MESOPOROUS SILICA NANOPARTICLES OF HESPERIDIN: SYNTHESIS AND *IN VITRO* CHARACTERIZATION

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ABSTRACT

This work represents an attempt to synthesize mesoporous silica nanoparticles and to take advantage of its surface area, pore size and pore volume for delivery of drug. In this work hesperidin, a bioflavonoid obtained from citrus fruit, was successfully loaded on silica nanoparticles by solvent immersion method. Hesperidin loaded nanoparticles were characterized for surface area, pore size, pore volume, *in vitro* dissolution, differential scanning calorimetry, x-ray diffractometry and scanning electron microscopy. The SEM and N_2 adsorption-desorption isotherm result demonstrated that both blank and hesperidin-MSNs possessed spherical surface with little aggregation. Mesoporous particles had surface area of 812.232 m^2 g^{-1}. It had pore size and volume was 2.242 nm and 0.635cm^3 g^{-1}, respectively. *In vitro* drug dissolution study showed slow release of hesperidin; which may be due to interaction between silanol function groups. This technique seems to produce particles with larger surface area and can be used as an effective carrier for drug delivery.

Keywords: Hesperidin, sol-gel method, mesoporous silica nanoparticles, surface area, N_2 adsorption-desorption

INTRODUCTION

Nanotechnology is a rapidly developing science, which helps to deliver the material at specific site with controlled manner. Due to the major challenges like poor water solubility, *in vivo* instability, low bioavailability and poor absorption in body, nanotechnology is used nowadays to improve the effectiveness of drugs.

Mesoporous silica nanoparticles have drawn great attention in recent research because of their adjustable physicochemical properties and their use as carrier for most of drugs, vitamins, proteins and genes[1]. Mesoporous silica nanoparticles (MSN) were first synthesized by Mobile Corporation Laboratories and named Mobile Crystalline Material (MCM)[2]. Mobile crystalline material system mainly consists of MCM-41, MCM-48, and MCM-50, having hexagonal, cubic and lamellar structures, respectively[3,4]. Among these three MCM systems, the MCM-41 is more stable and has large surface area. MSNs possess superior features like large surface area, tunable pore size, large pore volume, high stability and biocompatibility[5,6]. The porous nature of MSNs results in large surface area (1000 m^2 g^{-1}), uniform pore size, large pore volume (≈1 cm^3 g^{-1}) biocompatibility and high stability[7,8]. Stober's method is an effective method discovered in 1968 by Stober et. al., which gives uniform particles ranging from nanometers to few microns by controlling the reaction conditions[9,10]. The addition of silica source causes the expansion of size of MSN, by reacting preferentially with surface silanol groups on the existing particles rather than generating new particles and this is the reason why the method can generate uniform size ordered MSNs[11]. The synthesis mechanism involves hydrolysis and condensation of silica precursor followed by template removal by calcinations. USFDA approved the silica as "Generally Recognized as Safe material"[12]. The stability due to Si-O eliminates the use of external stabilizer which is essential in liposomes, niosomes and polymeric nanoparticles. The silica based mesoporous material is most commonly used but the carrier-drug interaction which takes place between silanol group located on surface of carrier matrix and functional groups of drug e.g. repaglinide[13], ibuprofen[14] etc. would have a strong effect on drug adsorption and release rates[15].

Hesperidin is a flavonoid glycoside derived from peels of citrus fruits family Rutacea and other citrus genera like Fabacea, Betulacea and Laminacea. Hesperidin (hesperitin 7-o-rutinoside) is comprised of

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aglycon hesperitin attached to disaccharide rutinose[16]. Hesperidin is poorly soluble in water (0.004 mg mL^{-1}) and freely soluble in organic solvents like DMSO and DMF. The antioxidant activity of hesperidin is due to its ability to quench oxidative radical chain reactions and helps to preserve neural health. It is absorbed from the small intestine but it act as a substrate for P-gp, and intestinal efflux protein. Poor transmembrane permeability and intestinal tight junction protein limits its intestinal absorption[17]. Hesperidin has been reported to possess anti-inflammatory, anti bacterial[18], hypolipidemic, diuretic activity and anti Parkinsonism activity[19] and also used in treatment of diabetic retinopathy. However, poor solubility, poor bioavailability and absorption pose restrictions on its therapeutic benefits[20,21]. Thus, the objective of this work was to load hesperidin in MCM 41 type of MSNs to determine the stability and *in vitro* release.

MATERIALS AND METHODS

Materials

Hesperidin was obtained from Meta Lab, Mumbai, India. Tetraethyl orthosilicate (TEOS %) was purchased from Sigma Aldrich, Bengaluru. Cetyltrimethyl ammonium bromide (CTAB), potassium dihydrogen orthophosphate and methanol were purchased from SD Fine Chemicals Ltd., Mumbai. All other chemicals and reagents used were of analytical reagent grade.

METHODS

Synthesis of blank mesoporous silica nanoparticles

Mesoporous silica nanoparticles were prepared by Stober's method, i.e. sol-gel method[22]. As reported by Vazquez et al., varying molar concentrations of surfactant was used for synthesis of MSN while molar ratio of ethanol, water, ammonium hydroxide (NH_4OH) and tetraethyl orthosilicate was kept constant[23]. The composition is given in Table I.

Table I: Composition of silica nanoparticles

Batch no.	Molar concentration of CTAB	Ethanol	Water	NH_4OH	TEOS
F1	0.1	20	45.6	10.4	1
F2	0.3	20	45.6	10.4	1
F3	0.5	20	45.6	10.4	1

CTAB was added to the mixture of ethanol and deionised water, alkalized by addition of ammonia solution. The mixture was stirred for 15 min at room temperature until clear solution was obtained. Then, TEOS was added drop wise under continuous stirring at room temperature for 2 h. The start of the reaction was confirmed by immediate formation of opaque solution. The former white precipitated silica particles were then recovered by filtration using 0.4 µm membrane filter. The product was washed with deionised water under same conditions and dried overnight. The particles were calcined at 550 °C for 4 h.

Drug loading

Hesperidin was loaded onto blank MSNs by solvent deposition method. Preliminary formulation batches of mesoporous silica nanoparticles (MSNs) were developed to decide the ratio of drug: blank MSN for hesperidin loading. They were used in the ratio from 1:1 (F1a, F2a and F3a) and 1:3 (F1b, F2b and F3b). On the basis of drug loading, it was decided to prepare the batches using the ratio of 1:1. Hesperidin was dissolved in methanol and then mixed with blank MSNs. The mixture was stirred for 24 h under ambient conditions, filtered, washed and dried at room temperature for 24 h[24].

Characterization of MSN

N_2 adsorption-desorption isotherm

Specific surface area, total pore volume and mean pore diameter were determined by nitrogen adsorption-desorption isotherm measurements using surface area analyzer (Quantachrom Nova station instrument, version 11.5) at -196 °C. Prior to analysis, samples were out gassed at 150 °C for 6 h. The specific surface area of the solid was determined according to Brunauer-Emmett-Teller (BET) method over a relative pressure range from 0 to 0.99. On the other hand, the pore volume and pore size distribution were determined from the adsorption isotherm by using non local density functional theory (NLDFT).

DSC study

Differential scanning calorimetric technique was used to study the physical state of the drug. The blank and hesperidin loaded samples were heated in hermetically sealed aluminum pans at a scanning rate of 10 °C min^{-1} from 30 to 350 °C using differential scanning calorimeter (DSC, PerkinElmer 4000).

PXRD analysis

X-ray diffraction patterns were recorded using X-ray diffractometer (Bruker D8 Advance, India). PXRD studies were performed on the samples by exposing them to

CuKα radiation (40 kV and 30 mA) and scanned from 10- 80^0 (2θ) angle.

SEM study

Morphological analysis of hesperidin loaded MSNs prepared by CTAB was done by scanning electron microscope (JEOL/JEM 2100, Japan). The particles were vacuum dried, coated with a thin gold-palladium layer with a sputter coater unit and observed microscopically at an accelerated voltage of 10 kv.

FT-IR spectroscopy

The FT-IR spectrum of formulation was recorded in order to predict the chances of drug and excipients interaction using Fourier Transform Infrared (FTIR Affinity-1) spectrophotometer. Small amount of the samples were placed onto the ATR (attenuated total reflection) crystal and scanned over a frequency range 4000-400 cm^{-1}.

% Drug loading capacity

Amount of hesperidin loaded in mesoporous particles was determined by suspending the particles (equivalent to 10 mg of hesperidin in 100 mL of phosphate buffer (pH 5) and stirred continuously on magnetic stirring for 24 h at room temperature. After stirring; the solution was filtered through cellulose acetate membrane (0.22 µm). The amount of drug in the filtrate was analyzed using UV spectrophotometer (UV 1801, Shimadzu, Japan) at 240 nm.

In vitro dissolution study

It was performed using USP dissolution test apparatus (type-II). Hesperidin loaded silica nanoparticles (equivalent to 10 mg of hesperidin) filled in empty capsule shells were put into the dissolution vessel containing 900 mL of phosphate buffer (pH 6.8) kept at 37±0.5 °C and stirred at 75 rpm. Aliquots of 10 mL samples were withdrawn at predetermined time intervals and fresh volume of dissolution medium was added to maintain sink condition. The amount of drug released was quantified using UV spectrophotometer (UV 1801, Shimadzu Japan) at 285 nm against phosphate buffer as a blank. The experiments were performed in triplicate for each batch.

RESULTS AND DISCUSSION

Drug loading

Hesperidin loading on blank mesoporous particles was affected by the concentration of CTAB used. It has provided larger surface area and pore volume as confirmed by BET analysis. The drug loading ranged from 73 to 88 % (Table II). One more reason for high loading of hesperidin is probably interaction of drug with surface silanol i.e. –OH group particularly through functional group -O- (ether); -COOH (acid) and –NH.

Dissolution study

The results of release of hesperidin from MSNs showed initial burst release from all formulations i.e. 25.32 to 33.15% at the end of 1 h. The study was conducted for total 8 h and the release was observed in the range of 49.46 to 76.11% (Table II). Highest release was obtained from F1a formulation where hesperidin: blank MSN was 1:1 and had low drug loading. The initial burst release was due to the adsorbed hesperidin on the larger surface area provided by the particles. The drug initially occupied into the pores with subsequent covering of the surface like a thin film of hesperidin on the surface. Here the drug loading was very high which has affected the release of hesperidin from the MSNs. The inverse relationship was observed between drug loading and drug release. The crystalline hesperidin could have blocked mesopores; therefore restricting the entry of aqueous media into the pores and may have hindered the release. Another possible cause for slow release could be strong interaction between surface silanol groups and –OH of hesperidin as more energy is required to break this bond. Among all formulations; batch F1a had shown highest release which may be due to the high surface area (as confirmed by N_2 adsorption-desorption isotherm), which gives greater area of contact and wettability. The release profile of all batches is shown in Fig. 1.

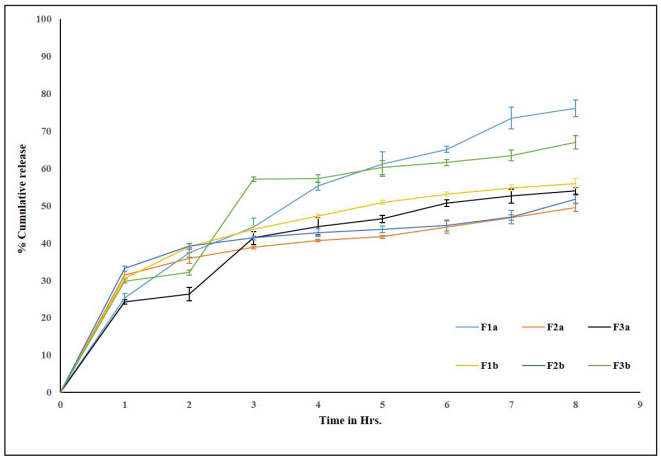

Fig. 1: Dissolution profile of hesperidin from all formulations

Table II: Results of drug loading and *in vitro* dissolution

Batch	% Drug loading (n=3)	% Drug release (n=3)
F1a	73±0.8	76.11±0.74
F2a	88±1.52	49.46±1.2
F3a	82±0.64	53.95±1.16
F1b	80±0.5	55.96±0.9
F2b	85±1.04	51.74±0.88
F3b	77±1.16	66.98±0.5

N_2 adsorption-desorption isotherm

Nitrogen adsorption desorption isotherm of the blank and hesperidin loaded MSNs are presented in Fig. 2A and 2B, respectively and type IV isotherm was observed i.e. hysteresis loop which confirms the presence of mesopores as well as micropores. The results of surface area, pore size and pore volume of blank and hesperidin adsorbed MSNs are given in Table III. The blank MSNs have shown high specific surface area and total pore volume. The mean pore diameter is at the beginning of mesoporous range. A major decline in a specific surface area was observed on drug loaded MSNs and it could be due to adsorption of hesperidin on the surface of nanoparticles. Also a decline in mean pore volume and increase in pore size was observed, indicating occupancy of space inside the pores by hesperidin[25].

Table III: Specific surface area, pore size and pore volume of blank and hesperidin loaded MSNs of 0.1 M CTAB (1:1)

Sample	S_{BET} ($m^2\ g^{-1}$)	Pore size (nm)	Pore volume ($cm^3\ g^{-1}$)
0.1 M CTAB MSNs (blank)	812.232	2.242	0.635
0.1 M CTAB MSNs (Hesp-MSNs)	153.123	3.451	0.146

Shape and morphology by SEM

Surface morphology of hesperidin loaded mesoporous silica nanoparticles is shown in Fig. 3.

In case of blank MSNs (Fig. 3A), they appear spherical in shape with smooth surface. The hesperidin loaded MSN's (Fig. 3B) were also spherical with little aggregation. The nanoparticles were obtained at low concentration

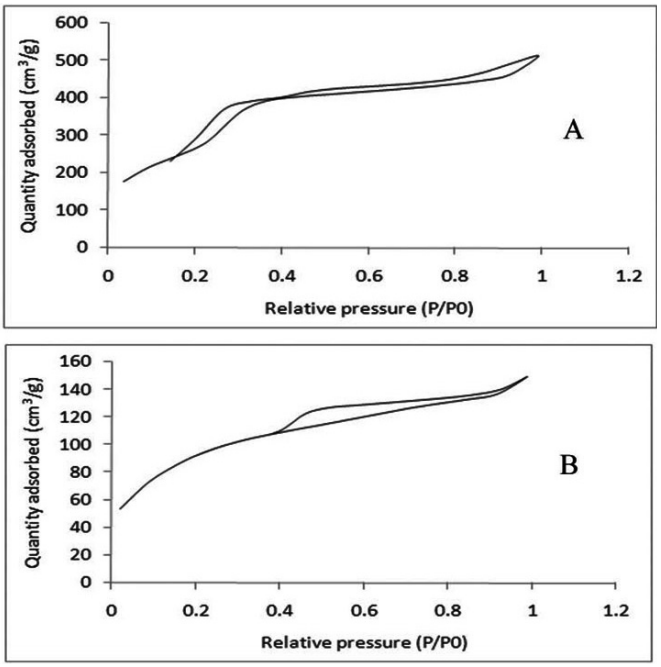

Fig 2: N_2 adsorption-desorption isotherm of blank (A) and Hesperidin loaded MSN (B)

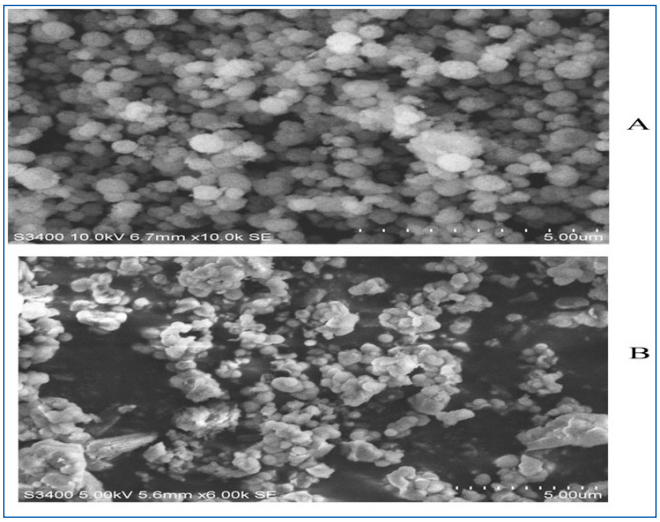

Fig. 3: Surface morphology of blank (A) and Hesperidin loaded MSN (B)

of CTAB. The blank MSNs seem to have similar particle size but the hesperidin loaded may have variation in size because of aggregation.

XRD analysis

The X ray diffraction pattern of pure hesperidin and its formulation are shown in Fig. 4 (A&B), respectively. It can be clearly seen from the diffraction pattern of hesperidin that the drug is crystalline and shows intense and characteristics diffraction peaks. The XRD pattern of pure hesperidin drug has highest peak at 2θ range

of 19.41° and other at 2θ range of 15.71°, 15.98° and 12.00°. The XRD pattern of drug loaded MSNs showed the peaks at same 2θ angle with little reduction in the intensity of peaks. It may be due to the presence of drug in crystalline form. This indicates the phase transition of drug from crystalline to amorphous has occurred to some extent only.

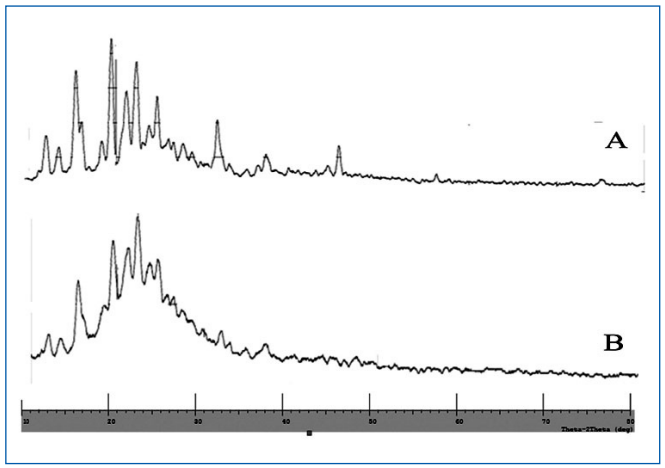

Fig. 4: XRD patterns of Hesperidin (A) and Hesperidin loaded MSN (B)

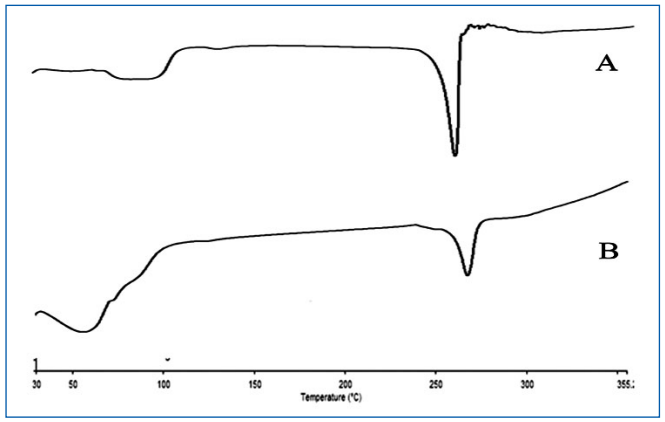

Fig. 5: DSC thermograms of Hesperidin (A) and Hesperidin loaded MSN (B)

Thermal analysis by DSC

The DSC thermograms of hesperidin loaded MSN and pure hesperidin are shown in Fig. 5A and 5B, respectively. A single sharp endothermic melting endotherm at 252.96 °C was clearly observed which was characteristic of hesperidin melting point (5B). Also a broader endotherm was observed at lower temperature range, which may be due to residual moisture/solvent loss.

In hesperidin loaded particles, the melting endotherm was observed at 261.74 °C (5A) which reveals the presence of drug. Apart from majority of drug being inside the pores it may be expected that some of the drug may be adsorbed on the surface which can easily be detected by these types of techniques. Here, the broad endotherm was slightly shifted to lower side of temperature. This study concluded that the drug was not in a completely encapsulated form.

CONCLUSION

Heseridin loaded mesoporous silica nanoparticles were synthesized successfully using Stober's method based on cationic surfactant CTAB as a template. The blank silica particles exhibited very large surface area, high pore volume and small internal pore size. After loading of hesperidin, the surface area was reduced with reduction in pore volume and increase in internal size due to the space occupied by material. During experimental work, inverse relation was observed between drug loading and drug release from the nanoparticles. This suggests that high drug loading and strong interaction between the functional groups of drug, surface silanol group and existence of hesperidin in crystalline form may be responsible for slow release. The MSNs had smooth surface with little aggregation. The drug loading and particle morphology affect the release behavior of drug. This study would help the researchers to use silica nanoparticles for delivery of drug for prolonged periods of time.

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