100 nm Mesoporous Silica Nanospheres
Drug Loading Protocol
Product Numbers SHSN100, SHAN100, SHSD100, SHAD100

1. INTRODUCTION

nanoComposix 100 nm MCM-41 Mesoporous Silica Nanospheres (MSN) have hexagonally-arranged pores that can be loaded with a variety of active pharmaceutical ingredients (API) via several different loading methods. Based on the desired API, MSN may be chemically modified inside and/or outside particle pores. Example experimental protocols are included for the following loading methodologies:

Solvent-based Methods

- Loading using dried particles
- Loading using suspended particles
- Incipient wetness impregnation

Solvent-free Methods

- Melt
- Co-milling

Other Methods

- Hydrotropy
- Solvent evaporation
- Adsorption/evaporation

Contact info@nanocomposix.com for inquiries regarding custom particle loading, technical support, or determining which nanoparticle and surface chemistry are right for your application.

2. PRELIMINARY CALCULATIONS

Regardless of the chosen loading methodology, calculations are required to determine theoretical loading of the MSN. These calculations are based on the surface area of the MSN and the size of the molecule to be loaded.

Example calculations for loading doxorubicin (Dox):

The typical pore volume of a 100 nm MCM-41 MSN is: V_p = 0.67 cm^3 g^{-1}

Consider a "sphere" representing the doxorubicin, with a diameter d = 1.6 nm and volume V_{Dox} = 2.14*10^{-21} cm^3

The pores of MCM-41 MSN can fit: $\frac{V_p}{V_{Dox}} = \frac{0.67}{2.14}*10^{21}$ = 3.13*10^{20} molecules/g

$$\rightarrow \frac{molecules/g}{N_A} = \frac{3.13}{6.022}*10^{-3} = 5.20*10^{-4} \text{ mol g}^{-1}$$

Molecular weight of doxorubicin hydrochloride: M_w = 543.525 g mol^{-1}

Total weight percent of doxorubicin inside MCM-41 MSN: 543.525 x 5.20*10^{-4} = 0.283 g/g → **28.3 wt%**
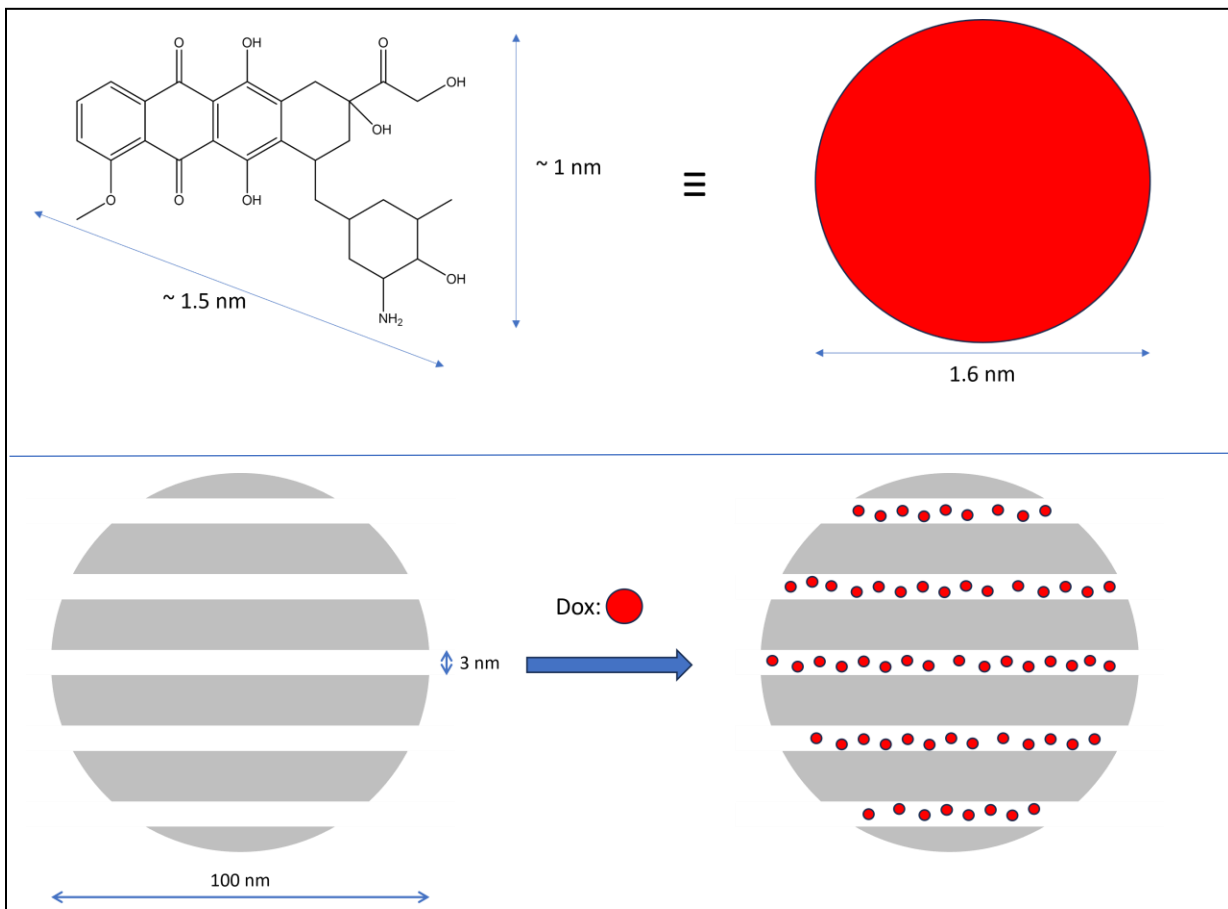
Figure 1

3. Loading using dried particles

The most commonly performed API-loading into mesoporous silica nanoparticles is to resuspend dried particles into a concentrated solution of the compound of interest. Upon solvation, the nanoparticles will uptake the active molecule that is dissolved in the solvent (generally an aqueous buffer for hydrophilic molecules), leading to high loading efficiency. This method primarily relies on the ability of the silica nanoparticles to suspend in the chosen solvent.

In addition to this passive mode of action, it is possible to exploit the structure of the API and increase its affinity for the silica matrix to enable better encapsulation efficiency. Under neutral pH, a drug molecule such as doxorubicin will be positively charged due to the primary amine present on its structure (see **Fig. 1**), as a result, negative charges of the deprotonated silanol groups present on MSN pores walls will inevitably attract the API during the loading process. Although this charge-charge interaction facilitates more efficient drug loading, this can hinder facile drug release during the delivery process. Starting MSN particles can be modified to better enable both effective loading and release. If, for example, API release needed to occur the lower pH conditions of the late endosomes or in cytosol (pH ~ 5-5.5), it would then be relevant to rely on carboxyl-functionalized pores rather than the original silanol. Carboxyl-modified pores will protonate at a higher pH than silanol, enabling the conditions required for both effective particle loading and controlled drug release in the desired pH range.

Pros:
- Low complexity
- Readily adaptable
- Hydrophilic/hydrophobic API compatible

Cons:
- Potential particle aggregation
- Relies on silica suspension in the solvent

Example Protocol: Loading doxorubicin in dried 100 nm MCM-41 nanoparticles (silanol surface)

1) Make an aqueous solution of doxorubicin by mixing 20 mg with 900 µL of deionized water
2) Add 100 µL of a 10x PBS buffer (pH 7.2-7.4) to ensure the neutral pH of the loading solution
3) Add 40 mg of dried particles and sonicate until homogeneous
4) Cover the container with aluminum foil and let it rotate overnight in the dark at room temperature or 4 °C
5) After incubation, centrifuge the particles, wash with same volume of water and repeat until a clear supernatant is obtained
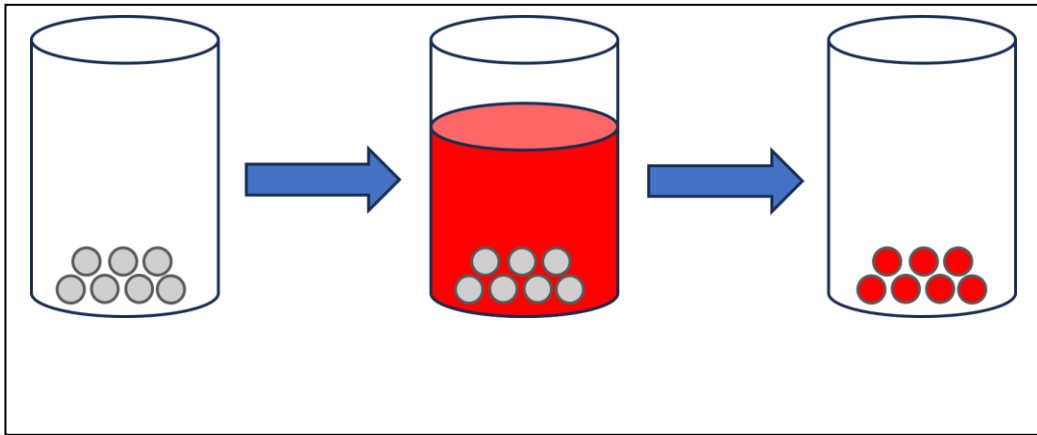
Figure 2

4. Loading using suspended particles

Due to potential for irreversible aggregation in the previous method, an alternative is to use nanoparticles already well suspended in a solvent and load the API in the same or a different solvent. In order to "force" the drug molecule into the already solvent-filed pores, it might be necessary to use a different solvent for the drug that is miscible with the particles' matrix.

Example Protocol: Loading doxorubicin in suspended 100 nm MCM-41 nanoparticles (silanol surface)

1) Make a solution of doxorubicin by dissolving the drug (20 mg) in 900 µL of DMSO
2) Use already suspended MCM-41 in the solvent of choice. Neutral or slightly acidic buffers would be preferred to ensure the protonation of doxorubicin and high interaction with the negatively charged pores (recommended: particles in DI water at 20 mg/mL, 2 mL)
3) Allow both solutions to mix in the dark for 24 h at room temperature or 4 °C
4) After incubation, centrifuge the particles, wash with same volume of water or neutral buffer and repeat until a clear supernatant is obtained

Pros:
- Low complexity
- Readily adaptable
- Hydrophilic/hydrophobic API compatible

Cons:
- Long process
- Low yield
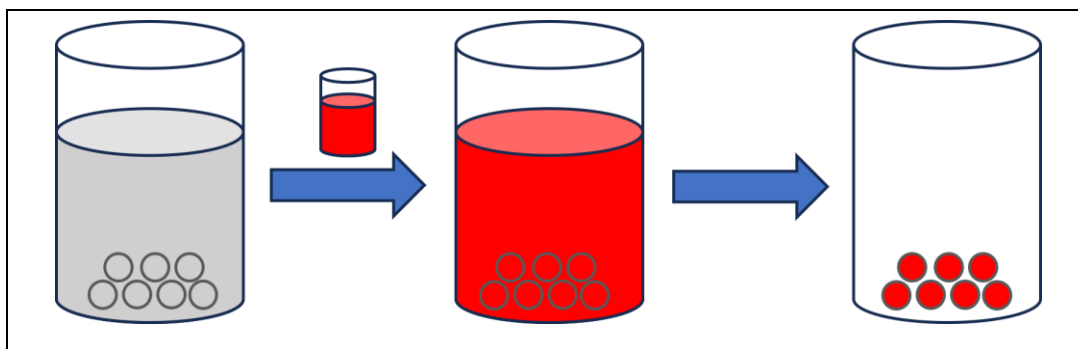
Figure 3

5. Incipient wetness impregnation

In the incipient wetness impregnation technique, a highly concentrated solution of API is slowly drop-casted onto dried nanoparticles, forcing the drug to enter the pores alongside the solvent. In order to avoid potential crystallization of the molecules on the surface of the material, small volumes are added at a time separated by drying periods. The use of volatile solvents in which the drug can be solubilized is necessary.

Example Protocol: Loading of ibuprofen in 100 nm MCM-41 nanoparticles (amine surface)

1) Prepare a concentrated solution of ibuprofen in dichloromethane (30 mg in 1 mL)
2) Add the ibuprofen solution dropwise (100 µL at a time) into 100 mg of dried particles, mix regularly to ensure wetting of whole powder
3) Repeat until entire solution is added
4) Dry in air for 24 h followed by reduced pressure for another 24 h

Pros:
- Good for hydrophobic APIs
- Low amounts of solvent required

Cons:
- Potential drug aggregation on MSN surface
- Tedious procedure
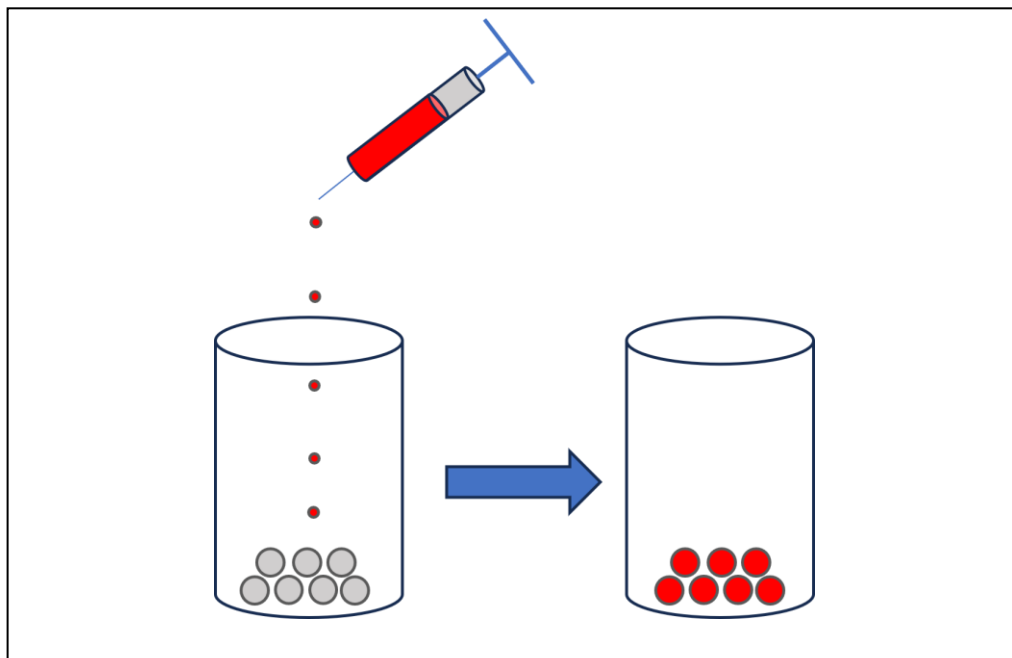
Figure 4

6. Melt

In the melt method, the particles and the drug of interest are mixed together and brought above the API's melting temperature for a few minutes, enabling API loading into MSN pores. The main advantages of the method are the absence of toxic solvents and the potential for high encapsulation yield.

Example Protocol: Loading ibuprofen into MCM-41 nanoparticles (amine surface)

1) Weigh 70 mg of MCM-41 MSN into a container suitable for temperatures above 100 °C
2) Add 30 mg of ibuprofen and physically mix with the particles
3) Heat the mixture to 100 °C for 5 min
4) Let mixture cool down

Pros:
- Good for any type of drug
- No toxic solvent required
- High yield of encapsulation

Cons:
- Potential API aggregation on particle surface
- Potential API degradation
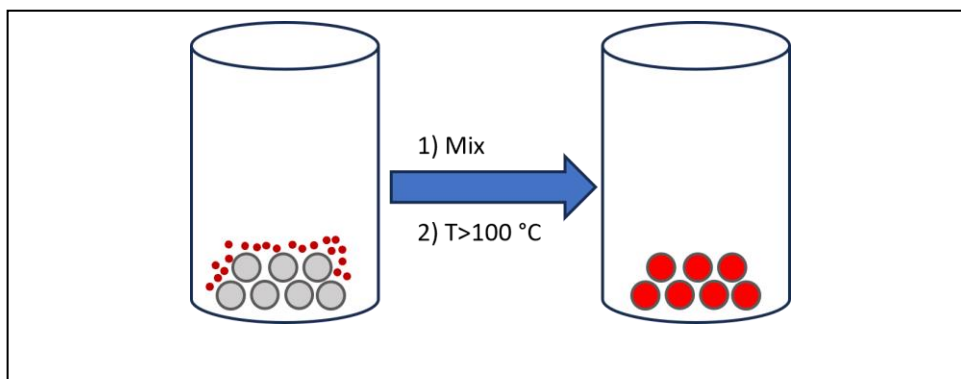
Figure 5

7. Co-milling

In the co-milling process, the drug and the mesoporous silica are intensively milled for periods of time to force drug molecules into MSN pores. This method can yield high encapsulation and avoids the potential temperature degradation of the melting method, but also yields material with some drug attachment to the particle surface. It also requires specific (milling) equipment.

<u>Example Protocol</u>: Loading ibuprofen into MCM-41 nanoparticles (amine surface)

1) Weigh 70 mg of MCM-41 and 30 mg of ibuprofen into an agate milling container in presence of an agate milling ball
2) Start milling cycles of 5 min "On" 2 min "Off" (if using a room temperature mill) at preferred frequency (for example 30 Hz), repeat 2-3 cycles

Pros:
- Good for any type of drug
- No toxic solvent required
- High yield of encapsulation

Cons:
- Potential aggregation on the surface
- Specific equipment
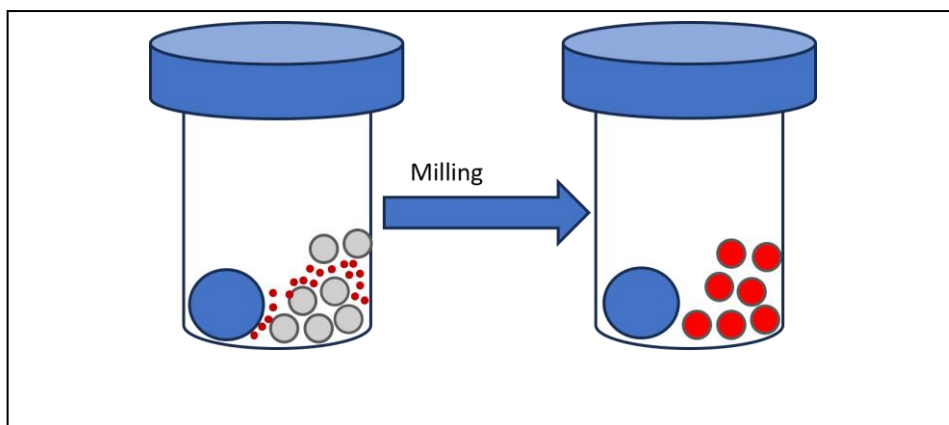
Figure 6

8. Hydrotropy

When facing poorly soluble drugs, it is possible to use chaperone molecules also known as "drug-coformers" to enhance their drug dispersibility and increase encapsulation into the silica nanoparticles. When the loading solvent is water, the chaperone molecule is called a hydrotrope.

Example Protocol: Loading of clofazimine in MCM-41 nanoparticles (silanol surface)

1) Prepare a solution of clofazimine in acetophenone (50 mM)
2) Add 10 mg of MCM-41 to 1 mL of the solution prepared in Step 1
3) Let stir for 24 h
4) Wash three times with water (centrifugation/resuspension cycles)

Pros:
- High yield of encapsulation
- No toxic solvent required

Cons:
- Requires optimization/screening

9. Solvent evaporation

A modification of the standard solvent-based method consists of a slow evaporation of an organic solvent in order to increase the relative concentration of the API inside the pores during the process. This method can still lead to significant adsorption of the drug onto the surface of the nanoparticles.

Pros:
- Low complexity
- Readily adaptable
- Hydrophilic/hydrophobic compatible

Cons:
- Long process

PROPRIETARY

10. Adsorption/evaporation

The DiSupLo (diffusion supported loading) process is a hybrid method that combines the physical mixing of the drug and nanoparticles with the slow diffusion of solvent inside the pores to create a highly concentrated slurry. The technique is easy to set up and only requires a shaker to homogenize the API/MSN mixture.

Example Protocol: Loading ibuprofen into MCM-41 nanoparticles (dried) (amine surface)

1) Physically mix 50 mg of MCM-41 MSN and 50 mg of ibuprofen using a shaker
2) Transfer the mixture into an open container and place it into a diffusion chamber containing 15 mL of ethanol
3) Let incubate for 3 h
4) Evaporate the ethanol from the particles by placing them in an oven at 50 °C overnight

Pros:
- Low complexity
- Readily adaptable
- Hydrophilic/hydrophobic API compatible

Cons:
- Long process
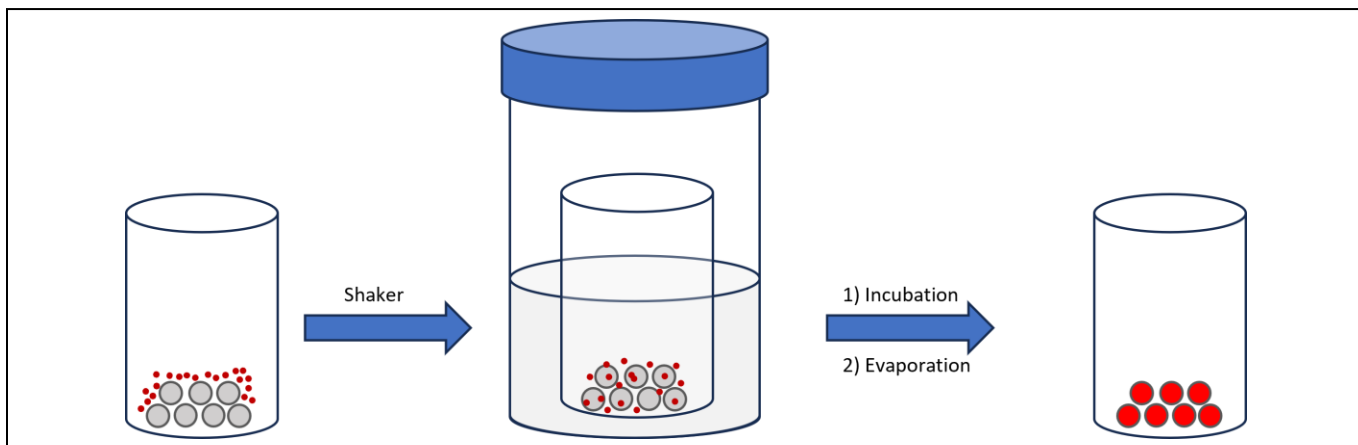
Figure 7

11. Additional resources

Learn more about mesoporous silica on our website (link mesoporous silica NCXU page; https://www.fortislife.com/products/articles/mesoporous-silica-nanoparticles/mesoporous-silica

For technical assistance, please contact (858) 565-4227 or email us at techsupport@nanocomposix.com.

12. Product use

nanoComposix nanoparticles and conjugation reagents are intended for research use only unless otherwise noted on the Certificate of Analysis (CoA) or Certificate of Conformance (CoC) for the product. Need a custom surface, conjugation, or cGMP-compliant material? Contact us at info@nanocomposix.com for additional information and pricing.

13. References

Chen, W.; Cheng, C.-A.; Lee, B.-Y.; Clemens, D. L.; Huang, W.-Y.; Horwitz, M. A.; Zink, J. I. Facile Strategy Enabling Both High Loading and High Release Amounts of the Water-Insoluble Drug Clofazimine Using Mesoporous Silica Nanoparticles. *ACS Appl. Mater. Interfaces* **2018**, *10* (38), 31870–31881. https://doi.org/10.1021/acsami.8b09069.

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