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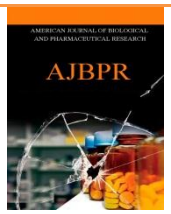

INCLUSION OF AMPICILLIN TRIHYDRATE IN HIGHLY ORDERED MESOPOROUS SILICA NANOPARTICLES

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ABSTRACT

Ampicillin was included in the mesoporous silica nanoparticle SBA-15 with pore diameter (8.8 nm) and high surface area (590 m^2/g) by the post impregnation method. These inorganic-organic composite were fully characterized by: UV-visible spectroscopy, thermo gravimetric analysis (TGA), X-ray Powder Diffraction (XRPD), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Nanocomposite shows decrease surface area from 737 to 114 m^2/g and pore diameter 8.8 to 6 nm, which showed that ampicillin had been successfully included in host SBA-15 pore channels with drug entrapment efficiency 72.6 % and drug loading efficiency 53%. Ampicillin loaded SBA-15 presents high loading and hence causes reduction in the toxicity and side effects of the drug which can also be an excellent and long term antimicrobial agent against various pathogenic bacteria's.

INTRODUCTION

MSN (mesoporous silica nanoparticles) have the potential to create NCDDS (novel and control drug delivery system). They have the pore diameter between 2-50nm. Highly ordered mesoporous silicates such as MCM (**M**obil **C**omposition of **M**atter No. 41, 48,) and SBA (**S**anta **B**arbara **A**morphous material No. 1, 3, 15) comes under this category. In 2001 the mesoporous materials MCM-41was proposed as a DDS due to ordered pore network, high pore volume, high surface area and a silanol containing group for drug loading.

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Acc. to pharmaceutical application SBA-15 [1] which is 2D hexagonal show ordered arrangements of channels and cavities of different geometry confined between walls built up from SiO2 units. Silicate drug-carriers (SiO_2) such as SBA-15, are biocompatible as well as biodegradable, because upon contact with body fluids, they slowly get degraded to orthosilicic acid [$Si(OH)_4$] and enter the blood or lymphatic system, which finally gets excreted through the kidneys. Although cytotoxic in high concentrations, the removal of Si $(OH)_4$ from the body is sufficiently fast, which apparently does not induce any toxic effects [2] They also show controlled drug release which minimize side effect by deliver the active agent on specific site or at the target and also decrease repetitive administration [3] .One more unique property of inorganic SBA-15 is the presence of surface silanol group whose first advantage is the weak interaction between drug and matrix so drug release is not controlled by diffusion and second is the matrix can be easily functionalize with different organic

molecules and controlled drug release under specific condition (pH, light or temperature sensitive) can be achieve [4,5].The oral bioavailability and controlled release rate of poorly water soluble drug can also be increase by functionalization it with different groups [6,7]

The aim of present study is to formulate a composite (SBA-Amp) by post impregnation method with high loading and in which drug is present in amorphous state.

EXPERIMENTAL

Reagents

Reagents used for the synthesis of SBA-15: Pluronic P123 [ethylene oxide–propylene oxide–ethylene oxide (EO20PO70EO20), Mw= 5800, Sigma–Aldrich], tetraethoxy orthosilicate [(C2H5O) 4Si, TEOS, Sigma–Aldrich], titanium tetra-chloride (TiCl4, Merck) and HCl (35%, Fisher Scientific). Ampicillin Trihydrate was purchased from sigma Aldrich.

Synthesis of SBA-15 matrix

The mesoporous silica SBA-15 was synthesized as before [9]: In a typical procedure, 2 g triblock copolymer, P123 was dissolved in 50 ml distilled water at 40 °C under highly acidic conditions created by addition of 10 ml HCl (2 M). A clear solution was obtained after 3 h of continued stirring of solution. Then, 7.8 ml TEOS was added to the above solution and kept at 40 °C under stirring for another 24 h. The aqueous solution thus obtained was transferred to a 200 ml capacity Teflon lined stain-less steel autoclave and hydrothermally treated at 100 °C for 24 h. After cooling to room temperature, the solid products were filtered, washed and dried at 70 °C and were calcined at 600 °C (heating ramp = 1 °C/min) for 4 h in air to remove organic templates and thus mesoporous powder SBA-15 was obtained

Drug Loading

For the preparation of SBA-15-ampicillin composite material, the post impregnation method was employed. SBA-15 molecular sieve white powder, which was synthesized by hydrothermal method, was used as a matrix to load ampicillin (Amp) in unmodified form at room temperature. Three concentrations was (Ampicillin: SBA-15) 1:5. Ampicillin was dissolved in water and was soaked with SBA-15, under stirring for 12 hours at 500 rpm. Determination of entrapment efficiency: The encapsulation efficiency and loading capacity of nanoparticles was determined by first separating the nanoparticles formed from the aqueous medium by ultracentrifugation at 15000 rpm for 30 min. The amount of free ampicillin Trihydrate in the supernatant was measured by UV spectrophotometry at 257nm (Elico, BL 198, Bio Spectrophotometer). The ampicillin Trihydrate entrapped in the nanoparticles was calculated as Eq 1.

Entrapment efficiency (%) = (Tp – Tf) 100/Tp … (1)

Where Tp is the total ampicillin Trihydrate used to prepare the nanoparticles and Tf is the free ampicillin Trihydrate in the supernatant [8].

Sample Characterization

Drug loading determination: The drug loading in porous SBA-15 was determined by UV spectrophotometry and thermo gravimetric (TGA) analyses.

Thermal properties (TGA)

TGA experiments were performed on a SDT Instruments Q-600 apparatus (DSC – TGA Instrument Inst serial 1364). Scans were recorded between 200 oC and 1190 ^{o}C at a heating rate of 20 ^{o}C/min. Dry nitrogen was used as pure gas (flow rate of 100 ml/min). All experiments were carried out in open, crimped aluminum pans (SDT Instruments). Sample size was 2.478 mg. All thermo grams were recorded in duplicate and all data handling was performed using the Universal Analysis 2000 software package (SDT Instruments).

UV-VIS Spectroscopy

UV-VIS Spectrophotometer (Shimadzu UV-2450, Milton Keynes, UK), equipped with 1.0 cm cell was used for spectrophotometric determination of the content of ampicillin in (SBA-15)-ampicillin composite material using equation (1).

XRD Analysis

Powder wide-angle patterns are obtained on a Bruker D8 advance diffactometer using CuKα monochromatic radiation (λ = 1.5418˚A) as X-ray source. For wide-angle analysis, signal is recorded for 2θ comprised between 15° and 70° with a step of 0.05° (step time of 2 s). Phase identification is made by comparison with JCPDS database.

SEM analysis

The morphology of the pure SBA-15 and composite materials was characterized by scanning electron microscope (FESEM, FEI QUANTA 200F). The samples for FESEM analysis were prepared by distributing the powder samples on a double-sided conducting adhesive tape.

HR-TEM Analysis

The pore morphology, as well as drug distribution inside the silica mesoporous framework, is evaluated by high-resolution transmission electron microscope (HRTEM). Images were obtained on a TECNAI G20 electron microscope at an accelerating voltage of 220 kV. The samples were prepared by finely dispersing the samples in acetone using sonication and were deposited uniformly on a 400 mesh copper grid coated with a holey carbon film and dried in air.

RESULTS AND DISCUSSION

Drug loading efficiency

The spectrophotometry was used for calculation of the drug entrapment (EE %) efficiency and loading efficiency (P_E) of ampicillin in composite material are summarized in Table 1. Chemical analysis results revealed that ampicillin has been entrapped in mesoporous SBA-15 matrix.

Drug loading efficiency was further confirmed using TGA [10]. The TGA thermogram of SBA-15, ampicillin and ampicillin loaded SBA-15 is shown in Fig. 2. The TGA (Fig. 1a) profile of SBA-15 shows a small mass loss up to 1000 ^oC (Maximum mass loss ~ 10%). The initial mass loss of SBA-15up to 200 ^oC attributed to thermos desorption of water from mesoporous structure of SBA-15.Beyond 200 ^oC, the small mass loss is attributed to condensation and dehydroxylation of the silanol groups of the material. The TGA (Fig. 1b) profile of the ampicillin drug shows almost no mass loss up to 250 ^oC, but beyond this temperature, it shows an abrupt mass loss of the sample up to 280 ^oC and thereafter it shows continuous mass loss up to 865 ^oC.

However, the TGA (Fig. 1c) profile of SBA-15/ampicillin composite material shows a significant mass loss. The initial mass loss of the composite material up to 215^oC is attributed to solvent removal. The composite material also shows two mass loss steps after dehydration. The mass loss observed between the temperatures 216–500^oC and 500–850$^oC$is attributed to the drug decomposition and release. The total mass loss detected in the temperature range of 215–865^oC is about 54.3%, which corresponds to the loading of 543 mg of the drug in1 g of the SBA-15-ampicillin composite.

Powder wide angle X-ray diffraction

Powder wide angle range XRD patterns of the ampicillin, SBA-15 and SBA-15-ampicillin composite are shown in Fig. 2. It is clear from the diffraction pattern of (Fig. 2a) ampicillin that the drug is crystalline in nature. The amorphous nature of SBA-15 (Fig. 2b) is in accordance with the previously reported results [11]. The XRD diffractograms of SBA-15/ampicillin composite shows no peaks which indicates that the drug in SBA-15 is in a non-crystalline state (Fig. 2c). This is in agreement with the previously reported results of others showing the state transition of the drug from crystalline to non-crystalline during the process of loading drugs in SBA-15 [12].

SEM and TEM analysis

Fig 3 shows the SEM images of SBA and composite. SBA-15 majorly presents fiber crystal which can be say rope like to wheat like macrostructure and average diameter 335nm and length. SBA-15-Ampicillin composite presents fiber crystal shape that is not changed with dimension. Fig. 4 shows the TEM image of SBA-15 and composite both shows 2D hexagonal p6 mm structure with relatively uniform pore width to uniformly distributed drug. The pores of rod like sub particle run parallel to the long axis of the rod and after the loading of drug inside the planar hexagonal mesoporous channel structure seemed to last. SEM and TEM results revealed that ampicillin has successfully been entrapped in mesoporous SBA-15 matrix.

Table 1. The drug entrapment (EE%) efficiency and loading efficiency (P_E) of Ampicillin in SBA-15 determined by UV-Visible Spectrophotometry and TGA.

Sample	UV-Visible Spectrophotometry		TGA (%)
	(EE %)	P_E (%)	
SBA-15- Ampicillin composite	72.6	53	54.3

Figure 1. The TGA thermogram of (a) SBA-15, (b) ampicillin and (c) ampicillin loaded SBA-15

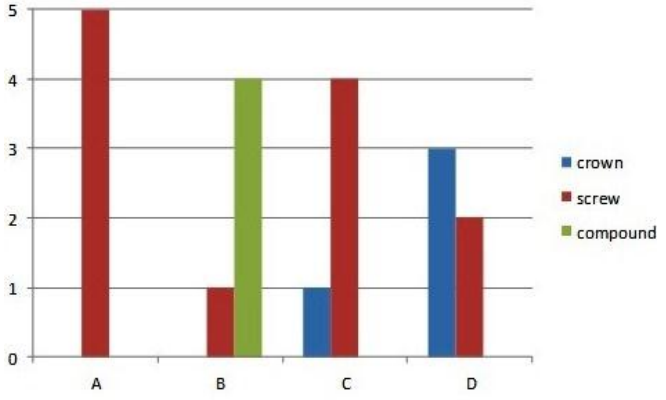


Figure 2. Powder wide angle range XRD patterns of (a) ampicillin, (b) SBA-15 and (c) SBA-15-ampicillin composite

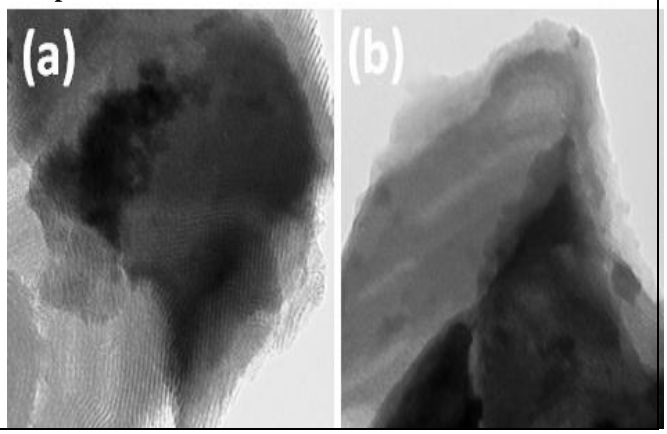


Figure 3. FESEM micrographs of (a) SBA-15 and (b) Ampicillin loaded SBA-15.

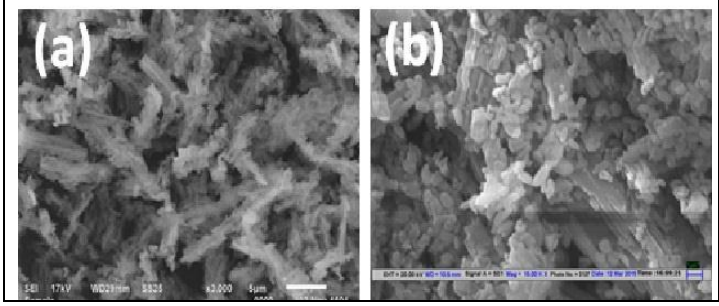
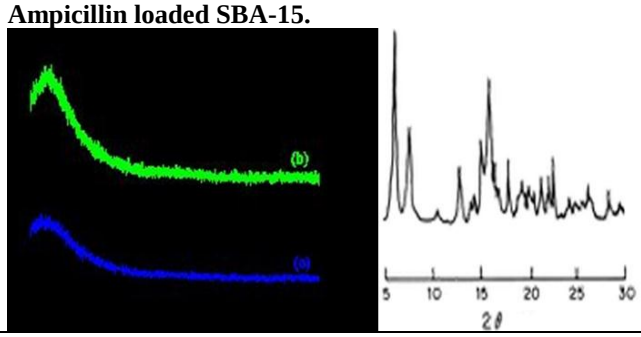


Figure 4. HRTEM micrographs of (a) SBA-15 and (b) Ampicillin loaded SBA-15.



CONCLUSION

Mesoporous SBA-15 material was synthesized and applied to load ampicillin drug. The drug was successfully incorporated in mesoporous silica by post impregnation method. UV-visible spectrophotometry revealed the maximum drug loading efficiency of 53% and encapsulated efficiency of 72.6%. The drug loading efficiency of 54.3% was observed by TGA analysis. Powder XRD and electron microscopy revealed the homogeneous dispersion of the drug inside the SBA-15 pore channels. The composite (SBA-15)-ampicillin is highly effective for releasing the drug in a controlled way to target site.

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CONFLICT OF INTEREST: None

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