SUMMARY

AND

CONCLUSION

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Two poorly water soluble antiretroviral drugs, ritonavir and lopinavir were selected for the present study, mainly because antiretroviral drugs having low solubility and bioavailability and require higher dose that could lead to higher toxicity effect and side effects in patients and therefore there is a need for an innovative formulation approach to enhance the bioavailability. Mesoporous silica nanoparticles have gained attention because of ability to increase the solubility as well as bioavailability of poorly water soluble drugs. Mesoporous silica nanoparticles have large surface area, tunable pore size property and high pore volume that features makes it the potential drug carrier. So in this study three different types of mesoporous silica nanoparticles were synthesized. Synthesized mesoporous silica materials have different pore size, structural properties and surface area and previously used in drug delivery system. MCM-41, MCM-48 and SBA-15 MSNs have some attractive features like porous interior with different structure and large surface area with tunable pore size can entrap a drug molecule easily. MCM-41 and MCM-48 come from M41S family and SBA-15 comes from SBA family. Mesoporous silica materials have good biocompatibility and tailored size of pores makes them promising drug carrier. Therefore in this study three different mesoporous silica nanoparticles with different structure, surface area and pore size (MCM-41NPs, MCM-48NPs & SBA-15NPs) were investigate to enhance the solubility and bioavailability of Ritonavir and lopinavir drugs.

Synthesis and characterization

Synthesis of different mesoporous silica nanoparticles were carried out by different synthesis schemes. MCM-41 is hexagonal with a pore diameter of 3.9 nm wherein while synthesis cationic surfactants (CTAB) were used as templates and fumed silica was used as silica precursor. MCM-41 is one of the most widely explored materials for drug delivery. Apart from this, one other materials of mesoporous nature belong from the same family have been synthesized by varying the starting precursors (TEOS) and reaction conditions. These may vary in their structural arrangement or the pore size. MCM-48 has a 3D cubic pore arrangement with smaller pore size 3.2nm than MCM-41. Likely Non-ionic triblock copolymers (P-123) have been used as a surfactant which has been designated as 2D hexagonal SBA-15 symmetry of the mesoporous structure. Highly ordered mesoporous structure of SBA-15 has also

been widely used for the biomedical purpose and this is different from other MCM mesopores, in that they possess larger pores of 5.9 nm and thicker silica walls. All the synthesized MSNs were characterised by different analytical techniques like SEM, TEM, P-XRD, and N2 adsorption-desorption.

The physical properties of their MSNs as derived from N2 adsorption-desorption, SEM, TEM and FTIR are shown in table 1

Table 1: Physical Properties of MSNs

Name of Carrier	Structure of carrier	BET surface area m^2/g	Pore size	Pore Volume
MCM-48	3D cubic sperical	1220.29 m^2/g	3.2 nm	0.96cm^3/g
MCM-41	2D Hexagonal	935.76 m^2/g	3.9 nm	0.82cm^3/g
SBA-15	2D Hexagonal	880.66 m^2/g	5.9 nm	0.89cm^3/g

RTV and LPV loading in MSNs and characterization

The solvent evaporation method was found to be most efficient for drug loading in the different MSNs. The entrapment efficiency (EE) of RTV and Loading efficiency (LE) of R-MSNs-NPs and L-MSNs-NPs as confirmed by UV spectrophotometric method and by Thermogravimetric analysis are summarize in table 2

Table 2: Entrapment efficiency and Loading efficiency of RTV, LPV and different MSNs by UV spectrophotometric method and by Thermogravimetric analysis

Name of compound	RTV			LPV		
	% LE		% EE	% LE		% EE
	UV method	TGA	UV method	UV method	TGA	UV method
MCM-48NPs	43.23%	45%	98.33%	46.83%	49%	98.13%
MCM-41NPs	37.12%	38%	84.26%	38.72%	40%	87.34%
SBA-15NPs	50.34%	53%	99.52%	54.14%	57%	99.45%

Both RTV and LPV could be maximally loaded in to SBA-15NPs which was confirmed by TGA and UV data and also by N_2adsorption desorption studies. It was mainly due to the large pore size of these nanoparticles and high amount of silanol groups present that adsorb large amount of drug. The Numerical data for MCM-48NPs, MCM-41NPs and SBA-15NPs (MSNs) and R-MSNs and L-MSNs is showing in below table 3 and 4

Table 3: BET surface area, Pore diameter and volume of MCM-48NPs, MCM-41NPs and SBA-15NPs before and after RTV loading

Name of compound	BET surface area	Pore volume	Pore diameter
MCM-48NPs	1220.29 m^2/g	0.96cm^3/g	3.2nm
R-MCM-48NPs	440.60 m^2/g	0.31cm3/g	2.7nm
MCM-41NPs	935.76 m^2/g	0.82cm^3/g	3.9nm
R-MCM-41NPs	380.15 m^2/g	0.46cm3/g	3.3nm
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

Table 4: BET surface area Pore diameter and volume of MCM-48NPs, MCM-41NPs and SBA-15NPs before and after LPV loading

Name of compound	BET surface area	Pore volume	Pore diameter
MCM-48NPs	1220.29 m^2/g	0.96cm^3/g	3.2nm
L-MCM-48NPs	405.87 m^2/g	0.25 cm^3/g	2.8nm
MCM-41NPs	935.46 m^2/g	0.82cm^3/g	3.9nm
L-MCM-41NPs	345.55 m^2/g	0.36 cm^3/g	3.5nm
SBA-15NPs	980.66 m^2/g	0.89cm^3/g	5.9nm
L-SBA-15NPs	293.65 m^2/g	0.32 cm^3/g	5.3nm

R-MSNs-NPs (RTV equi.100mg) were formulated in tablet form by performing direct compression method with standard excipients like L-HPC, cross Povidone, MCC, Lactose DCL and Mg. Stearate. Prepared tablets were characterized for various parameters like hardness, disintegration time; friability etc. and all parameters were within limits as per IP.

RTV *in-vitro and in-vivo* study

Dissolution tests were performed at different pH conditions with using minimum amount of surfactant at a particular pH in order to investigate the drug release behavior in different regions of gastrointestinal tract. The dissolution profiles show the superiority of R-MCM-48NPs and R-SBA-15NPs over R-MCM-41NPs, Pure RTV and MF in terms of % cumulative release of RTV. The RTV release profiles of R-MCM-48NP and R-SBA-15NPs showed more than 95 % drug release in all dissolution media within 45 min, whereas R-MCM-41NPs showed almost 72 %, 59.9% and 53% drug release in 0.1 N HCl, pH 4.5 acetate buffer with 0.75 % PLE and pH 6.8 phosphate buffer with 0.75 % PLE respectively. Pure RTV and RTV MF did not achieve complete dissolution in any of the selected media over the test period of 60 min. (Fig. 1) The enhancement in dissolution rate can be attributed to the fact that RTV may be in amorphous form after incorporation in mesopores of nanoparticles that improves the solubility rate and dissolution of RTV.

In vivo assessment demonstrated that R-MCM-48NP and R-SBA-15NPs exhibited better pharmacokinetic properties compared to R-MCM-41NP, Pure RTV and RTV MF. The relative oral bioavailability of RTV in Albino wistar rat resulted from R-MCM-48NPs was found **2.48 and 1.54 fold** greater than pure RTV and RTV MF, respectively. Likely from R-SBA-15NPs relative oral bioavailability of RTV was found **2.33-fold and 1.43-fold** greater than pure RTV and RTV MF. (Fig. 2 and Table 6) Thus it can be concluded that that using mesoporous silica nanoparticles for RTV; leads to improved dissolution properties and excellent oral bioavailability of RTV.

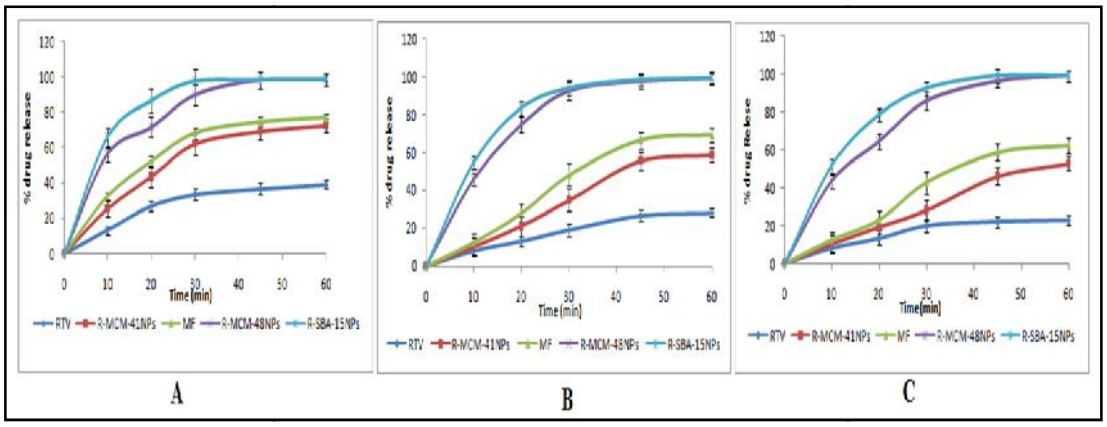

Figure:1 Release profile of RTV from pure RTV, R-MSNs and MF in (A) 0.1N HCl (B) pH 4.5 acetate buffer with 0.75 % PLE (C) pH 6.8 phosphate buffer with 0.75 % PLE

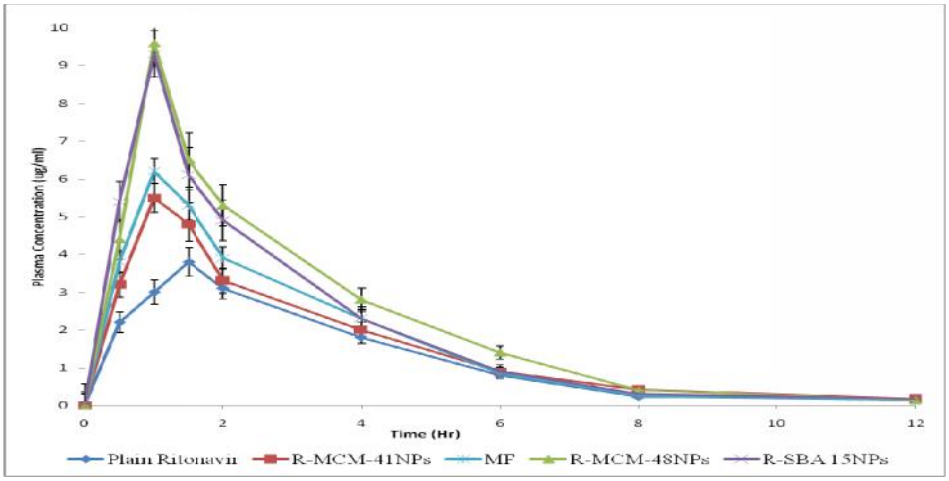

Figure: 2 Graphical Representation of RTV plasma profile for pure RTV, R-MSNs and MF of RTV in Albino wistar rat following oral administration

Table 6: Pharmacokinetic parameter of RTV with different MSNs

Parameter	Pure RTV	R-MCM-41NPs	R-MCM-48NPs	R-SBA-15NPs	MF
C_{max}	3.83±0.35 µg/ml/h	5.54±0.72 µg/ml/h	9.62±0.77 µg/ml/h	8.943 ±0.62 ug/ml/h	6.23±0.46 µg/ml/h
T_{max}	1.5 h	1 h	1 h	1h	1h
AUC_{0-t}	13.64±1.14 µg/mL*h	18.29±1.77 µg/mL*h	26.55±1.84 µg/mL*h	23.965±1.64 µg/ml*h	19.23±2.12 µg/mL*h
$T_{1/2}$	2.18 h	2.16 h	1.80 h	1.84h	2.06±0.16 h

LPV and LPV/RTV *in vitro and in vivo* study

The LPV release profiles of L-MCM-48NP and L-SBA-15NPs showed more than 95 % drug release in all dissolution media within 45 min, whereas L-MCM-41NP showed almost 56.3 %, 59.7% and 66.5% drug release in 0.1 N HCl with 0.75 % PLE, acetate buffer pH 4.5 with 0.75 % PLE and phosphate buffer pH 6.8 with 0.75 % PLE respectively. Pure LPV and LPV MF did not achieve complete dissolution in any of the selected media over the test period of 60 min. (Fig. 3) LPV was incorporated into the mesopores of nanoparticles that change the crystalline nature of LPV in an amorphous form that improves the solubility rate and dissolution of LPV.

LR-MCM-48NPs, LR-MCM-41NPs and LR-SBA-15NPs showed same results as given by L-MCM-48NP, L-MCM-41NPs and L-SBA-15NPs. (Fig. 4). The dissolution study of LPV/RTV formulation (LR-MSNs) did not show any major difference in dissolution profile of LPV in presence of RTV as compare to L-MSNs in all dissolution media respectively. That shows RTV did not show any role in in-vitro dissolution study of LPV.

In vivo assessment demonstrated that L-MCM-48NP and L-SBA-15NPs exhibited better pharmacokinetic properties compared to L-MCM-41NP, Pure LPV but not more than the LPV/RTV MF .

When L-MCM-48NPs, L-MCM-41NPs and L-SBA-15NPs administered orally in wistar rat, it shows slight enhancement in LPV plasma concentration, it was 0.81±0.19µg/ml/hr, 0.55±0.12µg/ml/hr and 0.75±0.16µg/ml/hr respectively but not more than the LPV concentration 1.58±0.25µg/ml/hr found in a combination of pure LPV/RTV. it is clearly shown that the absorption rate of LPV in combined pure LPV/RTV was higher than alone pure LPV, when the combination of pure LPV/RTV administered orally it shows more plasma concentration of lopinavir compare to the pure LPV. The reason behind that is when Lopinavir administered alone, it rapidly metabolise in the liver by CYP3A5 and CYP3A4. Ritonavir a widely used as pharmacokinetic enhancer, that inhibits the CYP3A5 and CYP3A4

isoenzyme in the liver microsomes and therefore it increases the concentration of lopinavir in systemic circulation. Therefore we also used combination tablet formulation of LR-MSNs-NPs for in-vivo assessment.

In this study, the drug plasma concentration profile of LR-MCM-48Ps and LR-SBA-15NPs showed significant improvement in LPV absorption as compared to pure LPV, the combination of pure LPV/RTV and MF of LPV. The relative oral bioavailability of LPV in albino wistar rat resulted from LR-MCM-48NPs was about **11.27-fold, 3.35-fold and 1.51-fold** higher than that of pure LPV, combination of LPV/RTV and MF of LPV/RTV respectively. Likely from LR-SBA-15NPs relative oral bioavailability of LPV was found **10.95-fold, 3.25-fold and 1.47-folds** greater than that of pure LPV, combination of LPV/RTV and MF of LPV/RTV respectively. (Table 8) In-vitro and in-vivo profile of RTV and LPV shows MCM-48NPs showing better dissolution profile than MCM-41NPs is might be due to small pore size of nanoparticles and the high surface area.

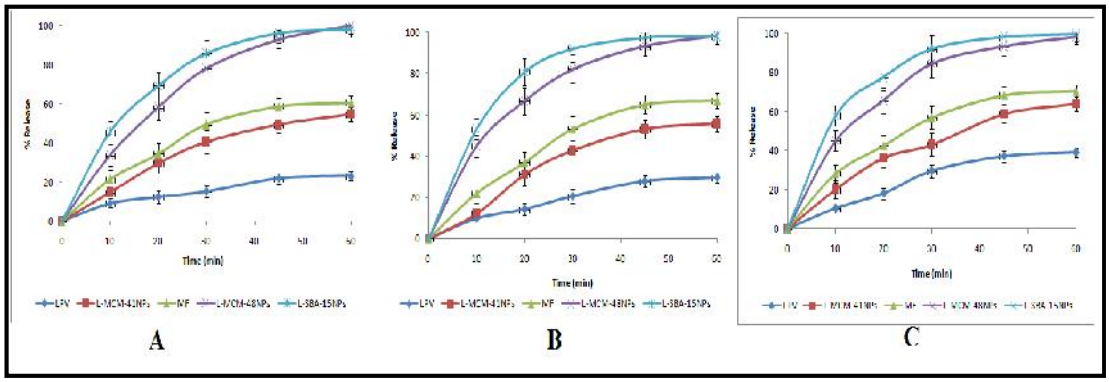

Figure: 3 Release profile of LPV from pure LPV, develop formulation of LPV with various mesoporous silica nanoparticles and marketed formulation in (A) 0.1N HCl with 0.75 % PLE; (B) pH 4.5 acetate buffer with 0.75% PLE; (C) pH 6.8 phosphate buffer with 0.75% PLE

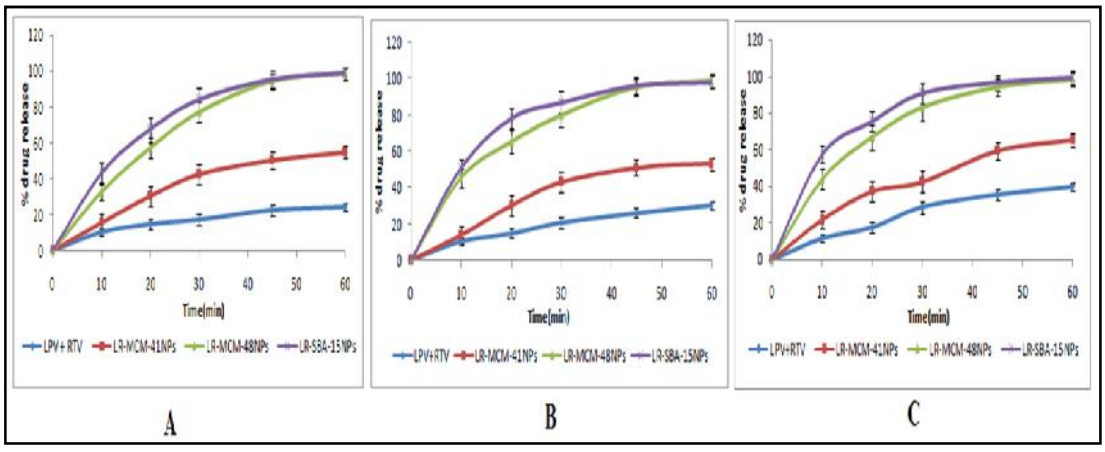

Figure: 4 Release profile of LPV from combination of LPV/RTV, LR-MSNs and MF in (A) 0.1N HCl with 0.75 % PLE; (B) pH 4.5 acetate buffer with 0.75% PLE; (C) pH 6.8 phosphate buffer with 0.75% PLE

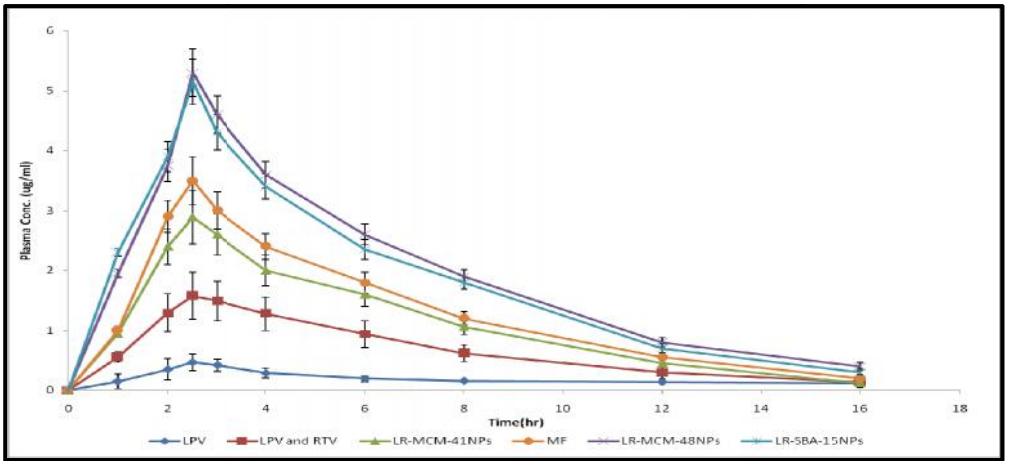

Figure 6.1 Graphical representation of LPV plasma profile for plain LPV, Combination of LPV/RTV, LP- MCM-41NPs, LP-MCM-48NPs, LP-SBA-15NPs and MF of LPV/RTV in Albino Wistar rat following oral administration

Table 8 Pharmacokinetic parameter of LPV with different MSNs

Parameter	Pure LPV	Pure LPV and RTV	LR-MCM-48NPs	LR-MCM-41NPs	LR-SBA-15NPs	MF
C_{max}	0.47± 0.10 µg/mL	1.58± 0.25 µg/mL	5.30± 0.34 µg/mL	2.90± 0.45 µg/mL	5.15 ±0.40 ug/mL	3.50± 0.36 ug/ml
T_{max}	2.5 h	2.5h	2.5 h	2.5 h	2.5h	2.5 h
AUC_{0-t}	3.59± 2.34 µg/mL*h	10.57± 3.23 µg/mL*h	33.64± 4.14 µg/mL*h	18.48± 4.74 µg/mL*h	31.95± 3.14 µg/mL*h	20.57± 3.22 µg/mL*h
$T_{1/2}$	3.73±0.24 h	3.72±0.19 h	3.95±0.32 h	3.86±0.21 h	3.56±0.29 h	3.38±0.26 h

The RTV and LPV molecules adsorbed in the large surface of the 3D interconnected MCM-48NPs gave rapid diffusion and faster dissolution in the media while, MCM-41NP has cylindrical pore 2D hexagonal structure, appeared to restrict the drug molecules in the pore channels from diffuse into the media that gives slow drug release. SBA-15NPs also shows better dissolution of RTV and LPV in all media, the reason of SBA-15NPs showing better dissolution profile than MCM-41NPs, is might be due to high amount of silanol group present in SBA-15NPs than MCM-41NPs that adsorb more amount of RTV and LPV molecules and also SI-OH groups on SBA-15NPs form very weak bonding with RTV and LPV molecules as compared to MCM-41NPs, that can be easily and quickly broken down in dissolution media. Due to all these reasons, the dissolution through SBA-15NPs was slightly faster than that of MCM-48NPs.

In-Vitro cell cytotoxicity

It was found that cell viability of caco-2 cells of R-MCM-48NPs, L-MCM-48NPs was more than the 85% respectively, Whereas more that 90% cell viable found in R-SBA-15NPs and L-SBA-15NPs respectively at the concentration 500 µg/ml concentration for 48hr. The result of cell cytotoxicity shows decrease in cell viability with MCM-48NPs as compare to the SBA-15NPs. The smaller size, larger the surface area, smaller particle size and cationic surfactant used are the factors which cause the slight decrease in the cell viability in MCM-48NPs.

Physical and Chemical Stability study

The stability of RTV and LPV molecules within the mesopores and integrity of mesostructure were determined by the physical stability. The drug molecules within the pore and mesoporosity of MSNs showed good physical stability after the 1, 3 and 6 month at 40^0c.

The Chemical stability of RTV and LPV loaded MSNs were checked by RP-HPLC. The chemical stability of drugs in MSNs was checked individually after kept in accelerated stability condition for 1, 3 and 6 months of samples. In all tested conditions, all the drug loaded MSNs show good chemical stability and found no changes in content and concentration of RTV and LPV.

The results of present investigations conclusively indicate the remarkable enhancement in oral bioavailability of selected drugs in prepared mesoporous silica nanoparticles. The study also confirms that size of mesoporous material is an important factor for efficient drug loading. RTV and LPV were more successfully loaded in to small/big sized MSNs. Hence the developed formulations of RTV and LPV/RTV can be potentially useful in clinical treatment of Acquired immunodeficiency syndrome. These formulations hold promise as better alternative to the conventional dosage forms and can be used as multidrug carriers also However, further investigations in human beings under clinical conditions are necessary before they can be commercially exploited.

Future Perspective of this study:

This study demonstrate significant improvement in oral bioavailability of Ritonavir and Lopinavir in prepared mesoporous silica nanoparticles and can provide a better alternative to existing formulation, once their toxicity or safety profile in human being are established. Further study can also be done with new ideas like:

1. Functionalization of surface of MSNs for targeting the antiretroviral drugs to lymphatic nodes

2. Development of sustain release or extended release formulation for antiretroviral drugs using the MSNs and examine in vivo permeability study of antiretroviral drugs loaded MSNs.