 was used for MSNs functionalization experiments
2. Mix 15 ml of deionized water, 7.5ml of glacial acetic, and 30ml of 75% ethanol.
3. Add 0.5 g MSN was stirred for 10 min
4. Add 0.5 ml of 98% APTES, dropwise and stirred for 24 h
5. Centrifuge with 50 mL pellets (15,000 rpm, 10 min)
6. Wash twice with DI water and 75% ethanol
7. Vacuum dried at 60°C for 24 h
8. The final mass MCM-41-NH_2 was 0.410 g

2.2.3 Cross-linking of aminated MSN and chitosan by glutaraldehyde

1. Mix 40 ml of deionized water and 0.4ml of glutaraldehyde
2. Add 0.41g MCM-41-NH_2 was stirred (4°C, 1 h)
3. Centrifuge with 50 mL pellets (15,000 rpm, 10 min)
4. Wash twice with DI water and 75% ethanol
5. Vacuum-dried at 25°C for 24 h and grinded into powder.
6. Add 50mg Chitosan to 30 ml of 0.1M HCl, stirring (25°C, 4 h)
7. Add MSN powder (404mg) in the chitosan solution,
8. Stirred at 25°C in 2 different periods: 24 h and 32 h
9. Centrifuge with 50 mL pellets (15,000 rpm, 10 min)
10. Wash twice with DI water and 75% ethanol
9. Vacuum-dried at 25°C for 24 h
10. The final mass MCM-41-NH_2 was 0.395 g

2.3 Characterization of Fabricated MSNs

2.3.1Scanning electron microscope (SEM)

Preparation of SEM samples:

1. Dissolve a tiny portion of powdered sample (MCM-41) into 5 mL of 95% ethanol. Use an ultrasonic water bath to help the powder disperse well until the solution is barely translucent.
2. Soak one silicon wafer with acetone for 5 minutes.
3. Dry the wafer with dust-free wipe.
4. Place the wafer on a piece of dust-free wipe. Then, use a 100 µL pipette, drop 1-2 drops of the sample onto the waver.
5. Stick a strip of carbon tape on a spherical metal stud. Then, use a tweezer to carefully stick the wafer onto the carbon tape.
6. Repeat steps 1-5 for all samples: MCM-41, MCM-41-NH2, MCM-41-CHIT (24 and 32 h), MCM-48, and SBA-15 (D3 and D7)
7. After sticking all the wafers on the carbon tape, vacuum-dry the metal stud (60°C, 24 h)
8. Coat the samples with platinum before SEM imaging

2.3.2 XRD

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

2.3.3 FTIR

Fourier transform infrared (FTIR) spectroscopy was used to confirm the synthesis of MSN-CTAB, MSN and the grafting of functional groups like MCM-41-NH_2, and MCM-41-CHIT. The frequency range of FTIR spectral scanning was between 4000 and 500 cm^{-1}.

2.3.4 BET Surface Analysis

BET surface analysis parameters

- The gas used was liquid nitrogen at -196°C.
- The degassing profile for MCM-41, MCM-41- NH_2, MCM-41-CHIT (24 and 32 h), MCM-48, and SBA-15 (D3 and D7)
    - Degassing process is a vacuum, high temperature treatment process

that drives water molecules away from the sample so nitrogen gas analysis can proceed.
    - Target temperature: 120°C, with 5°C/min heating rate
    - Soak time 960 minutes
    - All samples were measured at 20 mg for degassing.
- The analysis profile for MCM-41, MCM-41- NH_2, MCM-41-CHIT (24 and 32 h), MCM-48, and SBA-15 (D3 and D7)
    - Analysis temperature = 77.35 K
    - Analysis gas: nitrogen gas
    - Analysis type: physisorption
    - Analysis duration: 24 to 36 hours
    - After degassing, samples might decrease 1-2 mg in mass. Resulting around 18-19 mg

2.4. Drug loading and release

2.4.1 Dexamethasone loading

Dexamethasone (20mg) was added to 20 ml phosphate buffer saline (PBS) at pH 7.4. Three different MSN samples, MCM-41, MCM-41- NH_2, MCM-41-CHIT, (100mg) were dissolved in the DEX-PBS solution. Then, the solution was stirred (25 °C, 2 h) using a magnetic stirrer. Then the sample was centrifuged (9,000 rpm, 3 min 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 (9,000 rpm, 3min at 25 °C) for 5 min. Then, the supernatant was collected for further analysis of drug loading.

2.4.2. Measurement of dexamethasone content

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.

2.4.3. Calculate the drug loading capacity

To calculate the amount of dexamethasone encapsulated in MSNs, the prepared DEX@MCM-41, DEX@ MCM-41-NH_2, and DEX@ MCM-41-CHIT 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 absorption value of DEX in the supernatant was then measured.

The loading capacity (LC%) of DEX were calculated using the following equation:

$$\mathbf{LC\ (\%) = [\ (W0 - W1)/W\ ] \times 100\%}$$

where W0 is the total DEX mass added, W1 is the DEX mass in the supernatant, W is the total mass of drug-loaded MSNs

2.4.4. Dexamethasone release

All dexamethasone loaded samples were vacuum-dried at 60°C for 24 h. Three pellets of 30ml PBS (pH 7.4), each with 100mg of DEX@MCM-41, DEX@ MCM-41-NH_2, and DEX@ MCM-41-CHIT respectively, were 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 for further analysis. To maintain a constant volume, the withdrawn amount was returned, after each measurement, to the release media to keep a constant cumulative release percentage.

2.4.5. Calculate the drug cumulative release percentage

To calculate the amount of dexamethasone released from the prepared DEX@MCM-41, DEX@ MCM-41-NH_2, and DEX@ MCM-41-CHIT solutions, the collected sample solutions in different time periods were measured at 254 nm by UV–Vis spectrophotometer.

The cumulative release (CR%) of DEX was calculated using the following equation:

$$\mathbf{CR\ (\%) = (\ Wr\ /Wd\ ) \times 100\%}$$

where Wd is the total DEX mass loaded, Wr is the total DEX mass released

3. Results and Discussion

3.1. Fabrication and modification of mesoporous silica nanoparticles

3.1.1 Fabrication of MSNs

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. Then the surfactant CTAB was oxidized and disappeared after calcination (Figure 4). The synthesis of MSNs was schematically illustrated (Figure 5) .

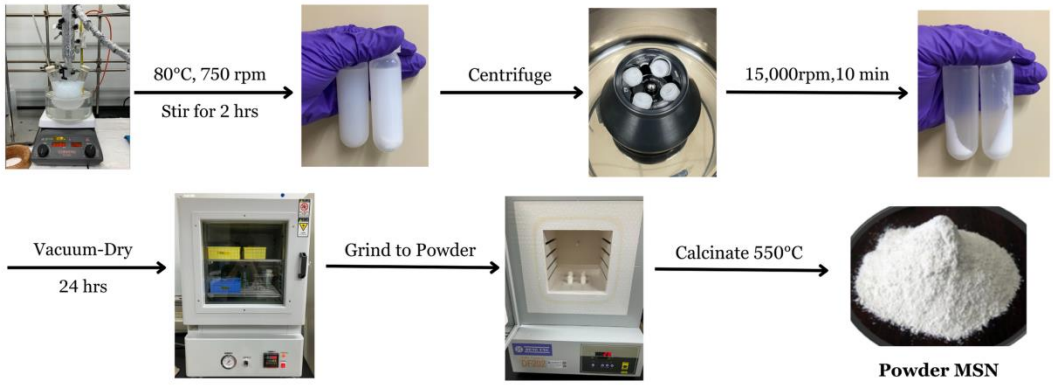


Figure 4. MSN precipitate after centrifugation and calcination .

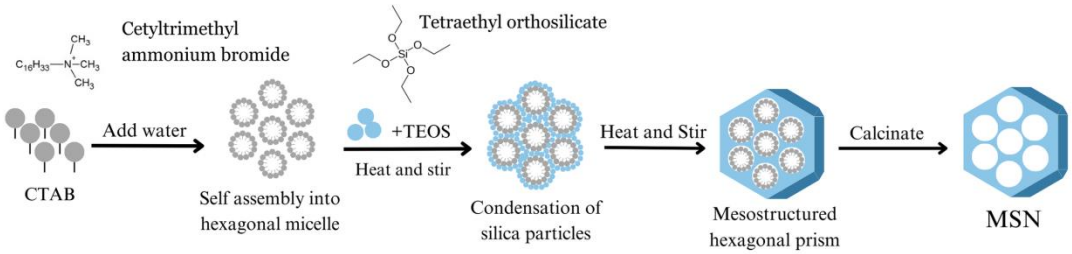


Figure 5 . Schematic illustration showing synthesis of MSN.

Tetraethyl orthosilicate (TEOS) was an organic silica compound (Figure 6), 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.

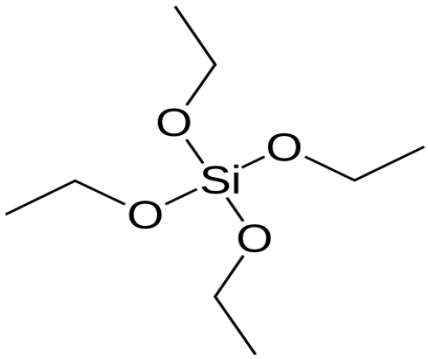


Figure 6. Chemical Structure of TEOS

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 7). 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.

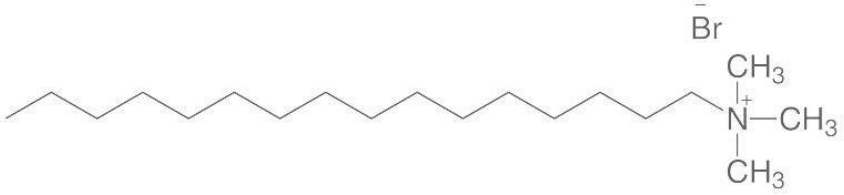


Figure 7. Chemical structure of CTAB.

Pluronic P123 wass a difunctional block copolymer surfactant (Figure 8) terminating in primary hydroxyl groups, consisting of hydrophilic polyethylene oxide (PEO) and hydrophobic polypropylene oxide (PPO) blocks arranged in an A-B-A tri-block structure: PEO-PPO-PEO. P123 could self-assemble into a spherical micelle structure constructed by ethylene oxide (EO) as a hydrophilic outer shell and propylene oxide

(PO) as a hydrophobic inner core.

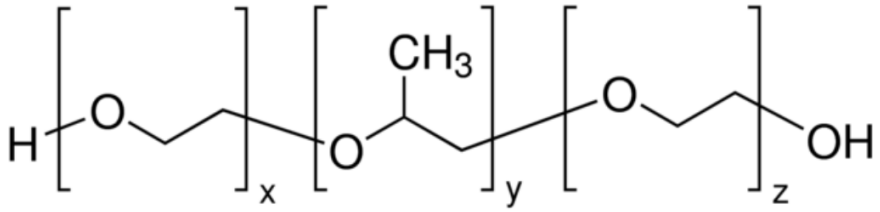


Figure 8. Chemical Structure of P123

3.1.2. Synthesis of aminated MSNs

The synthesis of MSN-NH_2 was schematically illustrated (Figure 9). The MCM-41 type MSN was used for amine modification in the experiment. The surface of MSNs 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. The aminated MSNs were with incorporation of amine groups on the exterior surface and internal pore surface.

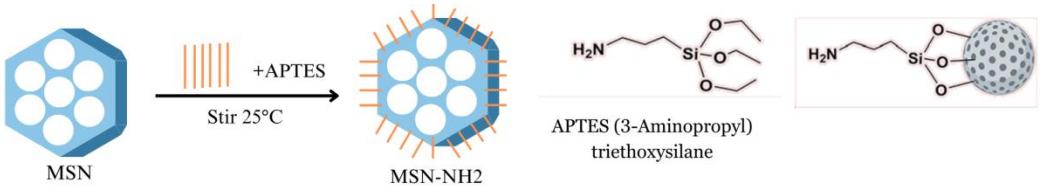


Figure 9. Amine groups modified the surfaces of MSNs

3.1.3. Synthesis of chitosan capped MSNs

The MCM-41 type MSNs were modified with amine groups and then followed with chitosan grafting. The synthesis of MSN-NH_2-CHIT was schematically illustrated (Figure 10). The exterior aminated MSNs were capped with chitosan mediated by glutaraldehyde as a cross-linker. 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. The process of chitosan modified on the surface of MSN was known as chitosan encapsulation. The amine groups on chitosan were bonded to the carbonyl groups of glutaraldehyde, forming a spherical cap on the surface of MSNs.

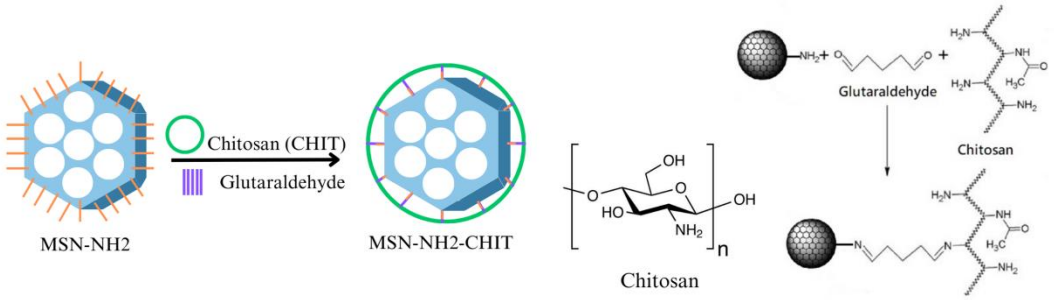


Figure 10. Chitosan modified MSNs

3.2. Characterization of the prepared MSNs

3.2.1. SEM

The particle size and the size distribution of synthesized MSNs were analyzed using SEM. The morphology of the MCM-41 using SEM technique showed spherical shapes (Figure 11). MCM-41 (60°C, 1 h) particles aggregated heavier and tighter than MCM-41 (80°C, 2 h).

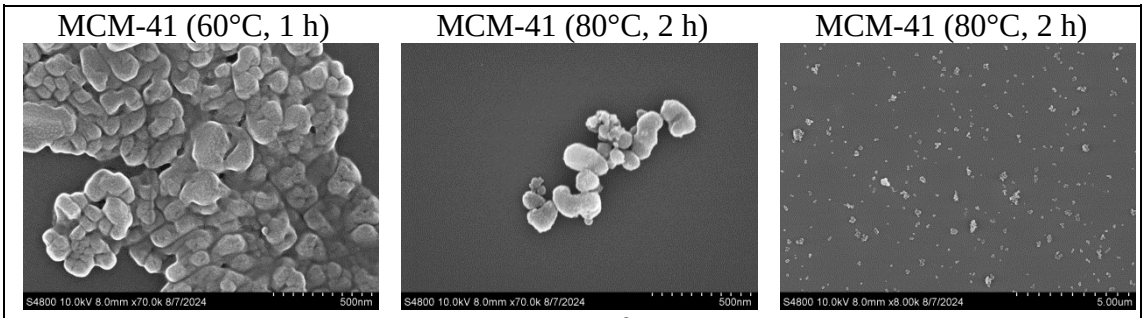


Figure 11. SEM of MCM-41

The particle size was measured by the SEM images (Figure 12). MCM-41 nanoparticles exhibited an average size of 95.1 nm. MCM-41-NH_2 nanoparticles exhibited an average size of 82.2 nm.

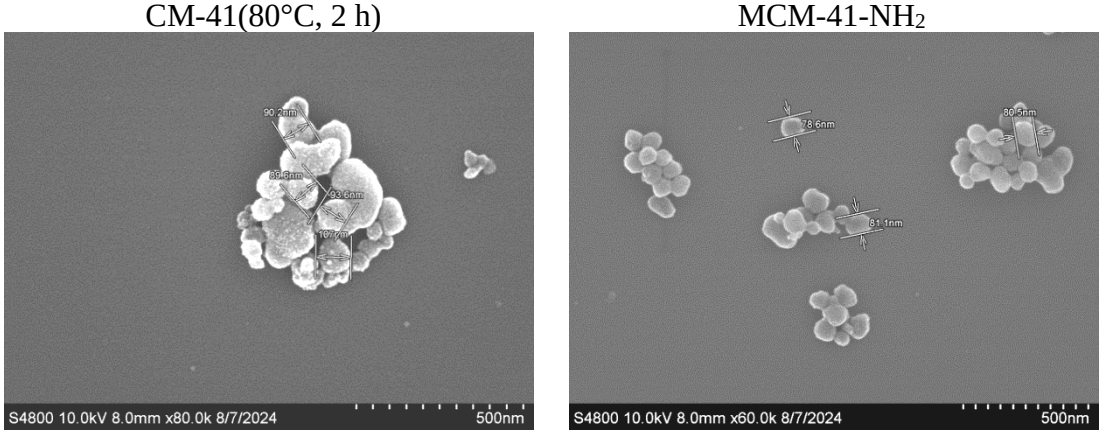


Figure 12. SEM of MCM-41 and MCM-41-NH_2

The MCM-41-CHIT synthesized under the conditions of 24 h and 32 h were spherical in shape and uniform in size (Figure 13). The average size was 110 nm.

MCM-41-CHIT (24 h) MCM-41-CHIT (32 h)

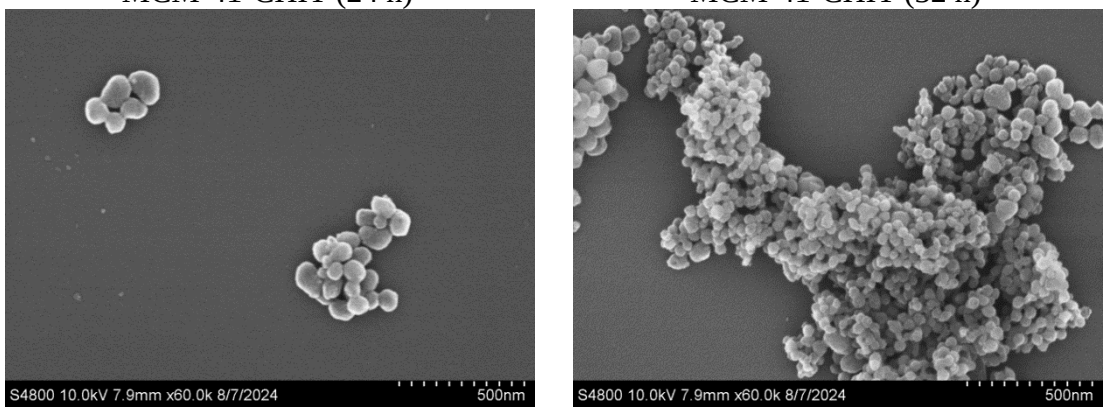


Figure 13. SEM of MCM-41-CHIT

From the SEM image shown (Figure 14), we noticed that MCM-48(32 h) had spherical morphology. These spheres were uniform with average sizes 570 nm.

MCM-48 (15 h) MCM-48 (32 h)

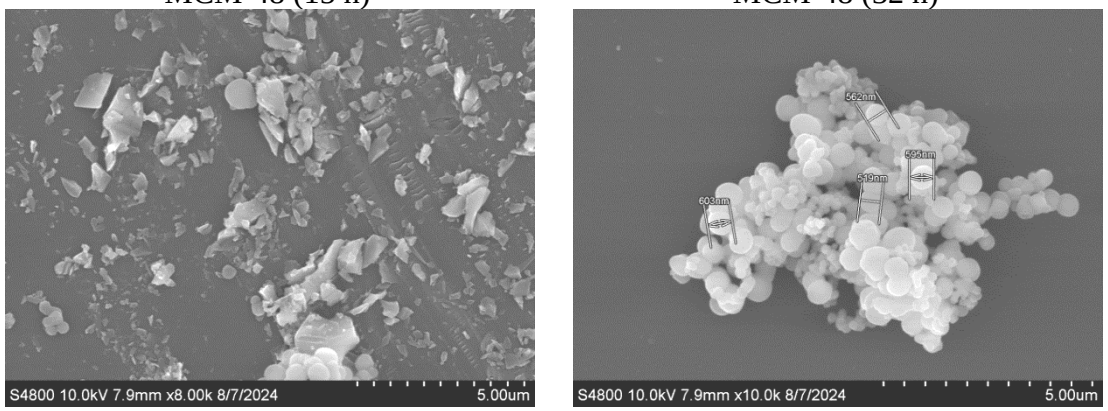
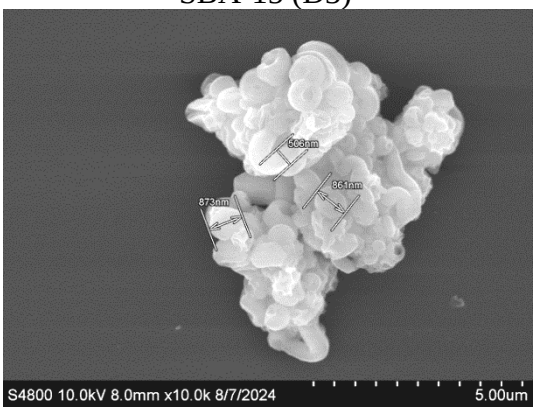


Figure 14. SEM of MCM-48

From the SEM image shown (Figure 15), the extracted SBA-15 materials were aggregated into wheatlike macrostructures. The particle diameter of SBA-15 was around 1μm. The particle size of SBA-15(D7) was relatively larger than SBA-15(D3).

SBA-15 (D3)



SBA-15 (D7)

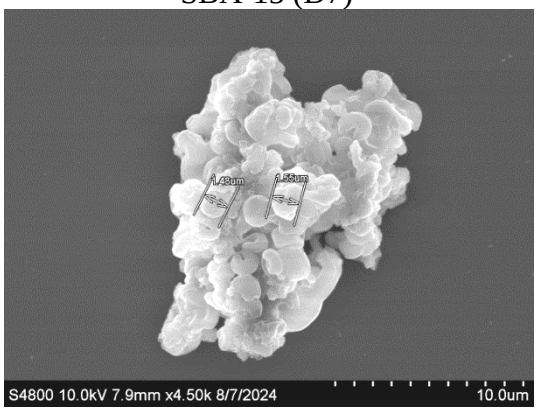


Figure 15. SEM of SBA-15

3.2.2. XRD pattern

In this work, the ordered mesoporous structure of the silica nanoparticles was confirmed by small angle XRD measurement. 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.

For MCM-41 (Figure 16), the findings revealed that a broad diffraction peak was emerged at $2\theta = 2.4^{o}$ representing the reflections of the ordered two-dimensional hexagonal mesoporous structure. Three well-defined diffraction peaks, attributing to the (100), (110) and (200) planes respectively, were clearly observed in the XRD pattern. The thickness of pore walls was correlated with the peak intensities. The decreased relative intensity of (200) peak to the (110) peak revealed the decrease in the thickness of the pore wall in MCM-41 as compared to MCM-41-CTAB. The diffraction pattern of MCM-41 type MSN represented the hexagonal mesoporous structure with 2-4 nm in pore size and 0.6-1.2 nm in wall thicknesses.

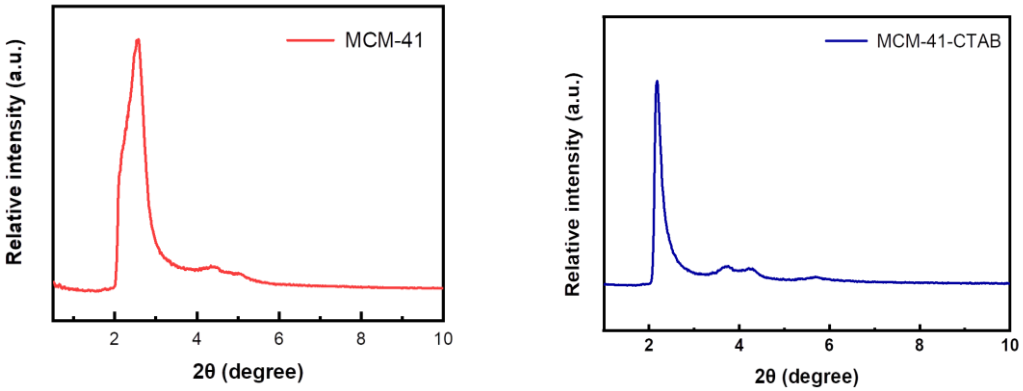


Figure 16. XRD patterns of MCM-41 and MCM-41-CTAB

For MCM-48, three well-defined diffraction peaks, attributing to the (100), (110) and (200) planes (Figure 17). The diffraction pattern of MCM-48 type MSN represented the reflections of the ordered two-dimensional hexagonal mesoporous structure,

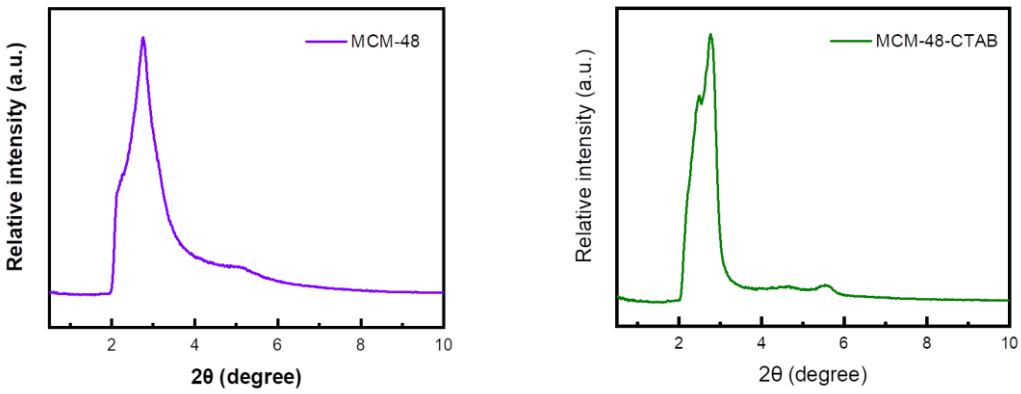


Figure 17. XRD patterns of MCM-48 and MCM-48-CTAB

For SBA-15, small-angle XRD confirms the hexagonally-ordered porous structure by displaying three well-resolved peaks assigned as the (100), (110) and (200) planes (Figure 18).

X-ray powder diffraction pattern could be analyzed by Bragg's law. The formula was as follows:

$$2d\sin\theta = n\lambda$$

(θ is the incident angle, d is the interplanar spacing, n is the diffraction order, and λ is the wavelength of the incident ray)

The d was calculated and accounted the pore size. The 2θ peak in SBA-15 was relatively smaller than MCM-41, so the pore size of SBA-15 was bigger than MCM-41.

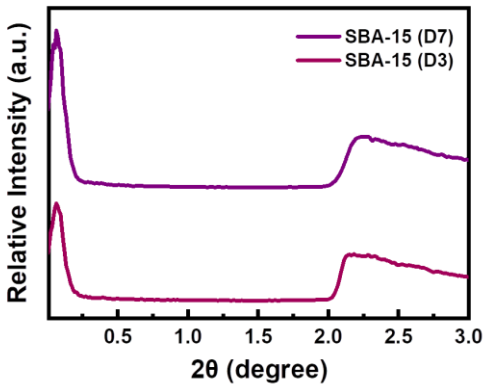


Figure 18. XRD patterns of SBA-15

3.2.3. Fourier transform infrared (FTIR) spectra

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.

FTIR spectra for MCM-41-CTAB (Figure 19) showed the band at 1084-1230 cm^{-1}, which demonstrated silica composite formation. 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}.

FTIR spectra for MCM-41, MCM-41-NH_2, and MCM-41-CHIT (Figure 20) displayed transmission peaks at about 1075 cm^{-1} and 800 cm^{-1}, which were assigned to the characteristic stretching vibrations of the Si-O bond. The corresponding fingerprints of aminopropyl functional groups of APTES was confirmed by the presence of a new band at around 1554 cm^{-1} related to asymmetric NH2 bending. In addition, the increased specific band of MCM-41-CHIT could be attributable to the alkyl groups of C-H bond by the weak C–H stretching vibrations at 2980 cm^{-1}. In reviewed literature, the spectrum of the MSN-APTES-chitosan sample would show two bands at 1467 cm^{-1} and 1633 cm^{-1} associated with amino groups in biopolymer chains with NH and CN boning. It showed trivial curve in our result.

The frequencies of each functional group were listed (Table 1).

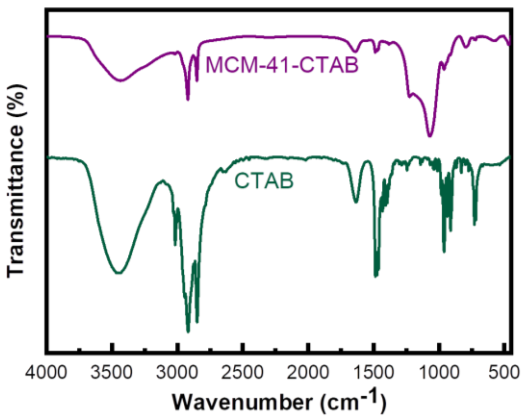


Figure 19. FTIR spectra of CTAB and MCM-41-CTAB

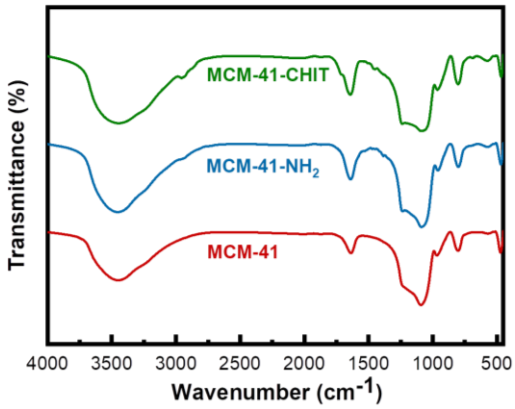


Figure 20. FTIR spectra of MCM-41, MCM-41-NH_2, and MCM-41-CHIT

Table 1. 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}

FTIR spectra for SBA-15(D3) and SBA-15 (D7) showed peaks of Si-O bonding (Figure 21). As for complete extraction of the surfactant, the disappearances of the C-H vibrations at 2970 and 2873 cm^{-1}, as well as those of the C-O-C vibrations at 1375 and 1456 cm^{-1}.

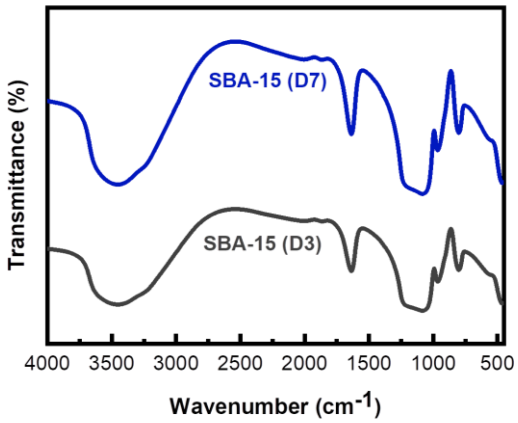


Figure 21. FTIR spectra of SBA-15

3.2.4. BET Surface Analysis

Nitrogen adsorption and desorption isotherms at -196°C were measured to analyze the pore volume and pore size by using Brunauer–Emmett–Teller (BET) surface area analysis method. The values for the BET surface area, the PDI, and the hydrodynamic diameters were given (Table 2). The nitrogen adsorption and desorption isotherms of MCM-41, MCM-41-NH_2, and MCM-41-CHIT were shown (Figure 22).

Analyses of the nitrogen adsorption/desorption isotherms yielded BET surface areas of approximately 896 m^2/g in MCM-41, 619 m^2/g in MCM-41-NH_2, and 519 m^2/g in MCM-41-CHIT. The pore volumes were 1.03 cm^3/g in MCM-41, 0.88 cm^3/g in MCM-41-NH_2, and 0.82 cm^3/g in MCM-41-CHIT.

After chitosan coatging, the N_2 adsorption isotherms of MCM-41-CHIT showed a relatively flat isotherm, accompanied by a loss of hysteresis loop. The surface area of MCM-41-CHIT decreased and the particle diameters increased. The hydrodynamic diameters of was 241 nm in MCM-41-CHIT (24 h) and increased to 424 in MCM-41-CHIT(32 h).

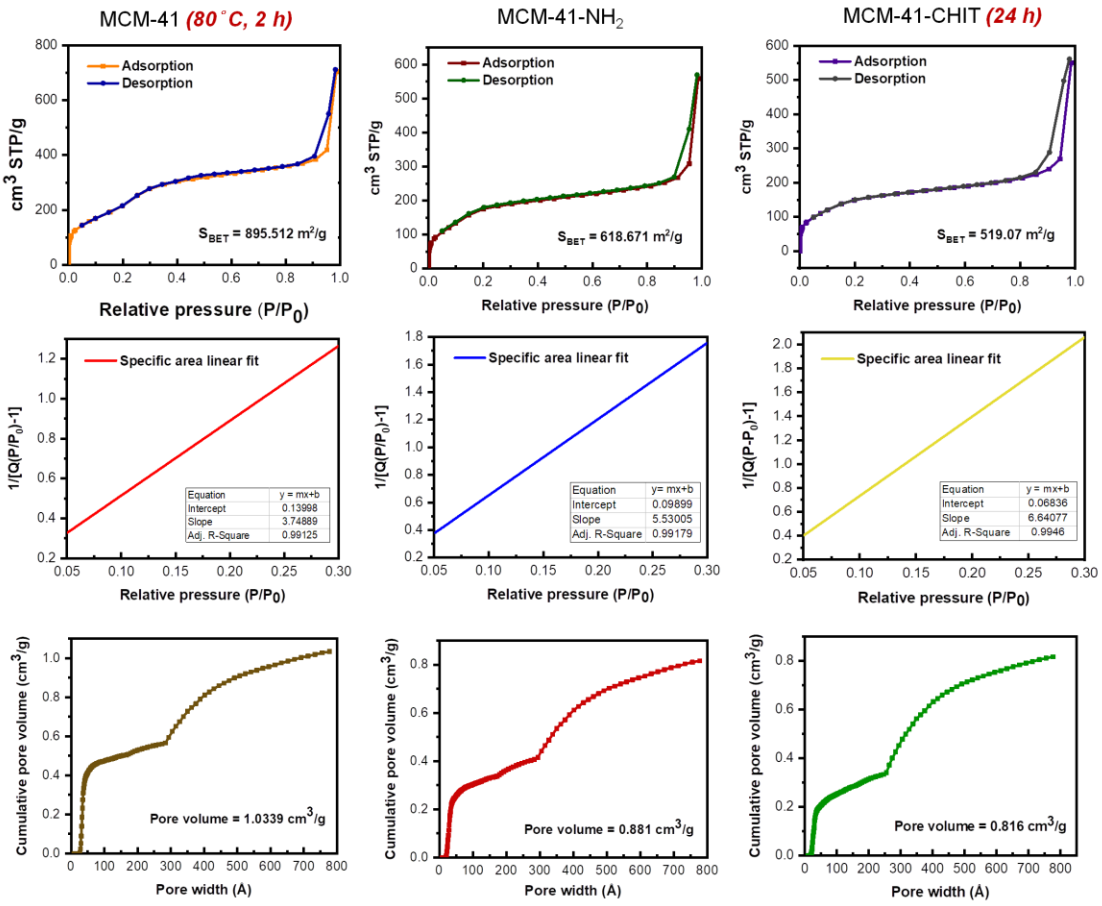


Figure 22. BET Surface Analysis of functionalized MCM-41

Table 2. BET analysis of MSNs

	BET surface area (m^2/g)	PDI	Hydrodynamic diameter (nm)
MCM-41 *(80°C, 2 h)*	12.537	0.375	131
MCM-41-NH_2	6.187	0.319	199
MCM-41-CHIT *(24 h)*	10.307	0.463	241
MCM-41-CHIT *(32 h)*	15.209	0.681	424

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 MCM-41, MCM-41-NH_2, and MCM-41-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. The wavelength of DEX was set at 254 nm.

The peaks present at 254 nm for DEX@MCM-41, DEX@ MCM-41-NH_2, and DEX@ MCM-41-CHIT indicated a successful loading of DEX (Figure 23).

The concentrations of DEX were calculated by Beer-Lambert Law (Table 3). The loading capacity of MCM-41-CHIT showed 54.5%, followed by 34.9% in MCM-41-NH_2 and 27.9% in MCM-41 (Figure 24).

The results demonstrated a promising effect of the chitosan modification which showed higher loading efficiency than non-modified MSN. The speculation below was in accordance with the experimental results. 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 chitosan could enhance the interaction with DEX molecules by forming multiple hydrogen bonds for stronger intermolecular forces.

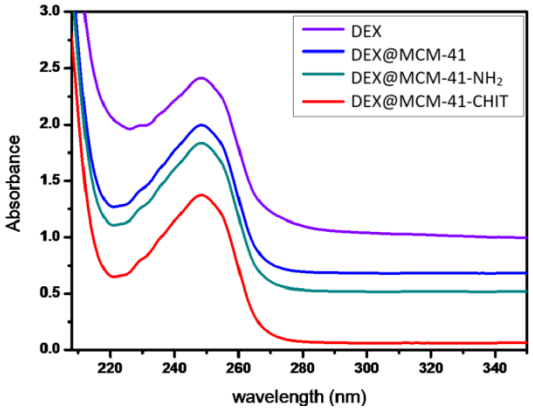


Figure 23. UV-Vis of DEX loading

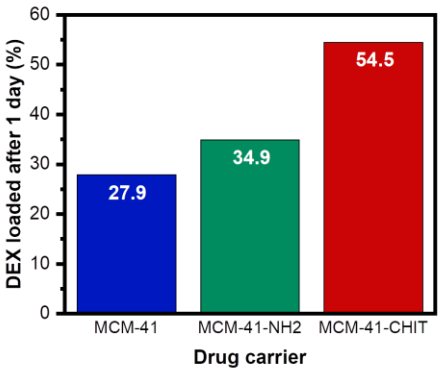


Figure 24. Loading capacity

Table 3. Amount of DEX in centrifuged supernatant

	DEX (μg/ml)
DEX	2.72
DEX@MCM-41	1.96
DEX@MCM-41-NH_2	1.77
DEX@MCM-41-CHIT	1.26

3.4. Dexamethasone releasing

DEX@MCM-41, DEX@ MCM-41-NH_2, and DEX@ MCM-41-CHIT

The release kinetics of dexamethasone from DEX@MCM-41, DEX@ MCM-41-NH_2, and DEX@ MCM-41-CHIT in PBS buffer at pH 7.4 were shown (Figure 25). The graphic pattern showed that the release in DEX@MCM-41 and DEX@ MCM-41-NH_2 already reached around 50% at 12 h and then around 80% at 48 h and revealed a fast drug release profile, so-called "burst release". 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, DEX@ MCM-41-CHIT released only 5% at 12 h and reached 10% at 48 h. The release was slowly sustained throughout the time intervals in 120h, which showed linearly constant release profile (Figure 26). The predictable drug release in constant rate was an essential factor for mucoadhesive drug delivery to maintain a stable therapeutic effect at target tissue site.

The amounts of DEX releasing after 120 h were shown (Table 4). The cumulative release from dispersed DEX@MCM-41, DEX@ MCM-41-NH_2, and DEX@ MCM-41-CHIT reached 90.7%, 87.3% and 19.3% respectively (Figure 27). In DEX@MCM-41 and DEX@ MCM-41-NH_2, the DEX molecules were loaded in the pores with weak intermolecular forces. The release began with the diffusion process of incorporated drug from the mesoporous channels toward the PBS media. This diffusion process was triggered immediately after the contact of the nanocarriers with the aqueous media. As a result, the DEX molecules easily diffused out of the pores, resulting in large amount premature release in short period of time.

In order to maintain drug release in slow and constant rate, chitosan was capped around MCM-41 to control the DEX release as so-called "gatekeeper". Chitosan formed a shell-like cap on the exterior MSN, grabbing DEX molecules and forming firm covalent bonds. DEX molecules embedded in the MSN pore channels were hindered by chitosan unlikely to escape with the effusion process.

Overall, these results suggested that the chitosan capped MSN could be a suitable candidate as DEX carrier for mucoahesive drug delivery.

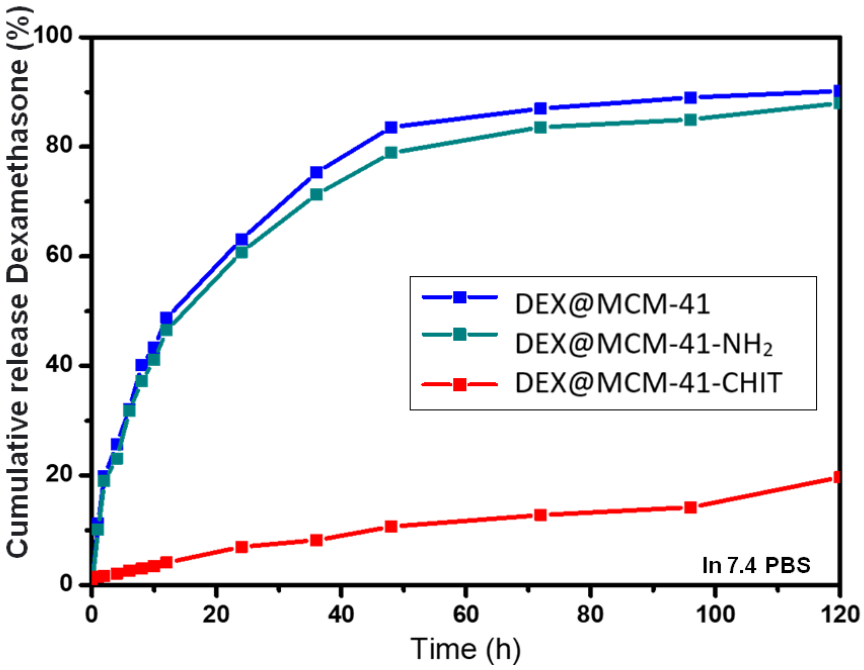


Figure 25. Kinetic release profile of DEX

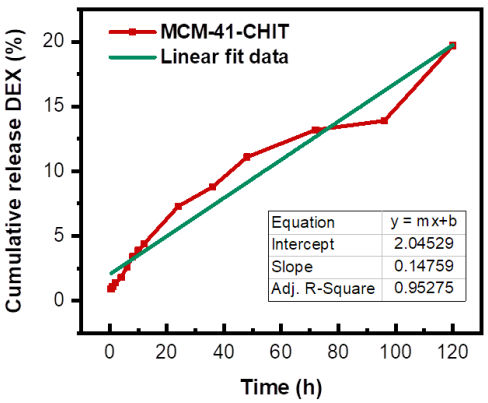


Figure 26. Linear regression of release

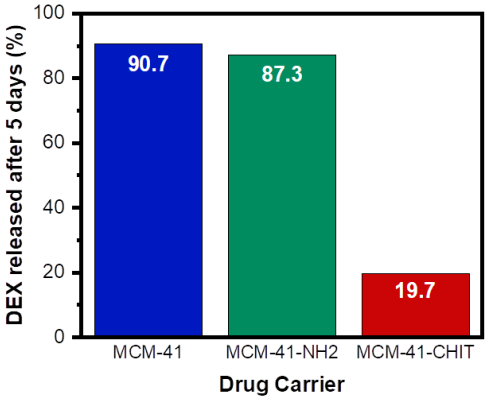


Figure 27. DEX release in 120 h

Table 4. DEX cumulative released after 120 h

	DEX (µg/ml)
MSN-DEX	0.69
MSN-NH2-DEX	0.83
MSN-NH2-CHIT-DEX	0.287

4. Conclusions

In this study, we achieved a successfuls approach to improve the dexamethasone treatment of lung disease via a smart mucoadhesive drug delivery system. The synthesis of chitosal capped MCM-41 type mesoporous silica nanoparticleswas optimized through the verification of characterization. Functionalization of MCM-41 with chitosan was proceeded through two-step conjugations of APTES amination and glutaraldehyde crosslinking. Dexamethasone was loaded in the chitosal capped MCM-41 and showed a high loading capacity of 53.7%. Accumulative release profile of dexamethasone in physiological solution showed the linear relationship of constant rate for a serial period of time. The release in 120 hours was 19.7% for MCM-41-CHIT.

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【評語】100030

此研究利用幾丁聚糖修飾中孔二氧化矽奈米顆粒作為藥物釋放系統，具有良好的應用潛力，符合當前藥物遞送技術的發展趨勢。建議進一步探索其在臨床上的應用，以及優化藥物釋放特性及研究其他藥物的遞送效果，擴展研究的應用範圍。