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Research Article

AMINOFUNCTIONALIZED MESOPOROUSSILICA NANOPARTICLESLOADED WITH 5-FLUOROURACIL

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A R T I C L E I N F O

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A B S T R A C T

We report the synthesis of Amino funtionalized and Mesoporous Silica Nanoparticles and loading of 5-fluorouracil. Themesosporous silica nanoparticles (MSNs) were synthesized viaSolgel process using Cetyltrimethyl ammonium bromide (CTAB) surfactant template. The prepared nanoparticles were amino functionalized by 3-Aminopropyltriethoxysilane (APTES) toimprove drug entrapment efficiency. The drug loading of 5- fluorouracil was done by backfilling approach. The prepared amino functionalized APT-MSNs were characterized by Fourier transform Infrared (FTIR) spectroscopy. Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) were used to study the structure and morphology of the composites.

INTRODUCTION

MSNs are solid materials, which contain hundredsof empty channels (mesopores) arranged in a 2D network of honeycomb-like porous structure. Due to their unique adjustable properties such ashigh surface area (>700 m2/g), pore volume (>0.9 cm3/g), and tuneable pore diameter (2–10 nm) MSNs can serve asversatiledrug delivery carriers. They present a stable and rigid framework with excellent chemical, thermal, and mechanical stability. Furthermore, both the exterior particleand interior pore surfaces can also be easily functionalized or site-specific delivery.

According to IUPAC classification, mesoporousmaterials will have a pore diameter ranging from 2 to 50 nm. Conventional MSN can load a dose of therapeuticdrug with 200-300 mg (maximally about 600 mg) drug/1 g silica [1]

MSN synthesisis done mainly by two methods

Sol-gel method: Four ingredients are necessary to formmesoporoussilica materials: a surfactant, silica source, an acid or base catalyst and a solvent like ethanol or water. This process requires two-step consideration: Hydrolysis and condensation. Hydrolysis produced colloidal particles in aqueous solution, which can be stimulated at alkaline and acidic pH. High concentration of amphipilic surfactant assembles into a spherical micelle in water and hydrophilic soluble precursor like polysilicic acid or silica acid. By electrostatic and hydrogen bonding interaction, the silica precursor is concentrated at the hydrophilic interface and form an amorphous silica, which is a mold of the mesoporousproduct. Removal of remaining surfactant can be done by calcination and extraction method [2].

Spray drying method [3]

The capacity to encapsulate drugs in nanoparticles can be improved by functionalization. The surface functionalizationis generally needed to load proper type of drug molecules (hydrophobic/hydrophilic or positive/negative charged).

In this work we have synthesized mesosporous silica nanoparticles bysolgel process using CTAB surfactant template. The prepared nanoparticles were amino functionalised for enhanced 5-FU loading using APTES.

LITERATURE REVIEW

1. Kummel AC *et al.* has done a review on the synthesis and surface functionalization of silica nanoparticles for nanomedicine.
2. Wang S *et al.* developed mesoporoussilica nanoparticles (MSNs) loaded with a poorly water-soluble drugtelmisartan, intended to be orally administered to improve the dissolution rate and enhance the drug loading capacity.
3. Xia T *et al.* modified the surface of MSN particles by a functional group that enhances cellular uptake and allows nucleic acid delivery in addition to traditional drug delivery.

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4. Lee C *et al*. developed intracellular pH-responsive mesoporous silica nanoparticles for the controlled release of anticancer chemotherapeutics.
5. Tummala S *et al.* formulated and characterized 5-Fluorouracil enteric coated nanoparticles for sustained and localized release in treating colorectal cancer.

MATERIALS

3-Aminopropyltriethoxysilane were purchased from Tokyo chemical industry Co. Ltd, Toshima, Japan. 5-Fluoruracil and Ninhydrin were purchased from Central drug house(P)Ltd., New Delhi, India.Dialysis Membrane (MWCO 12-14 kDa) were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India.

METHOD AND CHARACTERIZATION

MSN were synthesized using the method of Cai *et al* [4]. 1 g of CTAB was dissolved in a mixture of 3.5 ml of 2 M Sodium hydroxide and 480 ml ultrapure water. The mixture was heated to 65^{0}C and stirred at 1000 rpm while maintaining the temperature. To the homogenous liquid added 5 ml of TEOS. Stirring was continued for a period of 2 hrs. The white slurry produced is separated by centrifugation at 5500 rpm. Slurry was washed 3 times with water and dried at 75^{o}C. The dried slurry was then calcined at 600^{o}C for 4 hrs. The white powder produced was suspended in 50 ml of water with the aid of ultrasonication. Resuspended nanoparticles were freeze dried to get the free falling MSN.

Zeta potential was determined by the electrophoretic mobility of MSN in U- type tube at 25^{0}C, using Zetasizer Nano ZS (Malvern Instruments Ltd, Worcestershire, United Kingdom) after suitable dilution with water.

Morphology of MSN was characterized using scanning electron microscope JEOL model JSM – 6390 LV at 20kV
Particle size and structure of optimized MSN were studied using Transmission electron microscopy (TEM). The MSN was uniformly dispersed in ultrapure water, drop casted formvar coated copper grid and allowed to dry over night at room temperature. The grid was then imaged at 80kV using transmission electron microscope (Hitachi, H-7650)
Nitrogen adsorption–desorption isotherms were carried out on Micromeritics TriStar II 3020 analyzer at -195.800 °C under a continuous adsorption condition with equilibration interval of 5 seconds. All samples were pretreated for 12 hour at 200^{o}C under nitrogen before measurements. The pore size distribution was calculated from adsorption branches of isotherms by the Barrett–Joyner–Halenda method. Pore volume and specific surface area were calculated by using Barrett–Joyner–Halenda, Brunauer–Emmett–Teller and Langmuir methods, respectively.

Aminofunctionalization of MSN*[5,6,7,8]*

Aminogroupis attached to the surface MSN using 3-aminopropyltriethoxysilane (APTES). 500 mg of mesoporous silica nanoparticle was taken in a three necked round bottom flask placed on rotamantle and purged with nitrogen gas. Nanoparticles were dehydrated by heating at 100^{o}C for 6hrs. Mixture of 2 ml APTES and 50 ml dehydrated ethanol was added to the round bottom flask. Resulting mixture was reflexed at 77^{o}C under nitrogen environment for 10 hrs. Nanoparticle suspension was collected by centrifugation at 5500 rpm. Sediment was washed three times with water and ethanol to remove impurities and reactant and dried at 50^{0}C. Amino functionalized mesoporous silica nanoparticles (APT-MSN) are then resupended by ultrasonicationin ultrapure water. The suspension was then freeze dried.

Determination of degree of amino grafting on MSN [9]

Ninhydrin was applied to quantifythe primary amine groups on the surface of MSN forming the Ruhemann's purple in a weakly acidic solution. First of all APTES solutions of concentration 0.02, 0.04, 0.06 and 0.08millimolar were prepared in ultrapure water. To 1ml of each solution 3% ninhydrin reagent was added and heated in a boiling water bath for 15 minutes and cooled for 30 minutes. Then absorbance of each solution was taken at 570 nm using Jasco V-630 spectrophotometer. 10 mgof APT-MSN was added into 1 ml of ultrapure water and ultrasonicated.The suspension was mixed with 1 ml of pH 5 actetate buffer solution. 1 ml of 3% ninhydrin reagent was added to the above mixtureand it was heated at 100 ^{0} C for 30 minutes and cooled. The solution was centrifuged at 5000 rpm and absorbance of supernatant was measured at 570 nm by sing Jasco V-630 spectrophotometer against a blank reference without APT-MSN which was prepared simultaneously along with the sample preparation. By using the calibration plot, the corresponding concentration of amine group (M, mol/L) was determined. The molar quantity of amine grafting on the surface of MSNPs was calculated by the following formula:

$$Aminegraftingintermsofmol/gofMSN\ (A/G) = \frac{M_{APTES}}{W_{MSN}}$$

$$Aminegraftingintermsof Molecules/nm^2 = \frac{A/G \times 6.022 \times 10^5}{S_{BET}}$$

$$Aminegraftingtermsofweightpercentageof MSN = (A/G \times W_{AMP}) \times 100$$

$$Graftratio = \frac{A/G \times W_{MSN}}{Initialmolesof APTESaddedduringfucntionalization} \times 100$$

Where M_{APTES} is the moles of APTES determined from calibration plot, A/G is the moles of APTES grafted on per gram of MSN. S_{BET} is the specific surface area of MSN (m^2/g). W_{AMP}isthe molecular weight of aminopropylsilylgroup (86.1861g/mol). $W_{MSN}$is the weight of MSN added during functionalization.

FTIR scanning of APT-MSN was performed using Agilent technologies CARY 630 FTIR. FTIR spectra were taken at 4 cm^{-1} resolution, averaged over 32 scans in range of 650 to 4000cm^{-1}.

Zeta potential was determined by the electrophoretic mobility of APT-MSN in U- type tube at 25^{0}C, using Zetasizer Nano ZS (Malvern Instruments Ltd, Worcestershire, United Kingdom) after suitable dilution with water.

Drug loading

The drug loading of 5- fluorouracil was done by backfilling approach. 5-FU and APT-MSN were taken in the ratio 1:1. 2 mg/mlsolutionof 5-FU is prepared with ultrapure water. 40 mg ofAPT-MSN ultrasonically dispersed in 20 ml of 5-FU solution. The solutions were stirred under room temperature for 24 hour. The solution was then centrifuged at 6000 rpm and 1 ml of supernatant was collected. The amount of 5-FU in the supernatant was found out by UV spectrophotometricalyat

a wavelength of 266 nm to determine the unentrapped drug. The prepared nanoparticles were washed and freeze dried. They were subjected to FTIR spectroscopy to confirm drug entrapment. The drug loading capacity and drug entrapment efficiency wascalculated using the following equations.[10]

$$Drug\ loading\ efficiency\ (\%) = \frac{Weight\ of\ 5FU\ \ in\ APT - MSN}{weight\ of\ \ 5FU\ loaded\ APT - MSN} \times 100$$

$$Drug\ entrapment\ efficiency\ (\%) = \frac{\text{Weight of 5FU in APT} - \text{MSN}}{\text{Initial weight of 5FU}} \times 100$$

RESULTS AND DISCUSSIONS

Synthesis ofmesoporoussilica nanoparticles

The FTIR spectra of MSN was obtained and the spectrumindicates the peaks at 1630 cm^{-1} (stretching vibration –OH), 805 cm^{-1} (flexible vibration of Si-O) and 1090 cm^{-1} (stretching vibration Si-O-Si) 3300-3500 cm^{-1} (Si–O-H stretching vibrations) shown in (Fig1).

The mean zetapotential value obtained is -27.8±3.1 shown in (Fig 2). Zeta potential was uniform distributed. The negative zeta potential value was attributed to the silanolgroups present on the active surface of MSN.

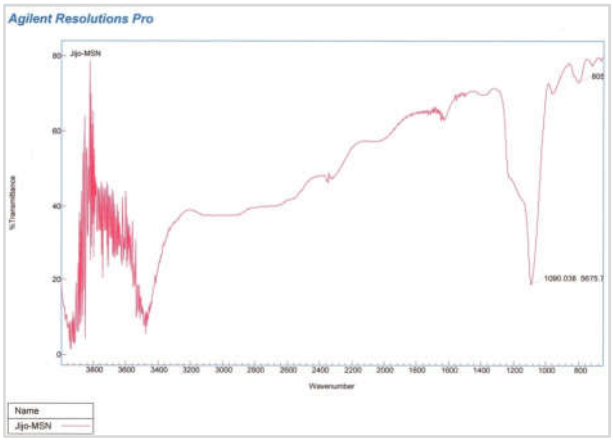

Fig. 1 FTIR spectrum for MSN

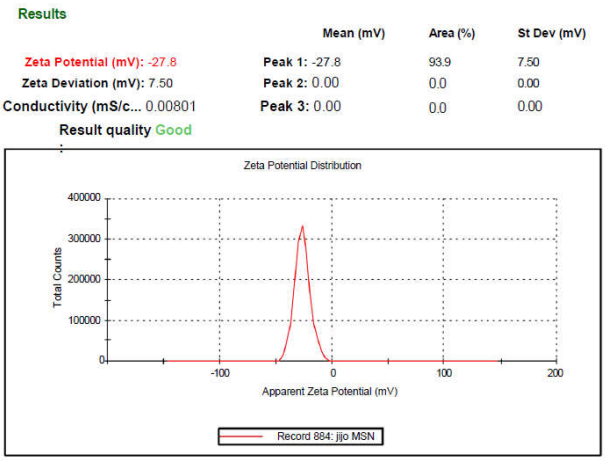

Fig 2 Zeta potential of MSN

The average diameter of the MSN was found to be 154.1±7.2 nm shown in (Fig 3). It was also confirmed thatMSN have uniform size distribution.

SEM images of the optimized MSN shown in (Fig 4). From the images it was confirmed that the particles have uniform size distribution and spherical shape.

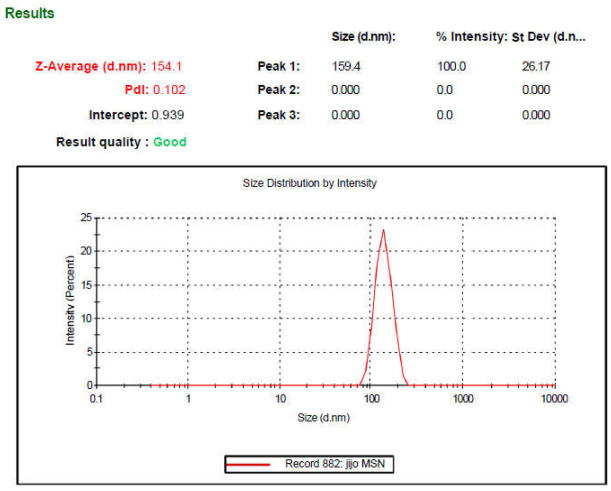

Figure 3 Particle size of MSN

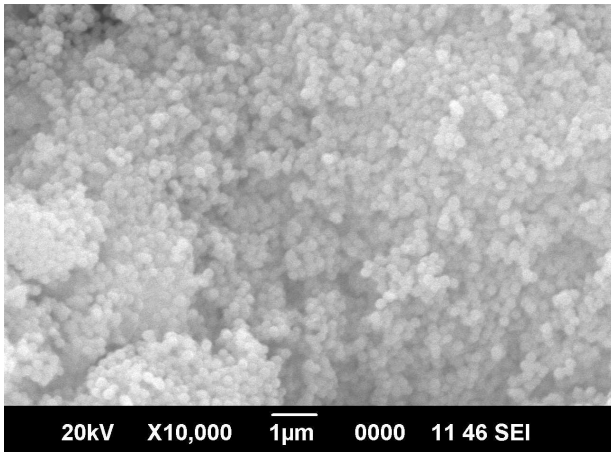

Fig. 4 SEM image of MSN

The TEM images confirm the spherical shape and uniform size distribution of the optimized MSN shown in (Fig 5). The mesoporous structure of MSN was also be confirmed from the image.

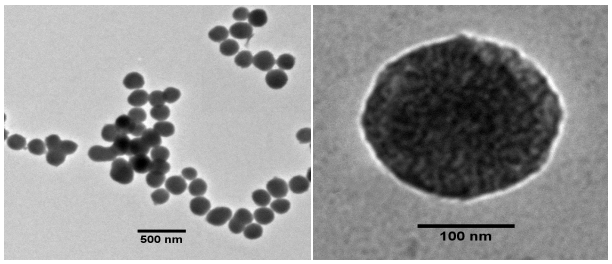

Fig 5 TEM image of MSN

From the surface area analysis it was confirmed MSN was highly porous in nature and it is having a BET surface area of MSN 860.1294 m^2/g. The single point adsorption total pore volume of pores less than 1,060.167 Å diameter at P/Po = 0.981148056 was found to be0.708318 cm^3/g. Average pore size of MSN was 2.15 nm (from adsorption pore distribution).

The amino functionalization of MSN by APTES was depicted in (Fig 6). The amino functionalization was clearly evidenced by the bands detected at around 1500 cm^{-1} due to

N-H bending and 3000 cm-1 attributed to C-N stretching vibrations shown in (Fig 7). The absorption peaks at 2875 cm^{-1} and 2945 cm^{-1} in the sspectrum were related to C-H stretching vibration of aminopropylgroup. The result observed was in close agreement with reported data.

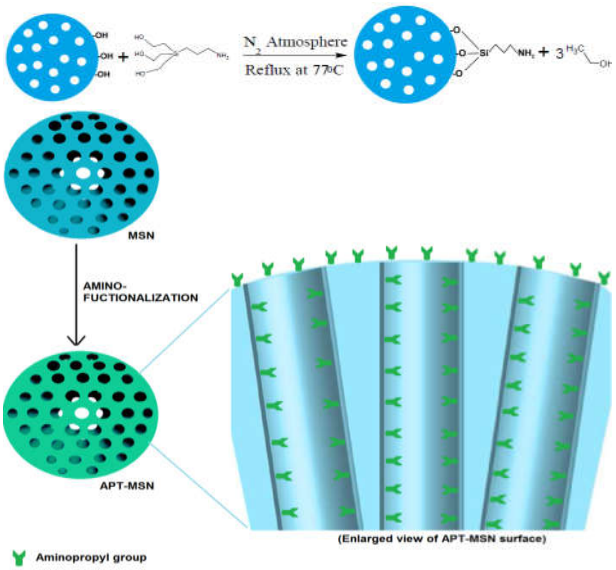

Fig 6 Amino functionalization of MSN

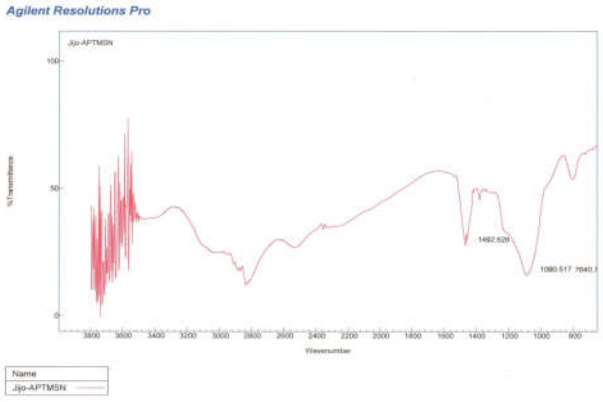

Fig. 7 FTIR spectrum of APT-MSN

Zeta potential of APT-MSN was found to be +6.4 shown in (Fig 8). In contrast to negative zeta potential of the precursor MSN, APT-MSN showed positive charge on the surface. It was attributed to the amino groups attached to the surface during functionalization. The amino groups undergo protonation which makes the surface charge positive.

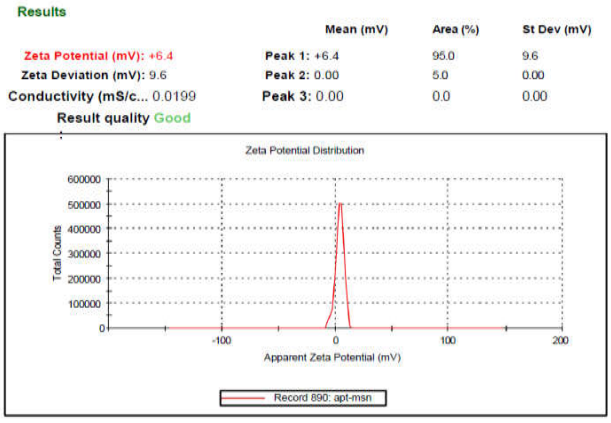

Fig 8 Zeta potential of APT-MSN

The mean particle size of APT-MSN was found to increase after functionalization, due to attachment of aminopropyl group on to the surface during the process shown in (Fig 9).

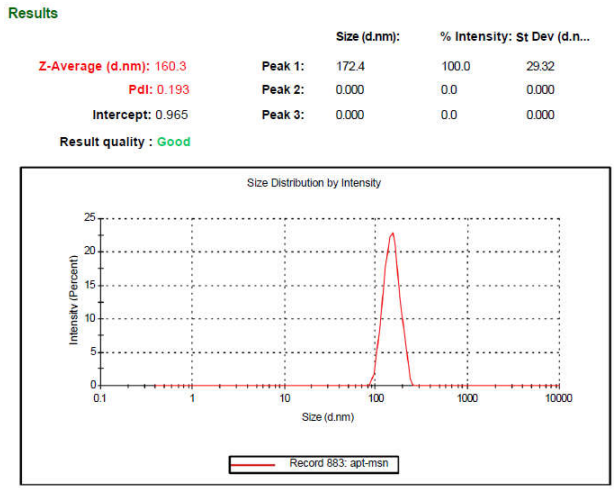

Fig.9 Particle size of APT-MSN

Determination of degree of amino grafting on MSN

Amine grafting in terms of mol/g of MSN (A/G) = 0.005841±0.000178mol/g
Amine grafting in terms of Molecules/nm^2 = 4.09= ~ 4
Amine grafting terms of weight percentage of MSN=50.34%
Graft ratio=30.71%

Zeta potential of APT-MSN was found to be +6.4 ±3.7 mV. In contrast to negative zeta potential of the precursor MSN, APT-MSN showed positive charge on the surface. It was attributed to the amino groups attached to the surface during functionalization.

Drug loading

The process of drug loading was depicted in (Fig 10). The APT-MSN showed significantly greater entrapment efficiency when compared to MSN. The $–NH_2$ groups present on the surface of APT-MSN can significantly increase the amount of 5-FU entrapped into nanoparticles due to the existence of positively charge provided by $-NH_2$ groups. Therefore, the control of the intensity of electrostatic force between nanoparticles and 5-FU may regulate drug loading capacity of MSN. Drug loading to APT-MSN was confirmed by FTIRspectroscopy shown in (Fig 11)

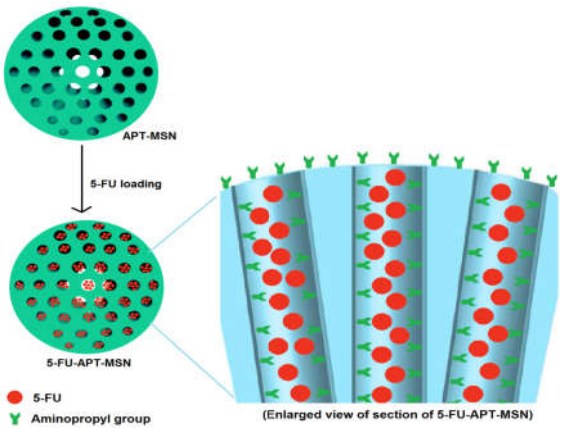

Fig.10 Representation of drug loading to APT-MSN

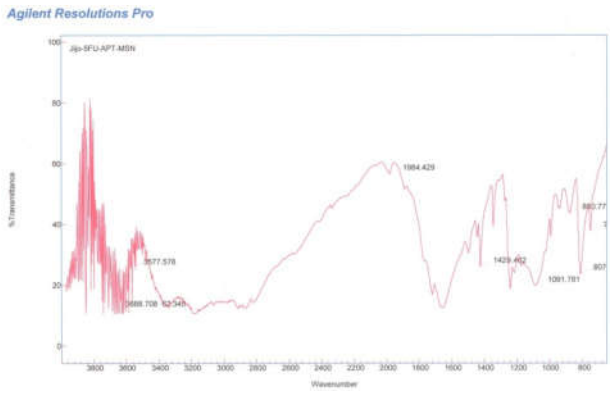

Fig.11 FTIR spectrum of 5-FU-APT-MSN

Entrapment efficiency of 75.47% was attained at pH 8.

CONCLUSION

The amino functionalized mesoporous silica nanoparticles were successfully synthesized and was proved for better drug loading of 5-fluorouracil. Its characterization were done and a greater entrapment efficiency of 75.47 was attained at PH 8.

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