CHAPTER 7: SUMMARY:

In the present study, the authors have successfully accomplished the encapsulation of poorly soluble RLX and BXR into mesoporous nanoframework. The various nano systems are available on the same drug for satisfying the purpose of solubility and bioavailability enhancement. But, the application of MSNs for the same is still scarce. Furthermore, novel application of 3D cubic structure of MCM-48 type of nano system still remains untouched. The comprehensive investigation of the 2D and 3D hexagonal and cubic structure has been represented in this research work. The comparative study between MCM-41 and MCM-48 NPs provided a firm platform for achieving solubility and bioavailability enhancement of poorly soluble drugs like RLF and BXR. However, the efficacy of the formulated nanoparticles remained limited due to scattering of drug throughout the body *i.e.* unfocused release of drugs to healthy and diseased cells due to lack of targeting efficiency of engineered formulation. Therefore, to avoid this issue pH dependent and receptor based targeted drug delivery system was formulated, which could have the potential of releasing drug exclusively to tumor cells. Further, the remarkable decrease in the release of drug to healthy cells might have potential to eliminate or diminish the adverse effect of drug to healthy cells. This concept was taken into consideration and chitosan (pH mediated drug delivery system) and folic acid-chitosan (folic acid receptor mediated drug delivery system) nanoparticles (MCM-41 and MCM-48) were formulated for RLX. On the other side, hyaluronic acid coated nano carrier systems were developed (MCM-41 and MCM-48) for BXR, which kills the tumor cells by acting as a dual responsive drug delivery system *i.e.* pH and receptor based nanosystem.

7.1. Summary for RLX

The journey of this research work was started with the synthesis of bare and surface decorated MSNs. The construction of MSNs begun with its basic skeletal *i.e.* MCM-41 and MCM-48 which was fabricated externally to get MSN-NH_2-41 and MSN-NH_2-48. Presence of amino group on the external surface was essential as it is a vital moiety to provide a platform where targeting ligand i.e. chitosan/folic acid could be attached easily. The success of surface decoration was checked by TGA in quantitative manner. The TGA data were in good agreement and showed 4.01%, 28.51% and 22.87% grafting of amine, chitosan and folate chitosan conjugates externally in MCM-41 type of nanosystem. Whereas, the figures were bit higher for MCM-48 type of nanoparticles *i.e.* 4.87, 29.57 and 25.68 respectively. Further,

inversion of zeta potential from the negative side to positive side support the formation of successful formation of amine decorated NPs. Further increment in positive charge for the final formulation indicated successful chitosan and folic acid coating.

A comprehensive BET study revealed obvious decline in surface area, pore size and pore volume from initial stage (bare MSNs) via intermediate stage (MSN-NH_2) and to a final destination (CHITO-MSN and FC-MSN). This successive reduction could be attributed to increase in particle size after each successive modification. Furthermore, uniformity in the pore size of the manufactured nanoparticles were envisaged by particles size distribution and TEM outcomes and the results were complementary to each other i.e. ranging from 90 to 150nm. Beside pore size uniformity analysis, TEM images exhibited synthesis of ordered mesoporous framework with 2D hexagonal assembly. Uniformity in particle size and increment in particle size after each modification is assigned to successive amine, chitosan, FC conjugates grafting. In other words, with respect to particle size, MSNs ended up with healthy CHITO-MSN and FC-MSN having a particle size in range of 130-150 nm in contrast to its initial size *i.e.* 90-96 nm for pristine MSNs moiety. This phenomenon was assessed by SEM and hydrodynamic size investigation. Furthermore, the appearance of three distinct and characteristic deflection peak in SXRD for MSN-41 types of nanosystem and four unique deflection peaks for MSN-48 types of nanosystem revealed successive formation of mesoporous silica assembly and again this was complementary to other aforesaid investigative techniques. However, the intense deflection peak was successively faded from amine grafted nanosystems to chitosan/FC attached nanosystems.

After comprehensive synthesis and characterization of nanoparticles, model drug RLX was encapsulated inside the pore by novel immersion-solvent evaporation technique which ensured maximum loading and entrapment efficiency. A nitrogen desorption investigation was performed and sharp reduction in surface area and the BJH pore size revealed occupation of pores by RLX. Furthermore, the existence of a hysteresis cycle with type IV absorption isotherm proved mesoporous structure remained unchanged after RLX loading also. Similarly, absence of prominent RLX peak appeared in FT-IR spectra of pure drug, absence of melting peak at 262 ºC in DSC thermograms, suggest the complete uptake of RLX within the pores and ruled out the possibility of extra drug present outside the surface. The conversion of crystalline RLX to amorphous RLX filled nanoparticles was proved by disappearance of characteristic WXRD peaks of RLX in WXRD. Besides this, RLX occupied NPs were subjected to SEM,

TEM, DLS analysis to confirm uniformity in particle size, pore size distribution and to check the integrity of the internal hexagonal structure after RLX encapsulation.

Furthermore i*n vitro* dissolution and diffusion study for nanoparticles were employed for oral and parenteral formulation respectively. The release data demonstrated higher and faster release of RLX from the MCM-41 and MCM-48 types of nanoparticles in SGF media as compared to its marketed formulation and RLX plain drug. Further, the *in vitro* release profile demonstrated superior behaviour of MCM-48 type of nanoparticles over MCM-41 nanosystem. Where, the former system demonstrated faster and immediate release as compared to latter. A similar investigation was performed for aminated nanosystems and inferred that RLX release was hindered by amine decorated nanosystems with respect to RLX release from pristine MSNs. Overall, the magnitude of solubility enhancement was 4, 4.4, 2.69 and 2.82 RLX-41, RLX-48, RLX-NH_2-41 and RLX-NH_2-48 respectively with respect to RLX. Moreover, an identical dissolution profile in fasted and fed state gastric and intestinal conditions suggested that the formulation could be administered either before or after meal.

The chitosan and FC based nanosystems were analysed for RLX release by carrying *in vitro* diffusion study at three different pH of PBS solution to envisage pH dependent release behaviour of chitosan coated MSNs. The diffusion data revealed a higher RLX release from RLX-CHITO-MSN at acidic pH, which was dedicated to unique property of chitosan which swells at acidic pH following the pore opening which leads to higher drug release in an acidic environment. In contrast, chitosan gets collapsed when we move away from acidic pH. This results into film formation, which covers the pores and ultimately exhibited significant fall in RLX release at higher pH. This phenomenon is solely attributed to unique pH responsive property of chitosan. Thus, being a polymer, chitosan provided controlled release of RLX with the evident pH selective release. Maximum RLX release from RLX-CHITO-41 in PBS-5.6 was achieved within 72h. Thus, pH responsive NPs were successfully fabricated to direct the drug release exclusively to cancer cells which are known to have an acidic environment. Further, the RLX release from RLX-FC-41 and RLX-FC-48 was lesser than RLX-CHITO-41 and RLX-CHITO-48 which could be due to presence of bulkier group on the external surface of former formulations compare to latter.

The MTT cell viability assay was performed on *in vitro* model of epithelial cells *i.e.* Caco-2 for oral formulations. The plain carrier and RLX loaded nanocarrier showed least toxicity on Caco-2 cell line. Further, the permeability study for oral formulations were carried

out on the same cell line and revealed 4.31 and 5.31 folds increment in the permeability for RLX-41 and RLX-48 respectively. The coefficient value declined for RLX-41-NH_2 and RLX-48-NH_2 *i.e.* 2.77 and 3.16 respectively as compared to RLX. Finally, the PK study for the oral formulations was performed on the female Swiss albino mice and the investigation demonstrated 3.33, 3.50, 2.77 and 2.55-folds increment in bioavailability of RLX with respect to plain RLX for RLX-41, RLX-48, RLX-NH_2-41 and RLX-NH_2-48.

The MTT cell viability assay was also performed on human breast cancer cell line i.e. MCF-7 for parenteral formulation. The MTT results demonstrated higher killing efficiency of chitosan and FC coated nanoparticles with respect to bare nanoparticles. Furthermore, this observation was strongly supported by qualitative and quantitative cellular uptake study performed on confocal microscope and FACS respectively. This investigation unveiled 2.1, 2.8, 2.3 and 3.3 times increment in cellular uptake for MCM-CHITO-41, MCM-FC-41, MCM-CHITO-48 and MCM-FC-48 respectively as compared to pristine nanoparticles. This could be due to FA receptor mediated drug release from FC coated nanoparticles. Further the selective drug release behaviour of chitosan at acidic condition enhanced the RLX availability to acidic cells (tumor cells). Later on, the apoptosis study for RLX-FC-41 and RLX-FC-48 exhibited a large number of apoptotic cell death compared to plain RLX. Finally, the PK and biodistribution study for the parenteral formulation exhibited higher drug release from the engineered formulation along with least toxicity to major organs as confirmed from the histological examination. The lack of histological evidence of any toxicity in the mice treated with RLX formulation concluded that the formulated nanoparticles are safe and devoid of any toxicity on the six major organs. Lastly, the 6-month stability study revealed lack of any unstability and also revealed that the mesoporous skeleton was well preserved.

7.2. Summary for BXR

Just like RLX, here also the experiment started with the synthesising bare and surface decorated MSNs. Large pore MSNs were synthesized for BXR, as it was not encapsulated in the previous nano carriers. The construction of MSNs begun with its basic skeletal *i.e.* MCM-41 and MCM-48 which was fabricated externally to get MSN-NH_2-41 and MSN-NH_2-48. Presence of amino group on the external surface was essential, as it is a vital moiety to provide a platform where targeting ligand *i.e.* HA could be attached easily. The success of attachment of ligand, formation of mesoporous assembly, complete encapsulation of BXR, particle size, pore size, pore volume assessment was done as carried for RLX using different characterization

techniques. Thus, the BXR free and BXR engulfed nanoparticles were evaluated in depth and characterization techniques gave promising results.

The i*n vitro* dissolution and diffusion study for nanoparticles was employed for oral and parenteral formulation respectively. The release data demonstrated higher and faster release of BXR from the MCM-41 and MCM-48 types of nanoparticles in SIF media (pH 6.8) as compared to its marketed formulation and BXR plain drug. The similar investigation was performed for aminated nanosystems and inferred the hindered BXR release by amine decorated nanosystems with respect to RLX release from pristine MSNs. This could be due to presence of amine moiety on the external surface. Overall, the dissolution release profile was improved by 3.33, 5, 2.3 and 3.12 times for BXR release from BXR-41, BXR-48, BXR-NH_2-41 and BXR-NH_2-48 respectively with respect to plain BXR. Moreover, the release pattern from fed and fasted state dissolution media exhibited higher drug release for FeSSIF condition in contrast to FaSSIF, where drug release was completed within 45 min in the former media for BXR-48 and it took almost 6 h for 85% BXR release from BXR-NH_2-48. Whereas, only 68% and 39% of release was achieved after 360 min for BXR-48 and BXR-NH_2-48 in FaSSIF. This again puts the weightage of having a higher release in the presence of food and it could be recommended to take a drug after meal.

The parenteral formulation *i.e.* HA coated nanoparticles were analysed for BXR release by carrying in vitro diffusion study at three different pH of PBS solution to envisage pH dependent release behaviour of chitosan coated MSNs. Further, diffusion data revealed the higher BXR release from BXR-HA-41 and BXR-HA-48 at acidic pH, which was dedicated to higher drug releasing tendency of HA in an acidic environment.

The MTT cell viability assay was performed on *in vitro* model of epithelial cells *i.e.* Caco-2 for oral formulations. The plain carrier and BXR loaded nanocarrier showed least toxicity on Caco-2 cell line. Further, the permeability study for oral formuations were carried out on the same cell line and revealed 3.79 and 4.66-folds increment in the permeability for BXR-41 and BXR-48 respectively. The coefficient value was declined for BXR-41-NH_2 and BXR-48-NH_2 with respect to bare nanoparticle but these were still greater as compared to permeability of pure BXR *i.e.* 2.28 and 2.71 respectively. Finally, the PK study for the oral formulations was performed on the female swiss albino mice and the investigation demonstrated 3.01, 3.31, 2.06 and 2.21 folds increment in bioavailability of BXR with respect to plain BXR.

The MTT cell viability assay was also performed on human breast cancer cell line i.e. MCF-7 for parenteral formulation. The MTT results demonstrated higher killing efficiency of HA coated nanoparticles with respect to bare nanoparticles. Furthermore, this observation was strongly supported by qualitative and quantitative cellular uptake study performed by confocal microscope and FACS respectively. This investigation unveiled 3.8 and 4.2 times increment in cellular uptake for MCM-HA-41 and MCM-HA-48 respectively as compared to pristine nanoparticles. Further, the selective drug release behaviour of HA at acidic condition along with CD-44 cell mediated BXR release made more drug available to tumor cells. Later, the apoptosis study for BXR-HA-41 and BXR-HA-48 exhibited a large number of cells undergoing apoptosis as compared to plain BXR. Finally, just like RLX, the PK and biodistribution study exhibited superior drug release pattern along with least toxicity to the major organ for the engineered formulation. This proved the safe nature of formulated nanoparticles. Lastly, the 6-month stability study revealed lack of any unstability and intactness of mesoporous nature.