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Research Article

DEVELOPMENT AND EVALUATION OF SBA-15 MESOPOROUS SILICA NANOPARTICLES FOR BIOAVAILABILITY ENHANCEMENT OF RITONAVIR

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ABSTRACT

Ritonavir (RTV) loaded SBA-15 mesoporous nanocarriers were prepared for improved drug dissolution and bioavailability of this HIV protease inhibitor drug categorized as BCS-class-II; indicate low solubility and high permeability. The SBA-15NPs and RTV loaded SBA-15NPs were characterized by different analytical techniques like UV spectrophotometry, differential scanning calorimetry, thermogravimetric analysis, FT-IR, N_2-adsorption-desorption technique, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and powder-XRD. Thermogravimetric analysis shows adsorption of ritonavir in SBA-15NPs was about 53%. Differential scanning calorimetry (DSC) and X-ray powder diffraction pattern confirm that, after RTV loading, no crystalline drug was present outside the carrier and structural arrangement of SBA-15NPs carrier was as it is. The N_2 adsorption-desorption technique was used to study the specific surface area, pore size and pore volume of plain SBA-15NPs and the RTV loaded SBA-15NPs. In drug loaded SBA-15NPs surface area and pore volume was decreased compared to plain SBA-15NPs indicating the RTV was entrapped inside the mesopores. The RTV loaded SBA-15NPs shows remarkable improvement in dissolution profile of RTV in comparison to the pure drug. In the pharmacokinetic study, the maximum peak plasma concentrations and AUC_{0-1} value of R-SBA-15NPs were found 2.32-fold and 1.75-fold higher respectively as compared to pure RTV. The results showed that SBA-15NPs enhance dissolution rate and improvement in bioavailability in comparison to the pure RTV.

Keywords: Ritonavir, SBA-15NPs, Ritonavir loading, Dissolution, Oral Bioavailability

INTRODUCTION

Human Immunodeficiency Virus (HIV) is the primary cause of Acquired Immune Deficiency Syndrome (AIDS) which still remains a serious cause of mortality globally. AIDS is the major health issue among the people world-wide.[1] According to the UNAIDS, there are about 36.9 million people suffering from HIV/AIDS worldwide in 2016.[2] The patients with HIV/AIDS are typically treated with High Activity Antiretroviral Therapy (HAART), which involves the chronic administration of at least three combined antiretroviral drugs. However, this therapy can only suppress viral replication temporarily.[3] Ritonavir (RTV) is protease inhibitor (PI) active against HIV which comes under BCS class II drug having low solubility and high permeability. Being poorly water soluble, it shows an extremely slow dissolution rate and pH-dependent solubility which could show limited absorption.[4,5] Single PI has limited success in pharmacokinetic (PK) aspects such as high dosing and low and variable bioavailability. The higher dose could lead to more toxic effect in patients and because of their high dose and side effects; [6,7] there is a need for an innovative formulation approach to enhance the bioavailability. In recent years, for improvement in the solubility and bioavailability of RTV, various techniques have been used like solid self-micro emulsifying drug delivery,[8] amorphous nanoparticles,[9,10] inclusion complex,[11,12] solid dispersion,[13,14] etc. Now a day's inorganic matrices are also being used as drug carriers for providing a controlled/targeted delivery of drugs, enhancement of solubility or bioavailability enhancement and improvement in stability of the compound in unfriendly environments. Among various mesoporous silica materials, MCM-41NPs are widely studies; [15-17] SBA-15NPs is also inviting attention due to its large pore volume and diameter with the highly accessible large surface area which capacitates high drug loading. In addition, SBA-15NPs having thick pore wall than frequently used M41S mesoporous family material which makes it more hydrothermally stable. Because of SBA-15Nps have large pore diameter, hydrothermal stability and high drug loading capacity make them suitable carrier for improving the dissolution and furthermore bioavailability of low solubility drugs.[18,19]

The SBA-15NPs is simply synthesized using Pluronic P-123 non-ionic triblock copolymer as a structure directing agent and tetraethyl orthosilicate as silica source in strongly acidic condition.[18] As correlated with MCM-41NPs, the SBA-15NPs having a high amount of silanol group that makes it more hydrophilic than MCM-41NPs. Because of more hydrophilic nature of SBA-15NPs, it forms weak hydrogen bond interaction with many compounds. Therefore, SBA-15NPs having the capacity to adsorb drug molecules easily and because of weak bonding with drug molecules, it can be easily broken down in a biological system and gives fast drug release.[20,21] Due to this attractive feature and no literature is available for RTV loaded in mesoporous silica nanoparticles till date. SBA-15NPs was used as a drug carrier to improve the dissolution rate and furthermore in bioavailability of poorly water-soluble drug RTV in the preferred absorptive region.

The major aims of this study were: (i) synthesis and characterization of SBA-15NPs (ii) loading of RTV in SBA-15NPs by solvent evaporation method (iii) preparation and evaluation of tablet formulation of RTV loaded SBA-15NPs (iv) performed *in-vitro* dissolution study of prepared formulation and (v) performed *in-vivo* PK study of prepared formulation in Albino Wistar rats.

MATERIALS AND METHODS

The active pharmaceutical agent RTV was received as gift sample from Hetero drug limited (Hyderabad, India). Tetraethyl orthosilicate (TEOS; ≥ 99 %), polyoxyethylene 10 Lauryl Ether (PLE) and Pluronic P-123 were purchased from Sigma-Aldrich (USA). Hydrochloride acid (HCl) and methanol (HPLC and AR grade) were purchased from Rankem (India). All other materials and solvents AR grade were used without any purification. Distilled water was used in the synthesis of SBA-15NPs.

SYNTHESIS OF SBA-15NPS

SBA-15NPs was synthesized as per the procedure reported in the literature.[18] Accurately weighed 8g of P-123 was added in 260 g of deionized water and stirred for 15 min. Then 40 ml HCl (37%) was added into the surfactant solution of P-123 with constant stirring. The mixture was stirred for 1h. Then 18.28 ml of TEOS was added slowly and stirred continued for 8h at 45°C. The obtained mixture was kept at 100°C for 48h in a reactor for hydrothermal crystallization treatment. After that; the solid product was recovered by filtration, washed with distilled water and kept the product in the oven at 80°C temp for overnight. Further removal of surfactant was carried out by calcination of the product in a muffle furnace at 550 °C for 6 h. The recovered final product was labeled as SBA-15NPs.

LOADING OF RTV IN SBA-15NPS

SBA-15NP was added in the methanol solution containing RTV (10mg/ml) at drug to carrier ratio 1:1.5 (w/w). Then, the mixture was stirred at ambient temperature for 1 h. Afterword, the methanol was removed by evaporation technique and recovered powder was dried at 40 °C in the oven for 24 h. and it was named as R-SBA-15NPs. The percentage loading efficiency of SBA-15NPs was determined using TGA.

PHYSICAL MIXTURE OF RTV AND MESOPOROUS SILICA NANOPARTICLES

The physical mixture (PM) was prepared by mixing of RTV and SBA-15NPs in a mortar and pastel, which were named as R-SBA-15NPs-PM.

CHARACTERIZATION OF SBA-15NPS AND RTV LOADED SBA-15NPS (R-SBA-15NPS)

Scanning electron microscopy (SEM)

The morphology of synthesized SBA-15NPs was examined by scanning electron microscopy (SEM) operated at an acceleration voltage of 15 kV. The SBA-15NPs were kept on aluminum stubs with double side adhesive carbon tape and gold coated with ion sputter MC1000. The samples were examined using a Hitachi-SU 1510 scanning electron microscope.

Transmission electron microscopy (TEM)

The porous structure and particle size of SBA-15NPs were confirmed by TEM Analysis. A TEM image of SBA-15NPs was taken with a TECHNAI-G2 Spirit-Biotwin, operated at 120 kV. The silica nanoparticles sample was dispersed in water using an ultrasonic bath for 10 minutes. A few drops were kept on 200 mesh, copper grid coated with a holey carbon film. The electron micrograph image was recorded on electron negative films and computer system attached to the TEM system.

Thermo-gravimetric and UV-Vis spectroscopy analysis for evaluation of drug loading efficiency

The loading efficiency of SBA-15NPs was calculated by thermogravimetric analysis (TGA) and the thermograms were carried out on Shimadzu thermogravimeter TGA-50. Around 3-10 mg sample of RTV and R-SBA-15NPs were mounted into the platinum pan respectively, then heated up to 500°C at a scanning rate of 10 °C/min under a nitrogen gas flow of 50 ml/min. The thermograms were analyzed using the TA-60 software. The loading efficiency was again confirmed by UV-Vis spectroscopy (UV-1700, Shimadzu) at 240 nm wavelength. A 10mg of R-SBA-15NPs were dispersed in a 10ml volume of methanol; so that the RTV gets easily solubilised into methanol and subsequent filtration of sample was carried out. The amount of drug was calculated with the help of the standard calibration curve. The percentage loading efficiency for SBA-15NPs was calculated by using a formula:

$$\% \text{ Loading Efficiency} = \frac{\text{Weight of RTV in nanoparticles}}{\text{Total weight of sample}} * 100$$

FT-IR analysis

FT-IR spectra of RTV, SBA-15NPs, R-SBA-15NPs-PM and R-SBA-15NPs were recorded on a Shimadzu IRAffinity-1, Miracle 10 single reflection ATR mode at room temperature. The samples were directly kept on the sample holder and spectra were taken in spectral region 4,000 to 700 cm^{-1}.

Differential Scanning Calorimetry (DSC)

The thermal behavior of RTV, SBA-15NPs, R-SBA-15NPs-PM and R-SBA-15NPs were checked by DSC on a Shimadzu DSC-60. Around 3-5 mg of sample was weighted and directly kept into an aluminum pan, crimped the pan with its lid and heated from room temperature to 300°C. The heating rate was 10°C/ minute and nitrogen was used as purging gas at flow rate 40 ml/min. Obtained data were analyzed using TA 60-WS software.

Powder X-ray diffraction (PXRD)

Small and wide-angle powder x-ray diffraction (S-PXRD and W-PXRD) patterns for pure RTV, SBA-15NPs and R-SBA-15NPs were collected using a powder X-ray diffractometer (EMPYREAN, PANalytical) using CuKα radiation beam operating at 40 kV and 30 mA. The results were obtained in S-PXRD 2θ range of 0.5–10 degrees and W-PXRD 2θ range 5–50 degrees in continuous mode at scanning speed 0.02 2θ/5s respectively.

N_2 Adsorption-desorption analysis

The N_2 adsorption-desorption isotherm of SBA-15NPs and R-SBA-15NPs were recorded using Micromeritics ASAP 2010 surface area and pore size analyzer at -196 °C. Prior to analysis,

SBA-15NPs were degassed under vacuum at 200 °C for 8 h and the R-SBA-15NPs were degassed at 40°C for overnight to avoid degradation of the drug. The specific surface area of SBA-15NPs was calculated by applied the BET method to the isotherm. The pore size of SBA-15NPs was calculated by applied Barrett–Joyner–Halenda (BJH) method to the isotherm.

FORMULATION OF TABLETS AND EVALUATION

R-SBA-15NPs were formulated in tablets by direct compression method. R-SBA-15NPs equivalent to 100 mg RTV and different excipients like cross-povidone, Low- Hydroxypropyl cellulose (L-HPC), microcrystalline cellulose, magnesium stearate and lactose monohydrate (SUPERTAB 11SD) were systematically blended and punched in single punch tablet machine which equipped with punches of 12 mm diameter with flat faced beveled edges. Prepared tablets were evaluated by several parameters like weight variation, friability, hardness and disintegration time.

***IN-VITRO* DISSOLUTION STUDY**

In-vitro dissolution and release study was performed in dissolution apparatus (Veego dissolution test apparatus). Six dissolution units were studied for *in-vitro* dissolution of the RTV pure drug, R-SBA-15NPs-PM and R-SBA-15NPs. R-SBA-15NPs equivalent to 100 mg tablets of RTV, PM and pure drug RTV were taken for the *in-vitro* dissolution study. *In-vitro* dissolution studies were conducted in the (a) pH 1.2±0.1 hydrochloric acid solution (0.1N HCl), (b) pH 4.5 acetate buffer with 0.75% PLE and (c) pH 6.8 phosphate buffer with 0.75% PLE media. The experiment was performed in USP dissolution apparatus II, 900 ml media volume at 37±0.5 °C temperature at rotation speed 50 rpm. Five ml of dissolution medium was collected from the vessels at a regular time interval and replacing the same amount with fresh dissolution medium. Samples were filtered through 0.22 µm syringe filter; RTV content was determined by UV spectrophotometry (λ= 240 nm).

***IN-VIVO* PHARMACOKINETIC STUDY**

The *in-vivo* studies were carried out for comparing the plasma profile of R-SBA-15NPs with pure RTV. In PK study, Albino Wistar rats (250–300 g) were used for given oral dose and prior experiment rats fasted for 12 h. The research protocol for the animal studies was approved by the Institutional Animal Ethics Committee (IAEC, file No. 1404), The Maharaja Sayajirao University of Baroda, Vadodara, India. In the experiments, animals were divided in to two groups and each group having three animals and the results are given in the mean ± standard deviation (SD). The bioavailability of R-SBA-15NPs was compared to the pure RTV. The pure RTV and R-SBA-15NPs equivalent to 10 mg/kg dose of RTV were dispersed in 2 ml of CMC solution (0.5 % w/v) and administrated orally to wistar rat. The rats were anesthetized by using ether before blood withdrawing. Blood samples (0.3 ml) were collected through the retro-orbital vein into 60 µl EDTA (0.5 %w/v) containing micro centrifuge tubes at 0, 0.5, 1, 1.5, 2, 4, 6, 8 and 12 h after administration. Collected blood samples were mixed with the anticoagulant by properly shaking and centrifuged at 5000 rpm for 10 min at 4 ºC using a high-speed centrifuge machine and then plasma samples were collected and stored at -20 °C. A simple protein precipitation method was used for extraction of RTV from collected plasma samples. Acetonitrile was used as a protein precipitating solvent. 100 µl of drug contain plasma samples were piped into a micro centrifuge tubes and 400 µl of acetonitrile was added into it and mixed onto vortex for 2 min. Further, the samples were centrifuged at 10000 rpm at 4-5 °C for 15 min. The supernatants of the centrifuged samples were transferred into a sample loading vials and which were injected into the HPLC system. In the PK study, parameters like C_{max}, t_{max}, $t_{1/2}$ and Area Under Curve (AUC) were calculated from plasma concentration Vs time profile curve and results were shown as mean±SD.

STORAGE STABILITY STUDIES

As per the European Agency for the Evaluation of Medicinal Products guideline,[22] stability study of R-SBA-15NPs was carried out at 40 ± 2°C and 75% ± 5% of relative humidity (RH) condition for 6 months. The samples were withdrawn at 1st, 3rd and 6th month and changes in R-SBA-15NPs were observed by differential scanning calorimetry (DSC) and P-XRD.

RESULTS AND DISCUSSION

SCANNING ELECTRON MICROSCOPY (SEM)

The morphology of SBA-15NPs was confirmed by SEM analysis. Fig. 1 showed the synthesized SBA-15NPs were uniform in shape and having a smooth surface.

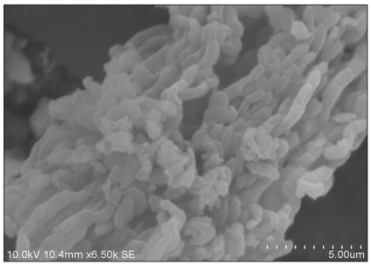

Figure 1: SEM image of SBA-15NPs

TRANSMISSION ELECTRON MICROSCOPY (TEM)

Fig. 2 Showed pore structure of SBA-15NPs and it clearly showed regular 2D hexagonal honeycomb like structure arrangement with well inter connected mesoporous structure and well ordered SBA-15NPs shows particle size in range 200-300nm.

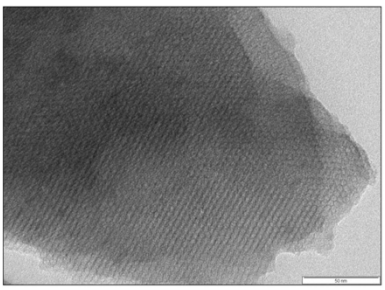

Figure 2: TEM image of SBA-15NPs

ANALYSIS OF DRUG LOADING METHODS

RTV was loaded in SBA-15NPs by the solvent evaporation method and the % loading efficiency was calculated by the thermal analysis of RTV and R-SBA-15NPs. In Fig. 3 TGA plots of RTV and R-SBA-15NPs show their single stepwise weight loss. In Fig. 3(a) shows RTV remain unchanged until the temperature of analysis reaches 215 °C. Then there was a first gradual reduction in weight between 215 to 320 °C and the second reduction between 330 and 410 °C due to final RTV decomposition. Likewise in Fig. 3(b) shows R-SBA-15NPs thermal behavior showed a gradual reduction in weight between

190 to 320 °C that was attributed to the second reduction between 320 to 440°C. No change was observed afterword in the sample. Therefore on the basis of weight loss (%), the loading capacity of SBA-15NPs was determined to be 53%. Also the loading efficiency was again determined by UV spectroscopic method and it was found to be 52.36%. Both analyses showed comparable results for % loading efficiency

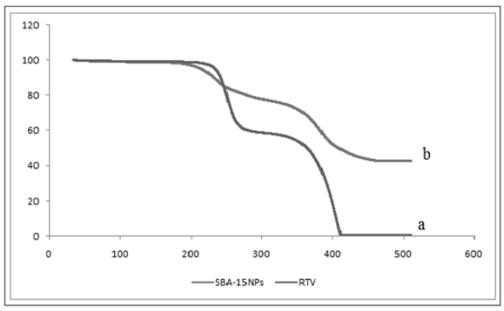

Figure 3: TGA thermogram of (a) RTV, (b) R-SBA-15NPs

FOURIER TRANSFORMS-INFRARED SPECTROSCOPY (FT-IR)

For functional group identification and confirming the compatibility between RTV and SBA-15NPs nanoparticles, FT-IR study was carried out. FT-IR spectra of pure RTV, SBA-15NPs, R-SBA-15NPs-PM and R-SBA-15NPs are shown in Fig. 4. The RTV spectrum shows peaks at 3327 cm^{-1} relatives to the N-H stretching of an amide group, 2962 cm^{-1} relative to hydrogen-bonded acid within the molecule, 1706 cm^{-1} relative to the ester group, 1660, 1611 and 1540 cm^{-1} relative to –C═C– stretching aromatic carbons in Fig. 4(a). In Fig. 4(b) the FT-IR spectrum of SBA-15NPs gave a broad peak between 3350-3500 cm^{-1} that indicating the presence of isolated terminal silanol groups. The stretching vibrations of Si-O-Si and Si-OH present at 1084 and 801 cm^{-1} respectively. In Fig.4(c) R-SBA-15NPs-PM spectra showed characteristic peaks of RTV and SBA-15NPs which proves compatibility between both drug and silica nanoparticles. On the other hand, in Fig. 4(d), R-SBA-15NPs spectrum showed a remarkable decrease of the peak at 2962 cm^{-1}, 1706 cm^{-1} and slight shifting of –C═C– stretching aromatic carbons with the disappearance of other peaks indicating that the complete entrapment of the RTV by SBA-15NPs. These changes suggested that the isolated terminal silanol group present in SBA-15NPs have some interactions with RTV functional groups.

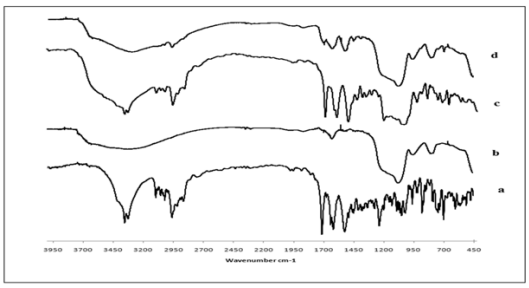

Figure 4: FT-IR (a) RTV, (b) SBA-15NPs (c) R-SBA-15NPs-PM and (d) R-SBA-15NPs

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The DSC thermogram of crystalline pure RTV, SBA-15NPs, R-SBA-15NPs-PM and R-SBA-15NPs are shown in Fig. 5. Crystalline RTV thermogram showed a sharp endothermic peak at 123 °C that corresponds to its fusion temperature point in Fig. 5(a). SBA-15NPs did not show any transition because the melting point of silica is very high and it is shown in Fig. 5(b). In physical mixtures, the sharp endothermic peak of RTV was present indicating the compatibility between RTV and SBA-15NPs in Fig 5(c). The R-SBA-15NPs thermogram did not show any sharp endothermic peak of RTV, it suggested that no drug was present on the outer surface of nanoparticles shown in Fig. 5(d) that confirmed successful loading of RTV.

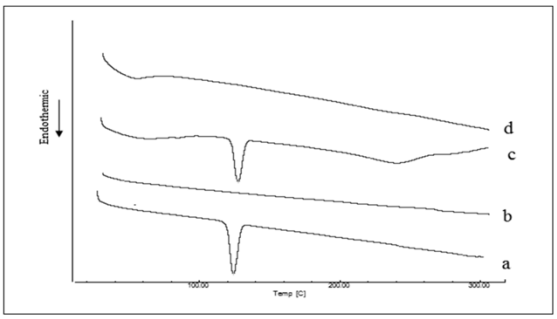

Figure 5: DSC thermogram of (a) RTV, (b) SBA-15NPs, (c) PM of RTV and SBA-15NPs, (d) R-SBA-15NPs

POWDER XRD (PXRD)

Small-angle powder XRD (S-PXRD) patterns of SBA-15NPs and R-SBA-15NPs are shown in Fig. 6(A). In Fig. 6A (a) SBA-15NPs diffractogram showed a strong diffraction peak at 100, and two small diffraction peaks at 110 and 200 between 1° to10° in the 2θ/5s region, that diffraction pattern confirms the formation of uniformly ordered 2D hexagonal mesostructure. In Fig. 6A (b) R-SBA-15NPs shows same diffraction peak pattern but peak intensity was slightly decreased that confirmed the drug was loaded in SBA-15NPs and no structural change observed in nanoparticles. Likewise, wide-angle powder XRD (W-PXRD) pattern of plain RTV is shown in Fig. 6B (a) showed several diffraction peaks at region 5-40° in the 2θ/5s region, i.e. identical to already reported reference standard pattern which confirmed that the RTV was in crystalline form. Whereas in Fig. 6B (b) showed a complete absence of RTV diffraction peaks in R-SBA-15NPs. That confirmed the drug was completely entrapped and no crystalline drug remains on the outer surface of nanoparticles.

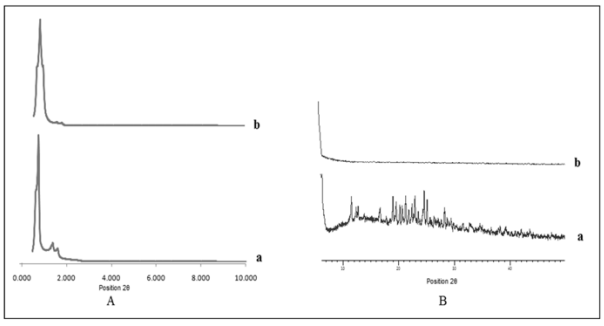

Figure 6: Low angle Powder-XRD (A) (a) SBA-15NPs, (b) R-SBA-15NPs; High angle Powder XRD (B) (a) RTV, (b) R-SBA-15NPs.

N_2 ADSORPTION ANALYSIS

N_2-adsorption–desorption isotherms relative to SBA-15NPs and R-SBA-15NPs are shown in Fig.7. N_2-adsorption–desorption isotherms gave data related to surface area, pore size and pore

volume of nanoparticles. N_2 adsorption-desorption isotherm of SBA-15NPs in Fig. 7a showed typical type IV isotherms and hysteresis loop that confirmed the nanoparticles have mesoporous property. After the drug loading in SBA-15NPs, Fig. 7b shows the type IV pattern and hysteresis loop of isotherm remain intact, reduction in surface area and also reduction in pore volume and pore diameter of R-SBA-15NPs as compared with SBA-15NPs was given in Fig. 8 and Table 1.

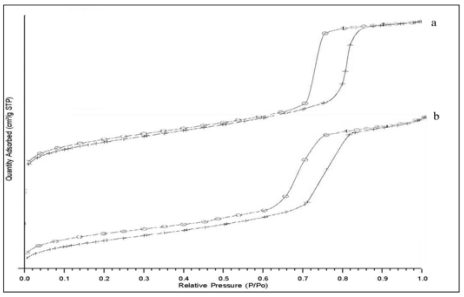

Figure 7: BET Isotherm of (a) SBA-15NPs, (b) R-SBA-15NPs

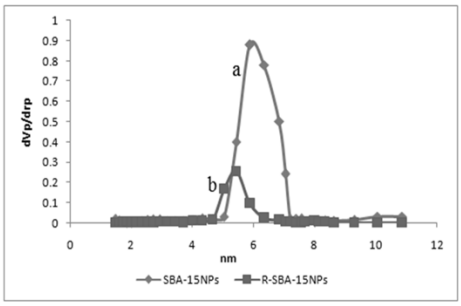

Figure 8: Pore size distributions of (a) SBA-15NPs and (b) R-SBA-15NPs.

Table 1 Evaluation parameter of R-MCM-41NPs and R-MCM-48NPs by N_2 adsorption-desorption

Name of compound	BET surface area	Pore volume	Pore diameter
SBA-15NPs	880.66 m^2/g	0.89cm^3/g	5.9nm
R-SBA-15NPs	243.90 m^2/g	0.27cm^3/g	5.4nm

EVALUATION OF RTV TABLETS

Formulated R-SBA-15NPs tablets were evaluated by various parameters like hardness, disintegration time, friability, and drug content (%). The total weight of prepare tablets were 500±5mg and the hardness of prepared tablet formulation was ranging from 6.9 to 7.2 kP, indicating that the hardness of tablets were good enough to withstand the external pressure. Tablets were prepared using common excipients which showed disintegration time 1±0.3 min and friability was less than 1 %. Percentage drug content values in prepared tablets were obtained in the range of 98.12 - 101.56 %.

IN-VITRO DISSOLUTION STUDY

Drug release studies were performed to see the release pattern of pure RTV and drug loaded nanoparticles using 0.1N HCl, pH 4.5 acetate buffer with 0.75 % PLE and pH 6.8 phosphate buffer with 0.75 % PLE. As the drug has a pH-dependent solubility, the minimum amount of surfactant was required to added in pH 4.5 acetate buffer and pH 6.8 phosphate buffer. The dissolution rate was significantly enhanced in the R-SBA-15NPs as compared to pure RTV and R-SBA-15NP-PM. The augment in dissolution rate was observed due to the changing of RTV into amorphous form after loading into SBA-15NPs. The RTV release profiles are shown in Fig. 9. R-SBA-15NPs showed more than 85 % drug release in all dissolution media within 45 min, whereas pure RTV and PM showed almost 39 % and 45% drug release in 0.1N HCl respectively in Fig. 9A, 29 % and 36% drug release respectively in pH 4.5 acetate buffer with 0.75 % PLE in Fig. 9B and 21 % and 29% drug release respectively in pH 6.8 phosphate buffer with 0.75% PLE in Fig. 9 C respectively. Physical mixture was revealed that some RTV molecules are adsorbed into the mesopores of MCM-41 lead to slightly enhance dissolution compare to pure drug. The reason of SBA-15NPs showing better dissolution profile than pure RTV, is might be due to the high surface area of SBA-15NPs improved the wettability of RTV, also RTV was incorporated into the small nanosize pore of SBA-15NPs that change the crystalline nature of RTV in an amorphous form that improves the solubility rate and dissolution. Because of the high amount of silanol group present in SBA-15NPs that having weak bonding with RTV molecules and that can be easily broken down in dissolution media. Therefore because of all these reasons SBA-15 NPs gave rapid dissolution as well as more diffusion in the dissolution medium.

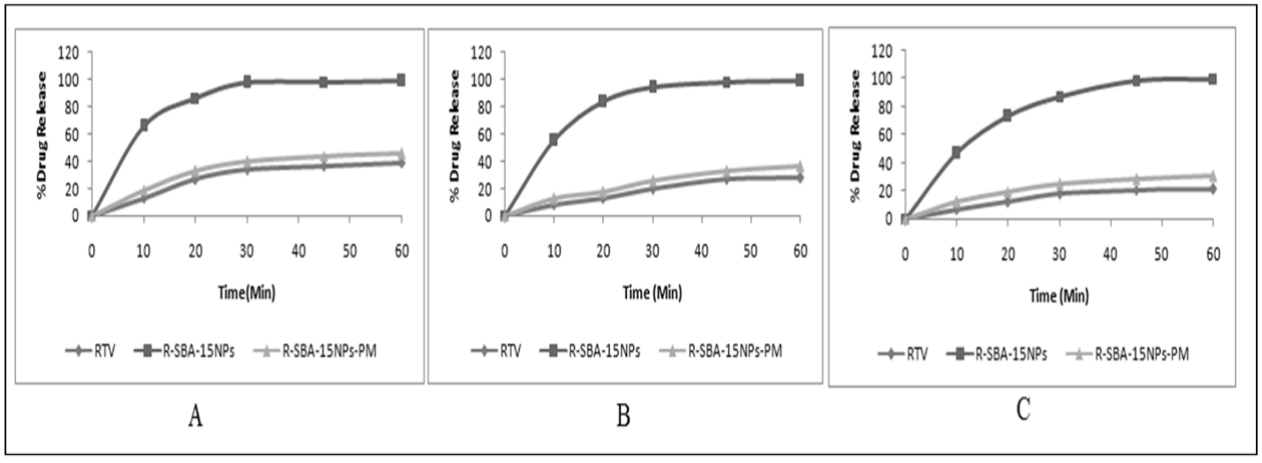

Figure 9: *In-vitro* dissolution study in (A) 0.1N HCl, (B) pH 4.5 acetate buffer with 0.75% PLE (C) pH 6.8 phosphate buffer with 0.75% PLE

IN-VIVO STUDY

The PK studies in wistar rat were carried out to investigate enhancement in oral bioavailability of RTV in SBA-15NPs. The Plasma drug concentration–time profiles of pure RTV and R-SBA-15NPs was seen after it was given orally in wistar rat and results are shown in Fig. 10. Plasma drug concentration profile of R-SBA-15NPs shows remarkable enhancement in drug absorption compared with pure RTV. An area under the concentration–time curve (AUC_{0-t}) of R-SBA-15NPs was 23.965±µg/ml*h which was 1.75-fold higher as compared with pure RTV 13.645± µg/ml*h. Peak Plasma concentration (C_{max}) of R-SBA-15NPs was 8.943 µg/ml around 2.32-fold greater than pure RTV 3.852 µg/ml. Time to reach maximum plasma concentration (t_{max}) of R-SBA-15NPs and pure RTV was found to be 1 and 1.5 h respectively. Elimination half-life ($t_{1/2}$) of R-SBA-15NPs and pure RTV was found to be 1.84 ± 0.10 and 2.19 ± 0.47 h respectively. The PK parameter C_{max}, AUC_{0-t} and decreased t_{max} of SBA-15NPs loaded RTV clearly shows in Table 2 improvement in oral bioavailability compared to pure RTV. Overall, *in vitro* and *in vivo* studies admitted that the SBA-15NPs as a carrier for RTV shows enhancement in drug dissolution rate and also gives faster absorption rate.

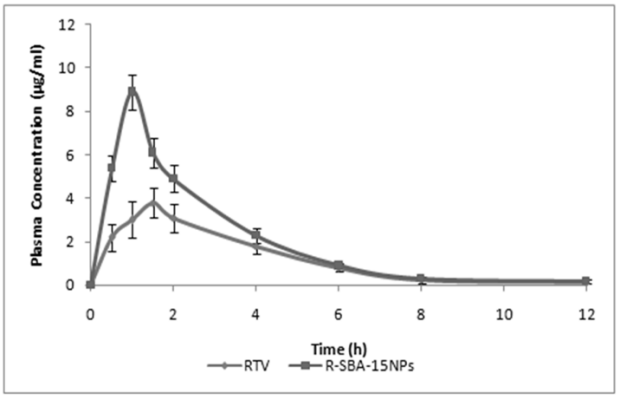

Figure 10: Plasma concentration–time profiles of RTV after oral administration at a dose of 10 mg/kg in male rats (n=3).

Table 2 Pharmacokinetic parameters of pure drug and prepared formulation

Parameter	RTV	R-SBA-15NPs
C_{max}	3.852±0.35ug/ml	8.943 ±0.62ug/ml
t_{max}	1.5h	1h
AUC_{0-t}	13.645±1.14 µg/ml*h	23.965±1.64µg/ml*h
$t_{1/2}$	2.19h	1.84h

STABILITY STUDY

The stored R-SBA-15NPs were found stable at 40°C ± 2 and 75% ± 5 over the period of 6 months. Both analysis DSC and W-PXRD show no evidence of drug crystal were detected as shown in Fig 11 A and B respectively.

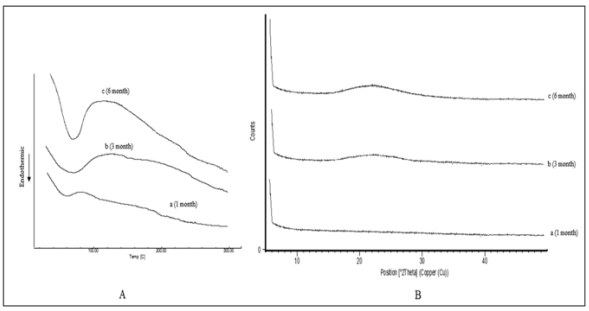

Figure 11: (A) DSC of stability samples and (B) and P-XRD of stability samples; a (1 month), b (3 month) and c (6 month) respectively.

CONCLUSION

In the present study, RTV was successfully entrapped in SBA-15NPs by the solvent evaporation method. SBA-15NPs have the high surface area, uniform 2D hexagonal pore size and large pore volumes that make it suitable drug carrier for drug delivery. *In vitro* and *in vivo* studies of RTV-loaded SBA-15NPs showed remarkable improvement in drug dissolution rate as well as enhanced the oral bioavailability of drug as compared with pure RTV. The study proves that well-ordered silica based mesoporous SBA-15NPs is a promising and suitable carrier for improvement in dissolution rate and bioavailability of poorly water-soluble RTV drugs. Therefore prepared mesoporous silica SBA-15NPs tablets may give a new approach for the development of oral formulations for poorly water-soluble drugs.

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