1

INTRODUCTION

1. Introduction

The drug administered by oral route has always been preferred by patients because of ease and convenience of administration. But for some drugs oral route administration may be an ineffective way of drug delivery.[1] Therapeutic effectiveness of drugs totally depends upon bioavailability, which is directly affected by the drug solubility. Poor solubility of drug gives the limited drug absorption resulting low bioavailability of drugs. To overcome the solubility problem to achieve maximum bioavailability of drugs by oral route has been a challenge to researchers. Solubility is most important factor to obtain the desired concentration of drugs in systemic circulation for better therapeutic effect. Drug solubility is defined as the maximum concentration of the drug solute dissolved in the solvent at specific pH, temperature and pressure.

Presently, only approximately 10% of new drug molecules have high solubility and high permeability and more than 90% of drugs approved since 1995 have poor solubility. The rate and amount of absorption of drug molecule is highly dependent on the bioavailability and it ultimately depends on solubility.[2-4]

According to Biopharmaceutical Classification System (BCS) Classification, drugs are categories in four class on their solubility and permeability characteristics. These classes are:

Class I- High solubility / High permeability, Class II- Low solubility / High permeability, Class III- High Solubility / Low Permeability, Class IV- Low Solubility / Low Permeability.[6]

Class II and IV drugs show slow dissolution rate and limited absorption of drugs giving poor bioavailability when administered orally Due to this solubility enhancement is an important parameter in formulation development for poor aqueous soluble drugs.

1.1 Bioavailability and its importance

Bioavailability expresses the rate and amount of the drug molecule absorbed from a dosage form and occur at the site of action. Bioavailability of a drug is regulated by three main aspects;[5]

- Rate and amount of drug release
- Consecutive absorption from the solution state
- Biotransformation during absorption process.

As per bioavailability description, when the drug is taken intravenously, its rate and amount of absorption are 100% means its bioavailability is 100%. But whenever drug is taken orally or by transdermal route its bioavailability is decreases because of its limited solubility or because of metabolise earlier or incomplete absorption of drugs. The amount of the drug present in the plasma at a fixed time intervals indirectly specify the rate and amount at which the drug molecules is absorbed from the dosage form and obtained at the site of action. Bioavailability is the vital factors in PK studies, for calculating dose for oral routes of administration. Bioavailability is expressed by two way i.e. absolute bioavailability and relative bioavailability.[7]

1.1.1 Techniques for improving oral Bioavailability

Compounds having poor solubility in aqueous media carry a higher risk of failure during discovery and development, and poor solubility may compromise pharmacodynamics and pharmacokinetics properties of drug and finally may affect the ability of the compound to develop as API.[8] Many strategies have been used for improvement in the solubility along with oral bioavailability of poorly soluble active substances and some commonly used techniques/methods for solubility enhancement are represented in Fig.1.1 Each of these approaches has its own advantages and disadvantages and search for the best technique is continuously being explored.

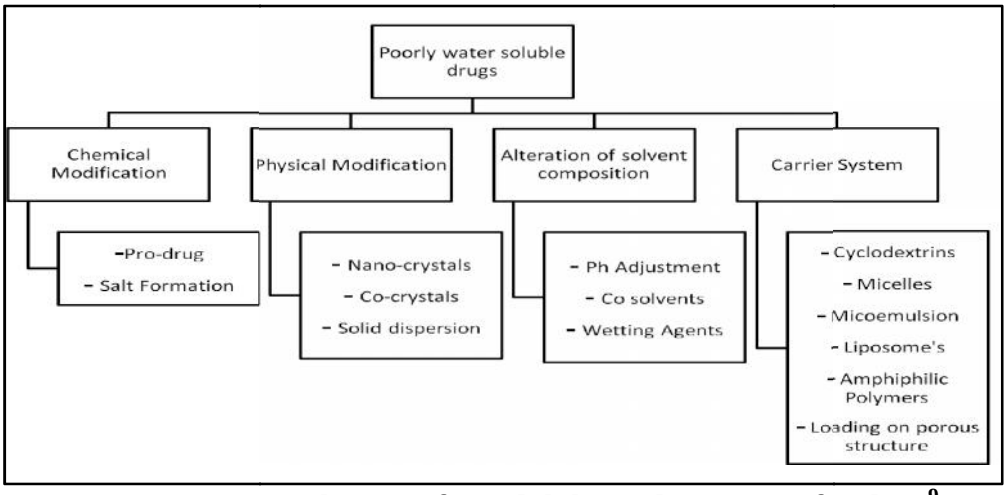

Figure1.1 Techniques for Solubility enhancement for drug[9]

1.1.2 Necessity to develop nanoparticle based drug delivery

The efficacy of drug delivery system is directly related with its particle size. Nanoparticle based drug delivery system are therefore being explored for efficient drug delivery. Because of drug nanoparticles are small in size and having the large surface area; it shows an increase in solubility and thus enhances the oral

bioavailability. Additionally because of nano size, there may be chances it easily crosses the blood-brain barrier (BBB) along with easy absorption by the tight junctions present around the endothelial cells.[15] Nanoparticles (NP); are a type of colloidal drug delivery system having particles size range from 1 to 1000 nm in diameter. Traditional way is not always suitable for manufacturing of oral or injectable formulation of each drug product. Products contain nucleic acid or proteins require a more inventive type of carrier system for protection from unwanted degradation and to improve their efficacy.[10,11] Generally, nanoparticles are made from synthetic or natural polymers that have received more attention because of (1) improvement in bioavailability by increasing solubility, (2) enhance the resistance time of drugs in systemic circulation (3) targeting the drug molecules at particular sites in body.[12]

Top-down and bottom-up methods are two types of approaches used in nano-synthesis. The top-down approach stand with larger objects and reduced their lateral dimension in order to achieve nano-scale material. The bottom-up approach produces a nanosize structure and assembled around the nano nuclease to get the larger nanostructure.

Bottom-up approaches include the miniaturization of materials elements with additional self aggregation process that leads to development of nanostructures. At the time of self-aggregation process, the physical forces at nanoscale are utilized to merge fundamental units into larger structures. Top-down approaches use larger initial structures; as it may be externally-controlled in the development of nanostructures. The bottom up approach is preferred due to proper control and uses block co-polymer, colloids amphiphilic and liquid crystals.[13]

1.1.3 Solubility problem for antiretroviral Drugs

The drugs having poor oral bioavailability fail to reach minimum effective concentration (MEC) in pharmacological action. Most of the antiretroviral drugs have poor solubility and bioavailability and usually require high amount of dose to achieve minimum effective concentration. The best example of this is antiretroviral drug Saquinavir. It is highly potent HIV protease inhibitor and its MEC is 100ng/ml.[14] but to reach up to this concentration 1200mg/per day oral dose is require. (600mg/tablet). Because of extensive hepatic first-pass metabolism done Saquinavir shows poor oral bioavailability. High dose of such drugs shows gastrointestinal side effects like nausea, dyspepsia, diarrhea, vomiting, abdominal

discomfort, flatulence and abdominal pain.[15] In general, high drug dose affect/harmful to the human and show some side effects and most of these drugs are very costly, high dose of such drugs is eventually shows its wastage that's not resonable.[16]

1.1.4 Nanoparticles and Antiretroviral Drugs

Acquired immunodeficiency syndrome (AIDS), is a disease where the immunue system of the patient is gradually destroyed.[17] earlier when AIDS treatment was first introduced, the patients were often prescribed 10–15 tablets per day. From the past two decades, researches have made it possible to reduce the tablet counts to make it just a few in a day.[18] Research shows that antiretroviral drugs more effectively reach intracellular as well as cross blood-brain barrier by developing polymeric nanoparticles of these drugs.[19] This technique also used in adjunct with vaccinations to prevent HIV infections.[20]. For aggressively opposing the HIV evolution a combination of two or three antiretroviral drugs of the various class is used, this known as highly effective antiretroviral therapy (HAART).[21] Nanotechnology has offered a very important role in drug delivery of anti-HIV drugs and increasing patient compliance.[22] Anti-HIV drugs should pass through the mucosal epithelial barrier while taken non-intravenous routes. Lymphoid tissues are chief places for HIV to thrive and infect. Number of research paper demonstrates that antiretroviral drugs loaded into nanoparticles are able to target macrophages and monocytes in vitro.[23, 24] The researchers have used poly(lactic-co-glycolic acid) (PLGA) to develop nanoparticles that entrapped three different anti-HIV drugs like efavirenz lopinavir and ritonavir.[25] The nanoparticle system exhibited extended drug release for over 4 weeks, whereas pure drugs were eliminated within 48 h. Central nervous system (CNS) is an additional site for HIV to inoculate and flourish resulting in serious HIV associated neurocognitive disorder (HAND).[26] Nanoparticles are known to be able to permeate BBB by phagocytosis or endocytosis and many articles occurs to reveals successful delivery of antiretroviral drugs.[27]

Need of Novel Formulation

The pharmaceutical companies are aspiring for development of new formulations for current drugs.[28] So as to provide benefit to the patients and boost the pharma market. It will also encourage to developing more potent drug delivery system. Another advantage of nanotechnology-based drug delivery system is it provide new opportunity to those drugs that could not be commercialized after designing and

development because of its solubility and the bioavailability issue and also those having side effects and high toxicity.[29,30] Inspite of various formulations available in the market using nanoparticles approaches newer approaches are continuously being explored to increase the solubility of poorly water soluble active substances. Another newly introduced path of nanotechnology for solubility enhancement is use of mesoporous silica nanoparticles as potential drug carriers.

1.2 Mesoporous Materials

Mesoporous materials are porous material having pore size between 20-500 A^{o}. Earlier in 1970's, zeolite was considered as the first representative of mesoporous material. Then in 1990's by Kuroda research team (Japan) and Mobil Corporation (USA) the first silica-based mesoporous material was produced almost simultaneously.[31,32] The combination of silica with amphiphilic surfactant (surface active agents) molecules lead to the production of mesoporous silica materials with large pores than the zeolite.[33]

Initially in the 1990s, ordered mesoporous materials were developed for catalysis application. Then, many researchers immediately perceived their potential for different applications in different research areas like optical material, photocatalysis, thermoelectrics magnetism, fuel cells, sensors, purification adsorption and also in medical application.[34] In 2001 first time, Maria Vallet-Regi et al uses mesoporous materials in drug delivery application.[35] Afterward, well ordered silica-based mesoporous materials has been developed and used because of their ability to host different guest molecules. The guest and host interaction would take place between the existing silanol groups present on/in the surface of the host matrices and the functional groups that present in the guest molecules. The host and guest interaction would have a strong effect on the drug adsorption and release properties of the carrier matrices which led an interest from the scientists to explore their applicability as drug carriers for targeted and sustained release formulations. Silica-based mesoporous nanoparticles have different shapes, most of these materials are 2D ordered arrays of cylindrical pores and hexagonal pores parallel to each other and also 3D cubic shape pore structure with uniform pore size and it is separated by the thin wall between them.[36-37]

1.2.1 Nomenclature of Mesoporous Materials

The MSNs are named after the company or research group introducing them or according to the structural characteristics of the developed material[38] some such

MSNs are shown in Table 1.1 all these mesoporous materials (MSNs) have ordered porous structure with a pore size 20-500A^0 Shown in Table 1.2. From amongst so many varieties of MSNs, the MCM and SBA have shown sufficient potential to host the drug molecules and most of the research of using MSNs as drug carriers is centred on these two carriers.

Table1.1 Different types of Mesoporous silica material

MSNs	Full name
MSU	Michigan State University
MCM	Mobil Composite Matter
OMS	Ordered Mesoporous Silica
MCF	Meso Cellular Form
KIT	Korean Advanced Institute of Technology
SBA	Santa Barbara Amorphous
HMS	Hollow Mesoporous Silica
TUD	Technische Universiteit Delft
FSM	Folded Sheet Mesoporous
AMS	Anionic Mesoporous silica

Table1.2 According to IUPAC Classification of Porous materials

Type of material	example	Pore size (A^0)
Mesoporous (20-500A^0)	Mesoporous alumina	20-30
	Diatom bio silica	20-450
	M41S	20-100
	SBA-15	50-150
	SBA-16	50
	Pillared layered Clays	100-200

1.2.2 Advantage of MSNs and as drug carriers

MSNs are promising carrier in drug delivery due to its unique and adjustable physicochemical properties like ordered nano sized pore network, large pores volume and large surface area. The MSNs have a distinguished property of high loading capacity and ability to carry poorly water soluble drug. A silanol group containing surface easily functionalizes from both internally and externally, that can extend the circulation time of drug in body, control the drug release profile, and provide the protection of drug against the enzymatic degradation in body and minimal blood–protein binding interaction. Many researchers found MSNs are biocompatible and biodegradable, have long term physical and chemical stability. [39-44]

Mesoporous silica materials contained hundred of empty channels arranged uniformly and also having large surface area with tunable pore size and pore volume. It also have excellent thermal, chemical and mechanical stability. Therefore MSNs can be used as a versatile drug delivery carrier in immediate and controlled drug delivery system. Compared with other nanomaterials mesoporous silica nanomaterials can easily be functionalized and have high drug loading capacity that may give immediate or controlled release of drug. Different MSNs like TUD, MCM-50, MSU-H, MCM-41, HMS and SBA-15 etc. have various excellent properties that are beneficial for drug delivery purposes. The small size of the pores restricts the space of a active substance and keeps the effects of surface interactions of the active substance and the pore wall. The surface chemistry and the pore size of mesopores may be easily changed and controlled.[45]

Silanol groups present on surface of silica material are an important and beneficial property of mesoporous material. Silanol group makes the mesoporous silica surface hydrophilic. It gives additional interaction points to the adsorbed drug substance. The relative amount of (Si-O-Si) siloxane and (Si-OH) silanol groups present on the mesoporous silica material. A larger number of siloxanes groups make the surface of material more hydrophobic. Therefore, the hydrophobicity of mesoporous material can necessarily be modified by thermal treatment. Three different types of silanol groups have been identified on the surface of mesoporous material: germinal, single and hydrogen bonded.[46] These silanol groups present on surface may interact via hydrogen bonds with functional groups of drug molecules hosted inside the mesopores. Nonetheless, these weak interactions between the adsorbed drug molecule and silanol may be broken in presence of water resulting in increasing the dissolution of active drug substance. Water penetrates into the pores during drug release process resulting in dislodging of guest drug molecules from the silica nanoparticles in external environment. Generally drug loading is executed under non-polar solvents to take full advantage of drug-carrier interactions.[47-48]

1.3 Synthesis of Mesoporous Silica Materials

Different types of mesoporous material are synthesized by different synthetic pathways, In 1992, Mobil corporation succeeded in synthesizing a new family of mesoporous materials M41S. Mobile crystalline company used the surfactant as structure directing agent around which an inorganic material can deposited through sol gel method. Sol-gel process is used to create nanoparticles with controlled

mesopores structure and different surface properties. This procedure is easy and does not require a lot of excipients, making it an economical procedure. There are two main steps important in this process: condensation and hydrolysis reactions.

Sol-gel is another bottom up approach often preferred and can be defined as the hydrolysis and condensation of liquid precursor to a solid. The whole procedure is stepwise procedure: formation of stable solution of surfactant and additives, hydrolysis and condensation reaction with the precursor, gelation of the porous network, formed ordered pore network, removal of liquid from the gel network at high temperature.[49]

Mesoporous silica nanoparticles are formed by liquid crystal templating (LCT) mechanism. (Fig 1.2) A common synthesis procedure for making template for mesoporous materials can be expressed as surfactants were dissolved in the solvent followed by subsequent inclusion of silica source. After that the mixture was stirred for a specific time at a definite temperature and pH allowing it to precondensation and hydrolysis. The temperature will be augmented in order to guide the condensation process. In the subsequent step, the products are removed by filtration, washed with specific solvent and dried at oven for given time. Ultimately, the surfactant was removed by calcination process, the product was cooled at room temperature to get mesoporous silica material for the further use.

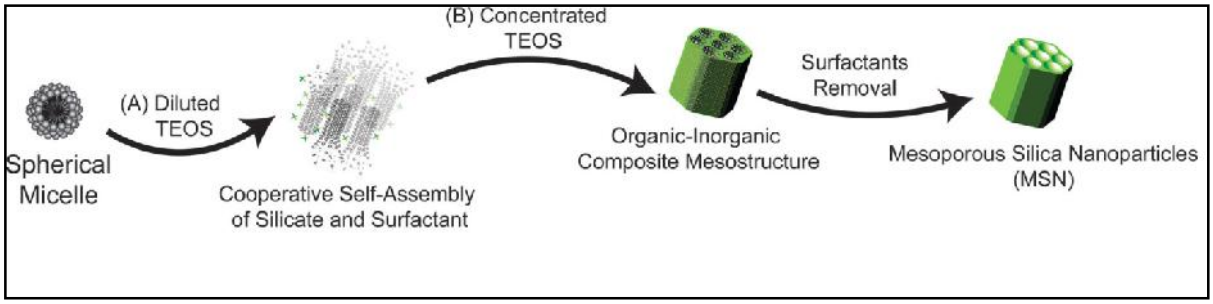

Figure1.2 Schematic representation of liquid crystal mechanism[50]

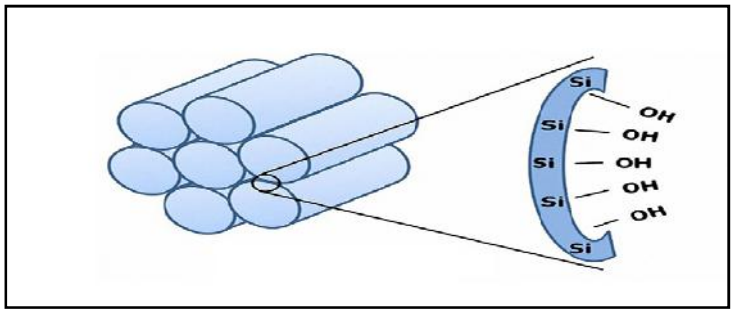

Figure1.3 Main features of the mesoporous silica materials[51]

The three main elements that form the MSNs include; silica source, surfactant used as a templating agent and a catalyst. Most commonly used silica precursors are tetraethyl orthosillicate (TEOS), tetramethoxyvinylsilane (TMVS), tetramethyl orthosillicate (TMOS), tetrakis (2-hydroxyethyl) orthosillicate (THEOS) and sodium meta-silicate. The concentration of silica precursor influenced the meso-structural ordering of the particles. High amount of silica source exhibit a disordered mesopores structure; whereas less amount was not enough to design a mesostructure.[52]

There is one common thing next to silica i.e. Surfactant. CTAB, CTAC, triblock copolymers P-123,P-127 are regularly used surfactant in synthesis of mesoporous nanoparticles. A surfactant is a structure-directing agent. When the concentration of surfactant is above critical micellar concentration (CMC), micelles are formed. At low concentration, surfactant will not lead to formation of micelles, thus the developing nanoparticles will be template deficient, and vice versa very high concentration of surfactants might consequence in a disordered mesostructure. Hence an ideal balance has to be maintained between all the reagents used. The shape and size of micelles build upon the type of surfactant, its concentration, presence of co-surfactant, pH of reaction and temperature.[53]

The Pore size of nanoparticles can be adjusted by selecting surfactant with varying lengths of the hydrophobic chain. The simple way to manage the pore size is to change the length of the surfactant micelle. Tetraalkylammonium salts are extensively used groups of surfactants for synthesised well structured mesoporous silica nanoparticles. The different length of alkyl chain of tetraalkylammonium salts affects on pore diameter of nanoparticles. Jana et al. investigate that the pore diameter of nanoparticles could expand from 1.6nm to 4.2nm by increasing chain length of surfactant from C8 to C22. This research shows the importance of surfactant chain length in pore size tailoring. [54] Pluronic surfactants are one another groups of surfactants with various molecular weights and hydrophobic segments used for synthesis of mesoporous nanoparticles. Generally, MSNs synthesized by the Pluronic surfactant micelles are larger pore size than prepared by alkyl ammonium surfactants because of increase molecular weights of hydrophobic segments.[55]

Likewise, Pore size also controlled by using auxiliary reagents such as hexane, TMB, DMHA can be added for tune the pore size.[49] In 2007 Kruk and Cao was successfully synthesised SBA-15 mesoporous nanoparticles using hexane as

auxiliary agent for adjusting the pore size in the range from 9.4 to 18.2 nm[56]It was observed that (Fig 1.4) increased amounts of surfactant in water/alcohol or water, will change the geometry of micelles. Enhancing the surfactant concentration, the rod-like micelles organize into hexagonal structure that builds the MCM-41 mesostructure. If the concentration of surfactant is elevated further then hexagonal structural phase converts into a cubic structure. And if surfactant concentrations increase at highest level, it turns into a lamellar liquid crystalline phase.[57]

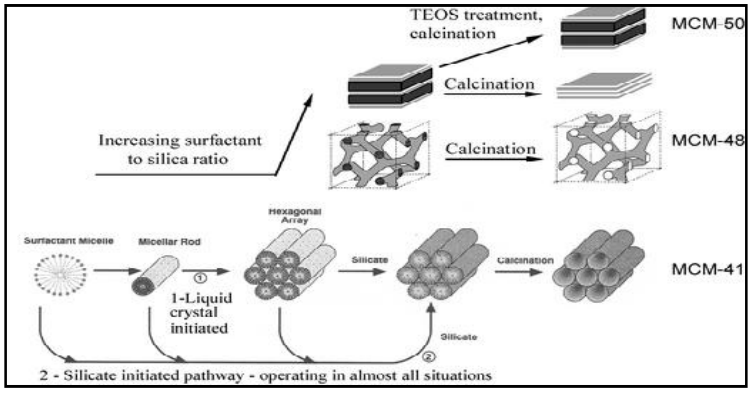

Figure1.4 The proposed LCT mechanism of formation of mesoporous silica material and effect on increasing the surfactant and silica ratio[57]

MSNs can be synthesized with different shapes like rod, cube, spheres and disk shaped.[58] The shape of the prepared MSNs is important physicochemical parameter for considering their biological application.[59-61] Yangs et al reported small changes in molar ratios of the reaction components and acidity can affect the shape of mesoporous nanoparticles.[62] Another example is Naik et al. reported condensation rate of silica is slower at lower pH, leads to lowering the local curvature energy, therefore formation of spheres and disk shape MSNs. They also showed that stirring rate in synthesis process may also change the morphology of mesoporous nanoparticle.[63]

A wide range of pore size and shapes can be obtained depending upon the nature of reactant and conditions. Carefully tuning of particle size of MSNs is a very important feature for the drug delivery. pH of the solutions plays a very important role in tuning the particle size of mesoporous materials.[62-65]

The facts are rate of hydrolysis of silane and condensation of siloxane both mainly rely upon pH of the solutions. Maintaining the pH is crucial factor for control the particle size of MSNs. Some researchers' shows rate of hydrolysis of TEOS increases with the pH increases. Therefore the rate of condensation of TEOS is not monotonic at pH 8.4.[64, 65] Then fast pH changing method was used for hydrolysis of

TEOS in synthesis of MSNs. In this method; sudde increases the pH from 2 (favorable for formation of more nuclei) to 6, gives fast silica condensation with strong electrostatic attraction between surfactants and silica may enhance the growth and formation of surfactant and silica nuclei.[66-70]

The particle size can also be controlled by addition of suitable additive agents like inorganic bases, alcohols, inorganic salts and amine. This agent changes the hydrolysis rate and condensation of silica sources. Also accelerate the reaction and thus resulting in small size of particles. Also decreases the pH value that leads to decrease in particle size.[71]

Another essential factor included in the synthesis of MSNs is reaction temperature. it has powerful impact on the particle size of mesoporous nanoparticles. When the temperature of reaction increases hydrolysis rate and polymerization of silica sources increases, thus growth of particles and construction of larger size of mesoporous nanoparticles.[72-74] Recently, Lv et al. shows when reaction temperature increases from 40 to 95°C, particle sizes of mesopores increased from 21nm to 38 nm. [75]

Once mesoporous carrier has been developed, its pores are filled with surfactant and for getting a completely empty mesoporous carrier the micelles/surfactants must be removed. Calcination is a simple method for removal of template. During this process template is completely remove out by keeping the material at 550-600^{0}c.[76] After the calcination procedure, the material cooled it at room temperature to get mesoporous silica material for the further use.

1.4 Characterization of Mesoporous silica nanoparticles

The MSNs are characterized for its pore morphology and particle size, surface and structural information using analytical techniques such as powder X-ray Diffraction (XRD), FT-IR spectroscopy, N_2-adsorption/desorption, Differential Scanning Calorimetry (DSC), Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM).

1.4.1 Powder X-ray Diffraction (PXRD)

Powder X-ray diffraction is most powerful technique to analyze the degree and nature of pore order of mesoporous material. The powder X-ray diffraction technique is based on scattering of X-rays by the electrons of atoms. If nature of the material is crystalline the sharp diffraction peaks observed in high angle XRD patterns. The same XRD can be carried out on mesoporous silica nanoparticles powder sample. The silica used in synthesis of mesoporous nanoparticles is in amorphous form so no

sharp peaks are observed in the high angle X-ray diffraction 2θ region in silica mesoporous sample. However, mesoporous silica shows reflection at the low angle X-ray diffraction 2θ region, typically in the region between 0.5°- 5° 2θ. The presence of peaks in XRD pattern is observed not because of a regular arrangement of atoms, but the systematic array of pores with very small nanometres range diameters.[77]

It define more accurately that it is an electrons that scatter the X-ray beams, it is the difference in electron density between the pore walls and the empty pore space which gives hike to these reflections. When some material is present inside the pores, this electron density variance is small and, likewise, the intensity of these low-angle diffraction peaks is reduces. In some cases, the intensity of diffraction peaks can be disappearing, even the fact that a perfectly ordered pore system is present.

1.4.2 Nitrogen Sorption Analysis

N2 adsorption/desorption analysis is a most reliable method to get information regarding the pore size, pore volume and surface area of mesoporous material. The exact information about the porous network is helpful to understand the nature of the material and its use for the future application. The surface area of porous material assists to find out such things as how solids, dissolve, react and burn with other materials. P.H. Emmett, Edward Teller and Stephen Brunauer have developed a method to calculate the surface area of the material. This method, known as Brunauer-Emmett-Teller (BET) is used for measuring the surface area by adsorption of non-polar gas like nitrogen argon, carbon dioxides).

Physical adsorption of a gas and subsequent calculation of adsorbate gas quantity allows estimation of specific surface area of MSNs. Weak forces like Vander Wal forces are mainly responsible for physical adsorption between adsorbate i.e. gas and adsorbent i.e. test powder. Liquid nitrogen is commonly employed for determination. The amount of gas adsorbed can be calculated by a continuous flow or volumetric procedure.

The data are treated according to the BET adsorption isotherm Equation 1:

$$\frac{1}{\left[V_a\left(\frac{P_0}{P}-1\right)\right]}=\frac{C-1}{V_m C}\times\frac{P}{P_0}+\frac{1}{V_m C} \quad \text{Equation 1}$$

P= Partial vapour pressure of adsorbate gas,

Po= Saturated pressure of adsorbate gas,

Va= Volume of gas adsorbed at standard pressure and temperature,

Vm= Volume of gas adsorbed at standard pressure and temperature

C= Dimensionless (BET) constant

Total Surface area (S_{BET}) of MSNs can be derived using the equation 2

$$S_{BET} = \frac{W_m N A_{cs}}{M} \quad \text{Equation 2}$$

N = Avagadro's number; M = Molecular weight of Adsorbate; Acs = Adsorbate cross sectional area

Nitrogen is most preferred because of its strong interaction and high purity with most solid materials. Commonly, interaction between solid and gaseous phases is weak; the solid material surface was cooled by using liquid nitrogen to get detectable amounts of adsorption. N2 adsorption at low temperature and with relative pressure (P/P0) range from 0.6 to 1 gives the full information about the mesoporosity of the material. Known quantity of nitrogen gas is slowly delivered into the sample cell. Generating condition of partial vaccum helps in achieving relative pressure less than atmospheric pressure. Accurate pressure transducers observe the changes in the pressure because of the adsorption process. After upon formation of adsorption layers, the sample is separated from the nitrogen atmosphere and heated to cause the adsorbed nitrogen to be released from the material and quantified. In some cases the desorption branch is different to the adsorption path, the isotherm then exhibiting a hysteresis loop. The shape of the hysteresis loop provides information about the size and the shape of the mesopores. (Fig1.6). Leslie G. Joyner, Paul P. Halenda and Elliot P. Barrett firstly developed calculation for pore volume and pore diameter. This is known as BJH calculation method. The BJH calculates a pore diameter distribution, outputs a histogram, and an average pore size with pore size distribution by gas adsorption is reported. Kelvin equation is used to determine pore size and its distribution. Kelvin equation (Equation 3) relates equilibrium vapor pressure (P) of a liquid contained in a capillary to equilibrium pressure of the same liquid over a free surface (Po) :

$$\ln \frac{P}{P_o} = \frac{-2\gamma V_{liq} \cos\theta}{rRT} \quad \text{Equation 3}$$

γ = surface tension of liquid nitrogen
θ = contact angle (usually zero for liquid N_2)
V_{liq} = the molar volume of liquid nitrogen
r = radius of pore
R = gas constant

As per the IUPAC six types of adsorption isotherms are illustrate in fig 1.5. Type I is micro porous; Type II, III and VI are nonporous or macroporous and Type IV and V are mesoporous. [78-83]

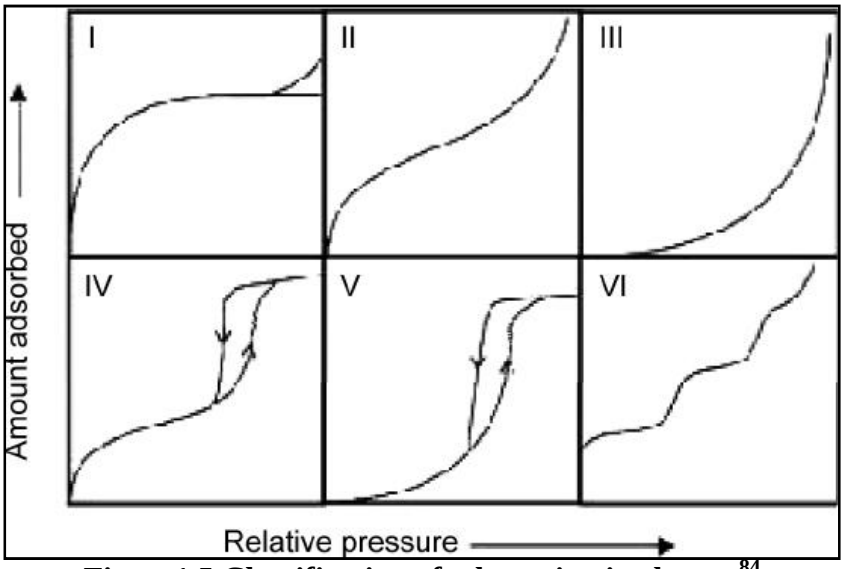

Figure1.5 Classification of adsorption isotherms[84]

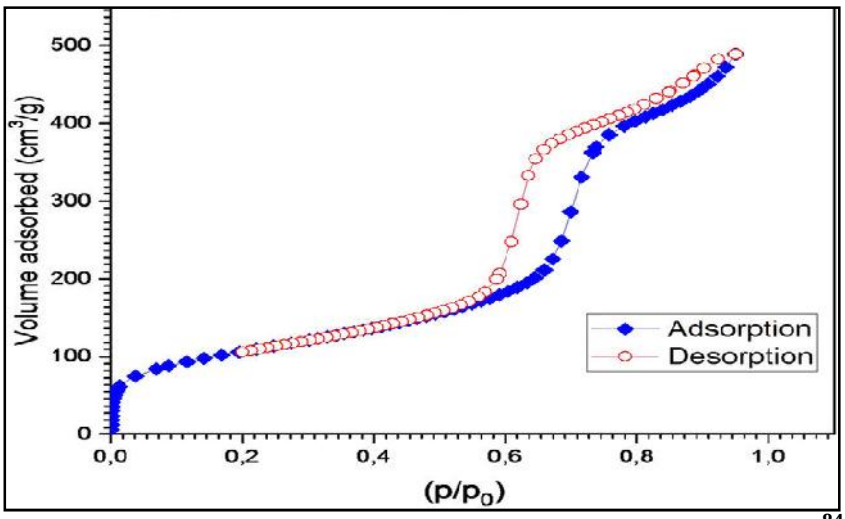

Figure1.6 Typical isotherm for a mesoporous Silica material[84]

1.4.3 Scanning Electron Microscopy (SEM)

SEM gives the primary information about the topography and morphology of solid material. Topographical features, particle agglomeration as well as shape and size of the porous materials can be observed. SEM analysis use of a low voltage electron

beam (<15 kV) and it is helpful for determine the particle morphology around 50,000x magnification. For SEM analysis of the sample, good electric conductance of the sample is needed for a clear image. Silica-based porous nanoparticles are non-conducting material and needs sputtering with thin layer of conducting material before analysis. Generally Au, C or gold can be used as conducting material for the sputtering[85]

1.4.4 Transmission Electron Microscopy (TEM)

TEM directly gives structural and particle size information of porous samples at very high resolution. TEM utilizes a high voltage beam of electrons (around 300 kV) that transmit through the porous samples to form the image of pores. The sample is dispersed in the solvent and several drops are kept on the copper grid coated with a holey carbon film. TEM image gives the strong and additional support to the powder XRD to prove the pore structure and order.[86]

1.4.5 Fourier transforms infrared spectroscopy (FTIR)

FT-IR is simple and sensitive analytical technique that gives the chemical structural information of materials. The FT-IR analysis is based on the principle that molecules have unique frequencies of internal vibrations. These frequencies occur in the infrared region of the electromagnetic spectrum. When infrared radiation passed through the sample, it will absorb IR at particular frequencies corresponding to molecular vibrational frequencies, which are measured in the infrared spectrometer. In case of silica-based porous material, the FT-IR spectra in the 400–1300 cm–1 region provides information about the structural details and silanol groups.[87, 88]

1.5 Mesoporous Materials and Drug

1.5.1 Drug Loading in Mesoporous drug carrier systems

Selection of appropriate drug loading method and loading conditions are important because drug molecule should not degrade during the loading treatment. For this proper selection of solvent and chemical surface properties of the mesoporous nanoparticles are important. In drug delivery, if fast release of drug molecules is needed, there must be a weak interaction between the drug molecules and mesoporous material. The pore size of the material must be optimized for the further diffusion of the drug molecules from the pores, that theoretically favor large pore material, but inversely small pore may be preferred, as they prevent the formation of ordered structures of the drug molecule.[89-90]

1.5.2 Drug Loading Methods

Loading Method affects the extent of drug being loaded into the mesopores, fitting of molecules into the pores and their distribution in the carrier. Various methods used for drug loading are:

1. Immersion Method: the most common method is immersion method where the mesoporous silica nanoparticles are immersed in concentrated drug solution. The drug molecules fill the pore of nanoparticles through capillary action followed by drug diffusion in mesoporous silica nanoparticle and adsorption on the pore wall. The drug loaded nanoparticles are collected by filtration. This methodology was applied for encapsulating various drugs such as paclitaxel[91], Methotrexate[92], Prednisolone[93] and Piroxicam[94] etc.

2. Incipient wetness impregnation method; a highly concentrated drug solution is used for loading of drug whose concentrations is close to its solubility. Once solvent get evaporated, the chances of recrystaliization of residual drug is high in this method. This approach has been applied for the encapsulation of various drugs such as Cinnarizine,[95] Itraconazole,[96] Glibenclamide[97] and Fenofibrate [98,99].

3. Melt method: A physical mixture of mesoporous silica nanoparticles and drug is prepared and it is ignited above the melting point of the active substance. A high chance of degradation of drug has limited its applicability as the mixture is heated above the melting point. E.g. fenofibrate[100]

4. Solvent evaporation method: A concentrated drug solution is prepared followed by addition of carrier and stirring for predefined stirring speed and time period which subjected to evaporation in rota evaporator. Indomethacin was the drug which has been successfully load by this method.[101, 102] The same approach has been applied to Atazanivir.[103]

5. In a Fluidized bed method, solvent is evaporated by spraying and heating the suspension with fluid bed machine. e.g. resveratrol[104]

Some other important factors affecting the properties of mesoporous silica material are listed below (fig 1.7):

1.5.2.1 Pore size

In drug delivery system, the pore size of silica nanoparticles is decisive factor when drug molecule has to be incorporated in to mesoporous material. Mesoporous material work as molecular sieves and determine how large drug molecule can incorporate in to the material. Generally drug loading is carried out by immersing

mesoporous material in highly concentrated drug solution when pores are filled due to capillary action method. Basically this process is based on adsorption property of mesoporous materials. The ratio of pore size and drug molecule size should ideally be >1 so the pores can easily adsorb the drug molecules. The pore size can be tuned from 2nm to several nanometers by changing the chain length of the surfactants, using polymeric structure directing agent. This tunable pore size property of mesoporous material allows hosting small or large molecule respectively.[108]

The concept of pore size as a kinetic-release controller is not only applicable for 2D hexagonal structures (MCM-41) but also applicable to 3D cubic structure mesoporous material (MCM-48). It has been shown that adsorbed ibuprofen into 3D cubic MCM-48 mesoporous material, which has a small pore size of 3.6 nm and larger pore size of 5.7nm, but with similar symmetry. The release of ibuprofen was faster from large pore size than small pore size mesoporous silica material.[105-107]

1.5.2.2 Surface area of mesoporous silica nanoparticles

Surface area of mesoporous material has the adsorptive property, an essential factor for drug loading method. Therefore surface area is most important factor for adsorption drug amount. Larger surface area gives higher loading of drug in the mesoporous materials.[106]

1.5.2.3 Pore volume of Mesoporous silica nanoparticles

Pore volume of mesoporous material is crucial factor which controls the drug loading. Pore volume of mesoporous materials vary from 0.8 cm3/g to 2.0 cm3/g. With larger pore volume, high drug amount can be absorbed into the surface of mesoporous material.[108]

1.5.2.4 Functionalization of Mesoporous Silica Nanoparticles

Surface area functionalization by using organic material is a recent achievement in the development of mesoporous silica nanoparticles.[109-113] Functionalized MSNs are mostly used for increasing/controlling drug absorption and drug release. Mesoporous silica nanoparticles exist of high amounts of silanol (Si-OH) on their surface that facilitate the covalent conjugation of a large variety of polar molecules like carboxylate groups, octadecyl, amine/phosphonate, carboxylate, polyethylene glycol, amine onto their surface. Large quantity of silanol groups present on the surface of the silica nanoparticles due to this high affinity for the polar molecules. Surface property of mesoporous silica nanoparticles very much affected by the functionalization, so this technique used to receive functional groups on the surface

of mesoporous silica particles for controlled drug delivery or targeted drug delivery.[114]

Generally mesoporous silica material are functionalizing by variety of techniques such as co-condensation, and post-synthetic grafting. Mesoporous silica nanoparticles functionality can be received by recast the silanol groups present both inside and outer surface of the pore. These groups are chemically accessible and can be facilely reacted with alkoxysilane derivatives to introduce organic functionality.

Increased drug and mesoporous silica nanoparticles surface interaction is most useful method for controlling the drug release in different media. For this, a strategy of modification with chemical groups on the surface of mesoporous silica nanoparticles which link the drug molecule through ionic bonds/ ester group is preferred which can increases/decreases the loading capacity of silica nanoparticles.[115] Some examples of functionalization of MSNs carriers used with drugs are shown in Table 1.3

Table1.3 Functionalization of MSNs and its effect on drug[116-120]

Drug Name	**Functionalize MSNs Carrier**	**Effect of functionalization**
Alendronate	NH2-SBA-15, NH2-MCM-41	Increase the loading capacity of carrier and slow release of Alendronate obtained
Ibuprofen	NH2-SBA-15	Slow release of Ibuprofen obtained
Ibuprofen	NH2-MCM-41	Slow release of Ibuprofen obtained
Gentamisin	PLGA-SiO_2	Reduce initial Burst and give sustained release
Erythromycin	TEOS-SBA-15, TMEOS-SBA-15	Sustained release of erythromycin
Aspirin	NH2-MCM-41	Sustained release of aspirin

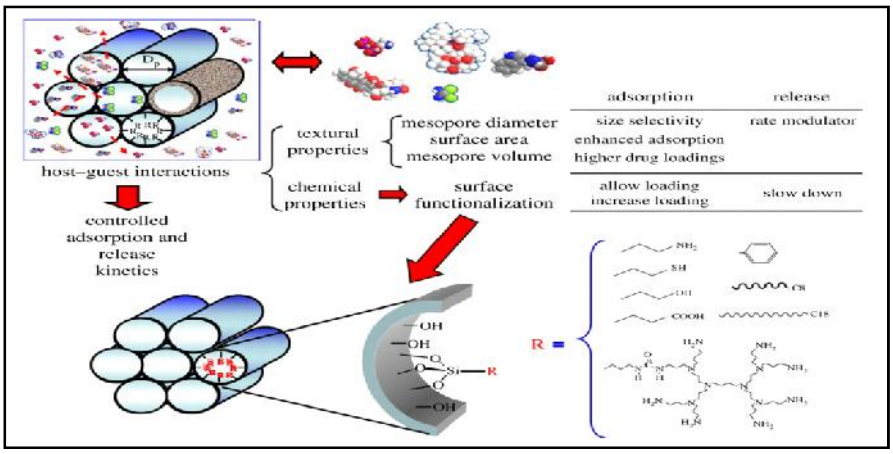

Figure1.7 Parameters that affect the loading and release rate of drug molecules in MSNs[119]

1.5.3 Confirmation of Drug Loading

Thermogravimetry (TG) and extraction are commonly employed techniques for estimation of drug loading. In extraction technique; drug released from the mesoporous nanoparticles in the medium is estimated. High performance liquid chromatography (HPLC) and UV are commonly employed analytical techniques for estimation drug and related degradants. In thermogravimetric method, the mass of the sample is estimated as a function of temperature. Weight of the entrapped drug can be estimated because the decomposition of the active pharmaceutical drug substance occurs at low temperatures compare to the carrier.

It is also necessary to check the mesostructure integrity after the drug loading procedure. Low angle powder XRD data taken prior and after drug loading gives necessary information. The same diffraction patterns are observed before and after drug loading with only slight decrease in the intensity of powder XRD peaks after the drug load in to mesopores. This confirms the drug loading as well as the no destruction of mesostructure. Transmission electron microscopy again confirms the structural intact of mesoporous materials after drug loading in mesopores.

Fourier-transform infrared (FTIR) spectroscopy taken before and after the drug loading can indicate whether drug is confined in the mesopores only or adsorbed on the surface of mesoporous material. Drug and mesoporous silica material respectively show particular absorption peaks at particular wavelength. After drug loading in mesoporous silica material, often decrease and slightly shifting of the drug peak confirms presence of drug with the mesopores.

DSC is used to confirm the drug loading in the pores of mesopores. Pure crystalline drug shows sharp endothermic peak at its fusion point. But after drug loading in

mesopores absence of endothermic fusion peak of drug indicated that drug molecules are successfully entrapped in mesopores.

N_2 adsorption/desorption analysis has been used to find out the inner morphology of the mesopores. This analysis gives the information about the surface area, pore size and pore volume of mesopores materials before and after the loading process. Generally after drug loading the pore volume and surface area of mesopores decrease that suggests the drug molecules are entrapped or inside in the mesopores. [121-125]

1.6 Mesoporous silica nanoparticles: Use as a Solubility Enhancer carrier

The mesoporous silica nanoparticles when used as a drug carrier, it can improve the solubility and dissolution rate of active substance, ultimately enhancing the oral bioavailability of drug. Augmentation in rate of dissolution of poorly water soluble drugs are based upon two main aspects: reducing the crystalline nature and increasing the surface area of drug.[126]

Changes or reducing in crystallinity of drug often leads to stability problems. Amorphous form exists in highly disordered state with higher energy and tends to revamp into a crystalline form of a lower energy state. The mesoporous silica material may avert these transformations by physically preserving the non crystalline form drug. Furthermore, loading of active into mesopores shows enhancement in permeation. It is reported that particle size of pore is an vital factor for stabilization of disordered drug. Generally the pores are adequately small, to prevent the formation of crystal structure inside the pores, therefore the loaded compound is forced to stay in the non-crystalline form and the phase transitions upon storage are averted. The carrier structure may also guard the loaded active drug substance from external environmental effects by creating steric hindrance.[127]

Dissolution of active from mesoporous nanoparticles is a little difficult process as compared to as such crystalline active substance. Type of carrier material determines the dissolution behaviour. From biodegradable carrier, dissolution occurs via decomposition of carrier and in other types of carriers; generally mass transfer from pores determines the rate of dissolution. Usually, stronger interactions are avoided where rapid dissolution is targeted. Porosity and surface modifications in certain carrier may benefit in modulating the dissolution. Porous materials having large surface pH may also change the dissolution pattern of pharmaceutical substances that ionize at different pH range. E.g. silica gel and calcium silicate are well known for

creating alkaline environment on surface in presence of adsorbed moisture so as to improve the solubility of an acidic drugs like ibuprofen.[128, 129]

The mesopores are tiny enough to provide reasonable protection of the loaded active substances; On the contrary, sufficient amount of drug can be transferred into it which is vital factor in both drug loading and dissolution. If diameters of mesopores are several times bigger than drug molecules, the crystallization inside the pores is not totally impossible.-Whenever pore size of carrier is many times bigger than drug there are chances of crystallization of active into carrier. This may lead to formation of nanosized crystals into carrier. However, solubility of these nano crystals is many times higher than crude bulk active which has ultimate advantage in absorption of active. Secondly, nano crystals also have higher stability over amorphous form.

MSNs have been vastly explored now a day for targeted drug release and probably few amalgations of porous materials and nanotechnology can be seen in future. Variegated types of carrier are available comprising mainly inorganic and polymeric types of which inorganic ones have higher stability. Siliceous materials have been deeply investigated in drug delivery of which ordered mesoporous silica have been emphasized a lot. Now a day, mesoporous silica nanoparticles have been recommended for various biomedical purposes including the oral delivery of poorly soluble pharmaceutical drug substances. Drugs of different therapeutic category were loaded in different mesoporous silica material to study their in vitro and in vivo behaviour. Some randomly selected references are shown in table 1.4

Table1.4 Different mesoporous silica material with different drugs [130-154]

Drug	Category	Carrier	%Loading	Reference
Ibuprofen	NSAID	MCM-41, SBA-15, APTES, Modified SBA-15, TUD-1,	10.5 50.4 16.9 37.2 41	130-133
Ezetamide	Antihypertensive	SBA-15	17.9	134
Atazanavir	Antiretroviral	NFM-1, AMS-6, STA-11	28.2 31.5 32.8	135
Efavirenz	Antiretroviral	MCM-41, SBA-15	---	136
Celecoxib	Non steroidal Anti-inflammatory	Carbon, SBA-15	28.5 29.5	137,138
Fenofibrate	Anticoagulant	SBA-15,	20	139

		MCM-41	20	
Furosemide	Diuretic	TCPSi, TOPSi	39 41.3	140,141
Atenolol	Anti-hypertensive	MCM-41, SBA-15	38, 42	142,143
Sodium alendronate	bisphosphonate drug	MCM-41, SBA-15	13, 17	144,145
Paclitaxel	Anticancer	MSNs	5-8	146
Gentamicin	Antibiotics	MSNs	20	147
Itraconazole	Antifungal	Syloid244, MCM-41, 3DOM, SBA-15	28.9 27 25-50 19.3	148-150
Telmisartan	Antihypertensive	MCM-41, SBA-15	27.5	151
Carbamazepine Cinnarizine Danazol Griseofulvin Ketoconazole Nifedipine Phenylbutazone	Anticholinergic Antihistamine Antiestrogen Antifungal Antifungal Antihypertensive Anti inflammatory	SBA-15	22.5 20.7 20.9 19.7 20.4 20.7 19.8	152
Cilostazol	Antiplatelete	MCM-41, MCM-48	26.4 32.5	153
Nimodipine	Antihypertensive	MCM-41, SBA-15	29, 20	154

1.7 Studies on effect on oral drug bioavailability

Mellaerts et al [155] first evaluated the in-vivo performance of Itraconazole from ordered mesoporous silica (pore size 7.3nm)(OMS) formulations and compared the results with the marketed product Sporanox and pure crystalline itraconazole. The bioavailability of the drug was boosted significantly. In rabbits, the area under the curve ($AUC_{0–24}$), determined within 24 h, from crystalline itraconazole was 521 ± 159 nM h and T_{max} was 9.8 ± 1.8 h. After itraconazole loaded silica administration, AUC0–24 increased up to 1069 ± 278 nM h with a T_{max} of 4.2 ± 1.8 h similar bioavailability was obtained after sporanox administration.

In another study (Wang et al [156]), carbamazepine bioavailability was evaluated from SBA-15 formulated as pellets. Carbamazepine was loaded in SBA-15 mesoporous silica material and pellets of Carbamazepine loaded in SBA-15 were prepared by extrusion process using proper excipients. After oral administration of pallets in beagle dogs plasma concentration profiles were determined with respect to time and

compared the obtained results with Carbamazepine commercial tablets. In PK study commercial tablet of carbamazepine shows Cmax, Tmax and AUC were 528.83±106.85 ng/mL, 65.00 ± 15.49 min and 72,580±25,283 ng h/mL respectively and carbamazepine-SBA-15 pellets shows 803.7±296.78 ng/mL, 65.00±12.25 min and 113,709±17,150 ng h/mL respectively. According to Student's t-test, whereas no significant difference in C_{max} ($P > 0.05$) was obtained, the difference in AUC was statistically significant.

Zhang et al [157] investigated impact of spherical shape mesoporous silica nanoparticles as on oral drug delivery system for enhance the bioavailability of telmisartan. They also study the effect on cellular uptake and permeability study of mesoporous silica material. The result shows higher drug release rate and the resulting higher concentration in the gastrointestinal fluids and blood as well as enhanced drug permeability through biological membranes. The mesoporous silica could significantly enhance the drug permeability in human colon carcinoma cell lines and reduce rate of P-gp mediated drug efflux Cellular uptake is strongly depend upon the concentration, time and size of mesoporous silica nanoparticles.

Telmisartan loaded mesoporous silica nanoparticles and marketed product Micardis its oral bioavailability was studied in beagle dogs. The $C_{max,}$ AUC_{0-24} of TEL-loaded MSN formulation and marketed formulation (MF) was 2315.17±150.20 ng/ml, 7432.70±1491.56 ng h/mL and 1891.65±272.81ng/ml, 4804.26±306 ng h/mL respectively. MSNs offer the potential to achieve enhanced oral bioavailability of poorly soluble drugs via improved drug dissolution rate and enhanced drug permeability. The result shows mesoporous silica nanoparticles have significant potential for novel delivery system for poorly soluble drugs

In 2016 Bukara et al[158] firstly report in-vivo pharmacokinetic study of ordered mesoporous silica material in healthy men volunteer. The study was carried out to assess the bioavailability-enhancing potential of ordered mesoporous silica in man. In this study single dose of Fenofibrate loaded ordered mesoporous silica formulation and MF Lipanthyl capsules given to 12 overnight fasted healthy volunteers respectively. Plasma concentrations of fenofibric acid, the pharmacologically active metabolite of fenofibrate, were monitored up to 96 h after giving dose. The Cmax increased by 77% and tmax reduced by 0.75 h and $AUC_{0–24h}$ increased by 54% of fenofibrate were significantly enhanced after administration of

Fenofibrate loaded ordered mesoporous silica based formulation. The results of this study serve as a proof of concept in man for this novel formulation approach.[159]

1.8 Physical and chemical stability of drug-loaded product

As compared to crystalline state amorphous form have high energy that gives greater molecular motion and thermodynamically unstable.[160] This property of amorphous state gives high solubility and higher dissolution rate compare to crystalline state. But sometimes recrystaliization of amorphous drug can be occurred spontaneously and this affects the solubility of drug. A major advantage of using MSNs as drug carrier is their strong physical and chemical stability. Mesoporous silica materials have small pore size and large surface area which gives high surface energy. Adsorption of drug molecules onto the silica allows the system to transfer to a lower free-energy state. The adsorbed molecules exist in an amorphous but physically stable state, due to a decrease in the Gibbs free energy of the drug/silica system. Re-crystallization of the drug can only occur if the thermodynamic state of mesoporous silica material is disrupted.[161] One more advantage of mesoporous silica systems is that it can stabilize the amorphous drug by size-confinement effects. Crystallization can occur automatically once a critical nucleation size is obtained. The drug molecules in the mesopores are obligate in such a way that they cannot reach this point and crystal growth is inhibited.[162]

A number of studies are available to show the long-term physical stability of mesoporous silica formulations. Ambrogi et al. analyze drug-loaded mesopores silica samples over 60 days at 75% RH and 40°C. They reported no change in physical characteristics of drugs and no recrystaliization of drug occurs. They proposed that this was due to drug molecules captured by/in the nanopores and the interaction between functional groups of drug and the silanol groups of mesoporous silica nanoparticles gives stability to the system. [163]

Van Speybroeck M reported that drug-loaded silica samples kept for 6 months at storage condition 25°C and 52% RH showed no recrystaliization of drug. And no changes in dissolution profile of drugs indicating physical and chemical stability of drug in mesoporous material. After storage pore diameter of silica material was retained, with slight decrease in surface area.[164] The drug-free silica samples had absorbed water over 3 months, but the drug-loaded samples showed little water uptake. This was attributed to the presence of hydrophobic drug molecules on the surface of the silica.

The chemical stability study of drugs that loaded in to mesoporous silica materials is also important. Some recent studies have described problematic chemical degradation of drug samples during storage under stressed conditions. [165]

1.9 Biocompatibility and Toxicity

1.9.1 Safety assessment of mesoporous materials

Biocompatibility and toxicity of MSNs has remained a major concern for researchers. Generally, with nanomaterials toxicity can be possible due to internalization of the particles by the cell and with micro-particles; toxicity is mainly due to membrane interactions. In vivo safety screening of potential formulation is performed on various animals before clinical trials to obtain information about immune response, toxicity, biodistribution, clearance, etc. The results obtained from in vitro and in vivo studies are not always consistent with each other, which is a common feature for many drug carriers of any type. This may be due to different experimental environment between in vitro and in vivo and non-standardized techniques when analyzing cytotoxicity and biocompatibility study. Silicon based mesoporous material are dissolved or degraded completely in aqueous solution in to silicic acid and it is the major form of silicon in the human body[166]. The same is valid for mesoporous silica based materials as the dissolution of silicon takes place through oxidation.[167]. For the biocompatibility of any material, chemical com-positions is important; biocompatibility is anatomically dependent and it is influenced also by the size, shape and structure of the material. Safety study of nano/micro material started with in vitro analysis per-formed on different cells of human body. To promote the practical application of mesoporous materials in drug delivery, biomedicine and other uses in medical field the long-term biocompatibility and cyto-toxicity of materials has already been systematically investigated. The NOAEL for dietary silica was determined to be 50,000 ppm (mg/L) demonstrating a huge margin of safety. In fact, this is equivalent to 2,500 mg/kg body weight/day for a rodent with the appropriately incorporated safety factors in the experimental design (100 fold). From this, the safe upper level for humans is calculated as 1,750 mg/day for a typical adult male (70 kg). In conclusion, many forms of silica exist in nature. Inhalation of crystalline silica is toxic, but consumption of water soluble silica as orthosilicic acid is not toxic even at very high levels. [168]

1.9.2 Toxicity of Mesoporous silica nanoparticles

After recognizing the application potential of mesoporous silica in drug delivery, the biocompatibility and cytotoxicity of the novel drug carrier have attracted much attention.[169]

The amorphous silica framework and surface chemistry,[170] in particular hydroxyl coverage and size and distribution of siloxane rings that comprise the framework structure can exhibit a wide range of configurations depending on processing conditions and environmental exposure.[166] Generally, there have been widely differing reports concerning the toxicity of mesoporous silica nanoparticles and amorphous silica.[171-173] However the cytotoxic effect is found to be dependant on particles size, surface chemistry and dose. A general consensus is that toxicity is associated in part with surface silanol (≡SiOH) groups which can hydrogen bond to membrane components or when dissociated to form SiO− (above the isoelectric point of silica ~pH 2-3), interact electrostatically with the positively charged tetraalkylammonium-containing phospholipids. Both processes leading to strong interactions, and possibly membranolysis. Such a process occurring at the cell surface could cause hemolysis. In support of the importance of silanols, it is known that treating the silica surface with polyvinylpyridine-N-oxide, aluminium salts, or surfactants can reduce or even switch-off hemolysis of red blood cells. Based on the high surface to volume ratio of silica nanoparticles, it might be anticipated that they would show in general higher toxicity compared to their bulk counterparts. However in the case of mesoporous silica nanoparticles, the reduced solid fraction of the mesoporous silica nanoparticles surface serves to reduce the surface area normalized hydroxyl coverage, and therefore the extent of hydrogen bonding and electrostatic interactions between the mesoporous silica nanoparticles and the cell membrane. Additionally, based on membrane curvature arguments, very small nanoparticles are less likely to disrupt and/or become internalized by the cell membrane because the membrane binding energy needed for the cell membrane to contact and fully envelop the nanoparticle scales quadratically with the NP curvature.[174, 175]

A second contribution to toxicity can be the reaction of radicals present on the silica surface with water to yield reactive oxygen species (ROS), in particular the hydroxy radical HO•, one of the most reactive species in nature. ROS can cause cell death by disrupting cell membranes or initiating programmed cell death. ROS can upregulate production of cytokines and other inflammatory mediators and can promote

mutagenesis and carcinogenesis. Although amorphous silicas can contain significant populations of strained three-membered siloxane rings. Strained siloxane rings could undergo homolytic cleavage to form surface associated radicals including the non-bridging oxygen hole center which react exothermically with water to form hydroxyl radicals. Notably, as-synthesized and surfactant-extracted mesoporous silica nanoparticles have a negligible concentration of three-membered rings. For amorphous silica nanoparticles in general, dissolution results in monosilicic acid or oligosilicic acid which have been shown to have no intrinsic toxicity. Although based on numerous recent studies it is generally observed that mesoporous silica nanoparticles have much lower toxicity than corresponding non-porous silica colloids.[176-179]

Most of studies have indicated that the safety of the mesoporous silica materials depended on the effects of their morphology, particles size, dosage concentration and surface function-alization and type of cells. Vallhov et al studied mesoporous silica nanoparticles (paricle size 270 nm) and microparticles (2.5 µm) were evaluated on human monocyte-derived dendritic cells in vitro. The smaller particles and lower concentrations possessed only minor effects as compared with the larger particles and higher concentrations, both in terms of cell viability, uptake and immune regulatory markers.[180]

Currently available information suggests that the shape of silica nanomaterials can affect their toxicity in two ways. First, the shape has an effect on the rate of its cellular uptake; and second, it can affect the extent of nano-material aggregation, altering its cytotoxic properties. Mesoporous silica nanoparticles with three different morphologies short rod-like, long rod like and spherical were studied in A375 cells at a particle concentration of 0.05 mg/ml, 0.2 mg/ml and 1 mg/ml. After 24 h, the cells treated with long rod-like nanoparticles were more susceptible to apoptosis than those cells treated with spherical nanoparticles.[181] Slowing et al did surface functionalization on mesoporous silica nanoparticles and studied extensively in the field of silica-based drug delivery. The effect of surface functionalization on the biocompatibility of mesoporous silica is also an important issue in their biological applications.[182] Mesoporous silica nanoparticles functionalized with aminosilanes by co-condensation method and studied on HeLa cell with concentration: 0.1 mg/ml, the cell growth profiles of HeLa cultures was not affected when exposed for 4 days as compared with the control sample without addition of nanoparticles. Furthermore, it

was found that organic functionalization of mesoporous silica by post-grafting can decrease the cytotoxicity of the materials.[183] Lin et al show surface functionalization of mesoporous silica with bioactive polymers, like PEG, can apparently enhance its biocompatibility. Concentrations above 200µg/ml caused a significant reduction in cell viability with unmodified MCM-41, whereas MCM-41 nanoparticles grafted with PEG did not influence the viabilities of either human endothelial or skin fibroblast cells even after 24 h expo-sure at 1000µg/ml.[184] He et al reported PEGylation was effectively improving the biocompatibility of mesoporous silica. This procedure can prevent nanoparticles from being captured by liver, spleen and lung, resulting in longer blood-circulation life-time and slower biodegradation.[185]

The toxicity is not only based on the amount or size of silica MSNs, but also on the type of cell line. Cancer cell lines (A 549, MKN-28) had a higher viability and resistance to silica MSNs than did normal cell lines (MRC-5, WS1 and CCD-966sk).[186] Similarly, a previous study showed that A549 cells were more resistant to the treatment of silica MSNs than were macrophages. The dopants, dye molecules, in general, have little effect on the toxicity of MSNs because they are isolated from the environment.[187, 188] However, if the dopants are photo sensitizers or some other specialized molecules, they can have an effect on the toxicity of the MSNs. For example, when a hydrophobic photo sensitizer, PS HPPH [2-devinyl-2- (1-hexyloxyethyl) pyropheophorbide], was doped inside organically modified silica MSNs, and the MSNs were delivered into cells (under irradiation), ROS were produced by the doped photo sensitizers and the cells were killed.[189]

Overall, silica nano-materials are low-toxicity materials, although their toxicity can be altered by surface modifications. Dose-dependent toxicity has frequently been observed in the study of nano-materials, with increasing doses of silica nano-materials invariably worsening their toxicity. Both, cell proliferation and viability were greatly hampered at higher doses observed in in-vitro and in vivo studies[187-193]

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