Synthesis and Characterization of Mesoporous SBA-15 and Modified SBA-15 for Application in Controlled Drug Delivery of Cloxacillin

A thesis submitted to the Department of Chemistry, School of Natural Sciences NUST, Islamabad, in partial fulfillment of the requirements for the degree of

Masters of Science (MS)

in

Chemistry

Submitted by: Sumia Gul

Reg No. NUST-201463663MSNS78214F

Supervisor: Dr. Habib Nasir

School of Natural Sciences (SNS)

National University of Sciences and Technology (NUST)

H-12 Campus Islamabad

2017

FORM TH-4

National University of Sciences & Technology

MS THESIS WORK

We hereby recommend that the dissertation prepared under our supervision by: SUMIA GUL, Regn No. NUST201463663MSNS78214F Titled: Synthesis of Mesoporous SBA-15 and Modified SBA-15 for Application in Controlled Drug Delivery of Cloxacillin be accepted in partial fulfillment of the requirements for the award of **MS** degree.

Examination Committee Members

1. Name: DR. M. FAHAD EHSAN Signature: _____

2. Name: DR. FAROHA LIAQAT Signature: _____

3. Name: _____ Signature: _____

External Examiner: DR. TARIQ MAHMOOD Signature: _____

Supervisor's Name: PROF. HABIB NASIR Signature: _____

_____ 18-10-17
Head of Department Date

COUNTERSINGED

Date: 18-10-17

Dean/Principal

THESIS ACCEPTANCE CERTIFICATE

Certified that final copy of MS thesis written by **Ms. Sumia Gul**, (Registration No. **NUST201463663MSNS78214F**), of **School of Natural Sciences** has been vetted by undersigned, found complete in all respects as per NUST statutes/regulations, is free of plagiarism, errors, and mistakes and is accepted as partial fulfillment for award of MS/M.Phil degree. It is further certified that necessary amendments as pointed out by GEC members and external examiner of the scholar have also been incorporated in the said thesis.

Signature: _____

Name of Supervisor: Prof. Habib Nasir

Date: 18-10-17

Signature (HoD): _____

Date: 18-10-17

Signature (Dean/Principal): _____

Date: 18-10-17

Dedication

DEDICATED TO MY BELOVED PARENTS, MY LOVING HUSBAND SOHAIB HASSAN NIAZI AND MY PIECE OF HEART,

SAAD HASSAN KHAN

Acknowledgments

All praises to **ALLAH** Almighty whose assistance helped me to accomplish this work. Foremost, I would like to express my sincere gratitude to my supervisor **Prof Dr. Habib Nasir** for the continuous support of my MS research project, for his patience, motivation, enthusiasm, and immense knowledge.

Besides my advisor, I would like to thank the rest of my guidance and examination committee **Dr. Muhammad Fahad Ehsan** and **Dr. Faroha Liaqat** for their encouragement, insightful comments, and guidance.

My sincere thanks go to **Dr. Fozia Rehman** for offering me to work in her lab and guiding me in each and every step. Without her assistance, I would not be able to complete my project on time.

I am very thankful to **Dr. Yaqoob Khan** from National Center of Physics for his support in characterization.

I would like to thank to all concerned people from School of Chemical and Materials Engineering (SCME), Institute of Environmental Science and Engineering (IESE) and COMSATS Institute of Information Technology Lahore for helping me to carry out all the characterization for my research project.

Special thanks to my best friend **Sadaf Khan** for providing me drug for the research project. I praise an enormous amount of help and love from my close friends, **Tehreema Nawaz**, **Zaib-un-Nisa** and **Saba Bashir**.

Finally, deepest and sincere gratitude to my family for their continuous love and support. I am thankful to my parents and sisters for their love and prayers. I am highly thankful to my loving husband **Sohaib Hassan** for his support, understanding and patience during my studies. I would also like to offer special thanks to my mother in law for her endless support.

Sumia Gul

Abstract

Drug delivery system has been used to control the release of drug within the body by carrying the drugs through drug matrices. SBA-15 being a mesoprous material has been effectively used for drug delivery system. The synthesized SBA-15 was functionalized with APTES by post grafting method to improve its drug release mechanism. For this purpose, Cloxacillin has been used for the very first time, as a model drug for loading on mesoporous materials.

Characterization techniques have been used for analysis includes small angle XRD, FTIR, SEM, BET for determining the surface morphology and surface area and porosity. These techniques confirmed the porosity and successful functionalization of silica. Surface functionalization caused the decrease in pore size from 5.1 nm to 4.6 nm, surface area from 534 m^2/g to 163 m^2/g and pore volume 0.036 cm^3/g to 0.015 cm^3/g and showed the slow and sustained release of cloxacillin as compared to pure silica. By measuring the concentration difference, drug loading and release was calculated. Results demonstrated that 5% of the drug has been loaded on pure SBA-15 and drug loading efficiency decreased up to 3% for modified material. The decrease in loading amount is because of the decreased in surface area and pore size as compared to pure sample. Drug Release experiments confirmed that modified material showed slow and sustained release than un-modified material.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

APTES	Amino propyl triethoxy silane
BET	Brunauer Emmett Teller
CTAB	Cetyl trimethyl ammonium bromide
EtOH	Ethanol
FTIR	Fourier transmittance infrared radiations
K_2HPO_4	Di potassium hydrogen phosphate
KH_2PO_4	Potassium di hydrogen phosphate
MCM	Mobil Composition of Matter
MSNs	Mesoporous silica nanoparticles
PBS	Phosphate buffer saline
P123	Pluronic 123
TEOS	Tetra ethyl orthosilicate
SIF	Simulated intestinal fluid
SBF	Stimulated body Fluid
SGF	Stimulated gastric fluid
SEM	Scanning Electron Microscopy
SAXS	Small angle X-Ray Scattering
SBA	Santa Barbara Amorphous
TMB	Trimethyl benzene
UV Vis	Ultra Violet Visible

Chapter 1: Introduction

"**Mold clay to form a bowl. It is the empty space which makes the bowl useful**."

Lao Tzu

This old Chinese proverb tells the importance of porous materials. These materials can be molded to any form, making pores of different sizes having various structures and then used its surface and porous voids for many applications.

Porous materials are differentiated on the basis of pore size. Materials having pore size less than 2 nm are considered as microporous materials, while, materials with pore size more than 50 nm are macroporous materials and the most interesting materials having pore size between 2-50 nm are mesoporous materials, to whom we are concerned about [1]. The shapes of pores may be spherical or cylindrical and can be transformed in various other forms.

1.1 Mesoporous materials

From porous materials, the most common are zeolites which are microscopic in nature and possess well-ordered pore size and high stability. They have many applications in the field of catalysis, sorption and membrane separation, but due to small pore size, their use is limited, so there was need to introduce new type of materials having larger pores.

Applications of mesoporous materials have been increased when the family of MCM materials by Mobil group researchers in 1992 came into existence. The main cause of interest is due to their extraordinary properties like, uniform pore size, well arranged structure, transformable pore size, greater surface area and good hydro thermal stability [2].

In addition, among all the various applications of mesoporous materials including separation technology, catalysis, sensors, hydrogen storage, separation and sorption and waste water treatment, recent studies showed that they can be used as devices used for drug delivery and as bio ceramics and regeneration in bone tissues [3]. Due to porous network of mesoporous materials, they can act as a carrier for the carrying various drug molecules because of their uniform pore size and highly

ordered nano channels. For the improvement in their application of drug delivery with controllable manner, an intensive research work is going on [4].

Two most important classes of mesoporous materials are MCM and SBA which are going to discuss in the next section. Other types of mesoporous silicas includes MSU, KIT, FDU and AMS which are synthesized by varying synthesis condition and surfactant types [5].

1.2 Chemistry of Porous Silica

To produce mesoporous silica particles with controlled surface properties and mesoporous structure a very simple and easy process is used, called sol-gel process. An inexpensive and convenient synthesis procedure is used due to its simplicity [6].

There are two main processes involved in the sol-gel process which includes hydrolysis and condensation reactions. Modification of pore structures and porous nature of mesoporous materials can be done by varying certain conditions such as pH of different buffer media, temperature, materials used in synthesis procedure, solvents of different polarity, precursor and additives. Sometimes this process is uncontrollable which leads to less ordered structures with broad pore size and distributions on the basis of molecular weight.

During polymerization, the alkoxides hydrolyses in aqueous solution and silica network is formed. After this, actual polymerization occurs by using water or alcohol via condensations.

(i) Hydrolysis

$$Si(OC_2H_5)_4 + H_2O \xrightarrow{\text{hydrolysis}} Si(OC_2H_5)_3OH + C_2H_5OH$$

(ii) Alcohol condensation

$$\equiv Si - OC_2H_5 + H - O - Si \equiv \xrightarrow{\text{Alcohol condensation}} \equiv Si - O - Si \equiv + C_2H_5OH$$

(iii) Water condensation

$$\equiv Si - O - H + H - O - Si \equiv \xrightarrow{\text{water condensation}} \equiv Si - O - Si \equiv + H_2O$$

Silanol groups are formed by hydrolysis of tetraethyl orthosilicate molecules (TEOS). An acidic or basic catalyst is required for the hydrolysis of TEOS and the rate of hydrolysis is dependent upon the concentration of acid or base. The two silanol groups or silanol group and ethoxy group condense and form siloxane bridges (Si–O–Si) forming whole silica structure [7].

1.3 Types

1.3.1 Mesoporous Silica 41 (M41S)

Kresge and coworkers of Mobil Oil Corporation discovered this family of silica materials for the first time [8]. Before their discovery, only microporous materials were known. M41S is named for many other forms of MCM (Mobil Composition of Matter) materials. Different pore structures of mesoporous silica are known, e.g. MCM-41 possess ordered pores with hexagonal structure, MCM-48 is known for cubic pore structure and MCM-50 has lamellar structure. The most commonly known member of the M41S family is the hexagonally ordered MCM-41 which was considered for drug delivery applications. These materials are synthesized under basic conditions by using cationic surfactants. M41S family is known for its ordered structure and uniform pores.

Molecular cavities of porous silica have been used in catalysis and adsorption process catalysts. Because of high surface area of porous silica and uniform pores, it is considered as advantageous in these applications.

M41S group has two most common types which includes MCM-41 and MCM-48.

1.3.2 MCM-41

MCM-41 is the mesoporous material of M41S family which has researched a lot. It contains uniform channels distributed in ordered way and has a hexagonal, amorphous structure. The greater surface area, pores having large volume and structure like honey comb makes it unique. Due to very thin pore walls, in the range of 1-1.5 nm, they show low hydrothermal and chemical stability [9].

The regular arrangement of MCM-41, with cylindrical pores forms one dimensional pore system. Their pore size is greater than zeolites and pore diameter between 2 to 6 nm. They have been used as catalysts in various chemical reactions.

1.3.3 MCM-48

MCM-48 has cubic and three dimensional structures. The ratio must be higher for surfactant to silica for the synthesis of MCM-48. Just like MCM-41, it also possesses similar pore size and surface properties. It is less studied for using complicated procedure of synthesis and less hydrothermal and chemical stability as compared to MCM-41 which possess good stability. However, as compared to one dimensional MCM-41, these materials are useful in different fields such as catalysis and separation technology [10]. The structures of both materials have been shown in the figure 1.1.

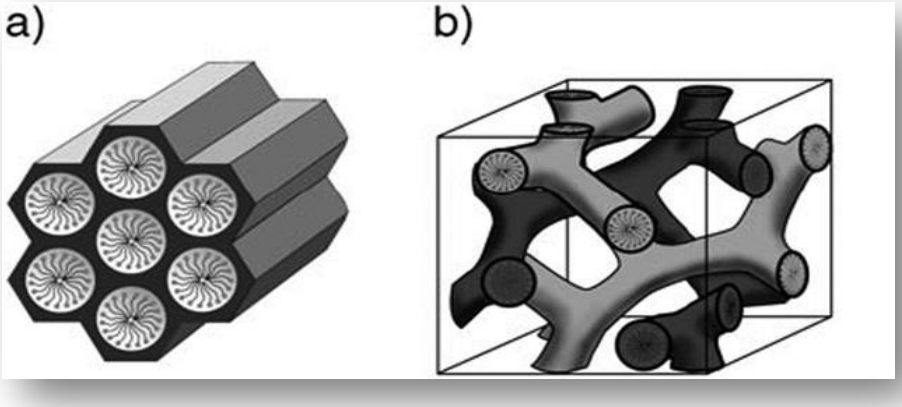

Figure 1.1. MCM-41(2D hexagonal) and MCM-48 (3 D cubic)

1.3.4 Santa Barbara Amorphous (SBA)

Due to poor wall thickness M41S materials has limited use, so there is need for new mesoporous materials with better properties. In 1998 non-ionic triblock copolymer was used to synthesize a new class of mesoporous silica materials under acidic conditions. SBA-X is abbreviated from Santa Barbara Amorphous type of materials where X is representation of a number showing the type of surfactant and particular pore shape. These were first reported in 1998 by Zhao et al. [11]. Pores having size between 2 nm up to 30 nm are considered as mesoporous and consist of many types such as SBA-11, SBA-14, SBA-15 and SBA-16. The most popular and best type is SBA-15 with desired properties.

1.3.5 SBA-15

The most common type of SBA family is SBA-15 which consists of structures having two dimensions, considered as non -crystalline and ordered pores with hexagonal shape containing micro and mesopores. In 1998, Zhao *et al.* reported it in the University of California, Santa Barbara. For synthesis process, nonionic tri-block copolymer (P123) is used as surfactant which consists of poly (ethylene glycol)-poly (propylene glycol) poly (ethylene glycol). Because of unique properties like greater surface area, pore size of large sizes and thickness of pore walls; they are considered as promising materials. Increased hydrothermal stability of SBA-15 than MCM-41 is due to thickness of pore walls. The structure of SBA-15 has been displayed in figure 1.2.

Figure 1.2. SBA-15 Structure

The tri-block copolymers P123 used as surfactant have two components, in which polyethylene oxide blocks helps in the formation of micro pores, and polypropylene oxide is responsible for mesopores. Moreover, the two blocks of surfactants affected directly the thickness of pore walls and small amount of micro pores and mesopores [12].

The removal of surfactant is necessary for the creation of pores, irrespective of the synthesis method and surfactant type. Surfactant can be removed either by solvent extraction process or calcination at high temperature. Although extraction method prevents the degradation of surfactant, but surfactant cannot be completely removed. In comparison to solvent extraction method, calcination results in complete removal of template, so this method is more accurate than first one [11]. SBA-15 synthesis chemistry has been displayed in figure 1.3.

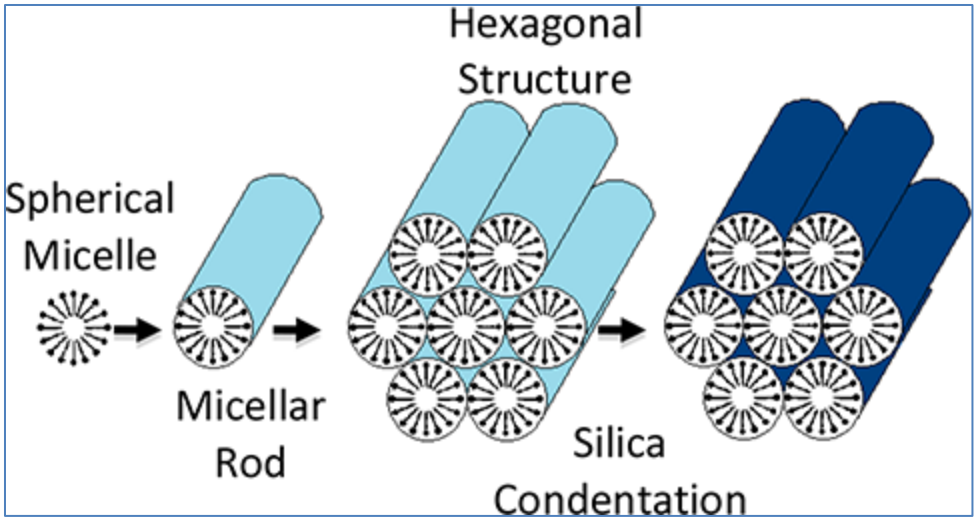

Figure 1.3. Schematic representation of SBA-15 [8]

The microporous network present around each mesopores is called corona. This network of microporous structure has role of connecting the mesopores side by side and causes to increase its surface area.

Moreover, some factors like pH, temperature, rate of stirring and strength of ionic interactions effects the morphology of particles of SBA-15. As these materials possess characteristic properties like pore size which be further tuned, ordered structure thickness of pore walls, high stability for temperature and cheap synthesis procedure, these materials are applied in various fields like, absorption, catalysis, sorption, photoluminescence, immobilization of enzymes, conductivity of proton and controlled drug delivery systems [13].

1.4 Applications

Mesoporous materials possess unique characteristics, for which these materials have been applied to many fields such as catalysis reactions as catalysts, sorption processes, separation medium, and sensing devices and delivery of many drugs. Repairing of bone defect processes has been done by them because of their biocompatible and bio erodible nature and potential host - guest applications [14]. By modifying with many organic compounds and combining with other types of materials, they are used in magnetic resonance imaging as a contrasting agent. Nowadays, materials having silica inside their structure, have been widely used as biomarkers, enzyme carriers and supporters and as sensors to identify the cause of diseases, pathogens detection and targeting of biological

compounds [15]. The highly ordered pore structures, controlling the pore size, its large surface areas and huge pore volumes and pore size of less distribution make it an ideal host for carrying many compounds including drug immobilization with slow and controlled release rate. In addition, more research is ongoing for their use as a system of delivering drug in sustainable and prolonged way [16].

1.5 Synthesis

SBA-15 can be prepared by using an easy process. Highly viscous surfactant along with silicate source is used for synthesizing particles of mesoporous silica by using sol gel method. For synthesis procedure of mesoporous silicates, the host-guest chemistry has major role. Moreover, it is affected by various factors so morphology can be changed by varying experimental parameters such as pH of different nature, total time for synthesis and temperature. Besides using other synthesis methods, with the change in surfactant type and factors like ratios of chemicals, additives, temperature, reaction time and buffers of different pH, same type of mesoporous material can be obtained [8].

1.6 Surface functionalization

As silanol groups are highly concentrated on inner and outer walls of materials, various organic and inorganic moieties are used for functionalization of ordered mesoporous silicates to get better drug loading, delivery on specific sites and to get better release mechanism. Surface functionalization controls the drug and surface affinity which controls drug release rate. To prevent premature release of drug molecules, mesoporous materials are functionalized with many types of organic and inorganic functional groups to reduce the release rate of molecular drugs [17]. The reason of reduced rate of drug release can be explained by the interactions of drug molecules with the surface of silica that occurs due to modification of mesoporous silicas with organo-functionalities that occurs by chemical and physical bonding [18].

Two types of methods are used for functionalization of surface:

1.6.1 One pot synthesis (co-condensation)

This is direct way of functionalization in which silica source is condensing with organotrialkoxysilane (R'-Si $(OR)_3$). This process occurs in one-step and helps for controlling the loading amount in a better way along with distribution of the organic groups, but sometimes less ordered mesoporous materials area produced in this way. The process can be completed in acidic,

basic and neutral environment, but conditions are dependent upon the type of the mesoporous material being synthesized. As a result, substitution of silanol group with other functional groups takes place on the walls of material [19].

1.6.2 Post grafting (silylation)

This is two step synthesis methods. First step includes the formation of silica precursor which is followed by reaction with organosilane and as a result organic groups are attached at the walls of silica by replacing the silanol groups. This method results in higher efficiency for exchanging the moieties with silanol groups. This method is good way for surface selectivity as compared to previous one [20]. Although well-ordered functionalized mesoporous materials are produced by post-grafting method but sometimes non-uniform distribution of organic groups occurs because the density of organic groups is higher on the pore channel opening and on the external wall [21].

1.6.3 Advantages and disadvantages of both methods

Although in both processes organic groups are substituted in place of silica, but, besides advantages they face some disadvantages. When we talk about cocondensation, then it is an easy process having good capacity for attachment of organosilane in a controlled way. It is observed that, concentration of functional groups is higher for one pot synthesis method while comparing to post-grafting method. Therefore, it seems that more time is required for post-grafting synthesis with less efficient product. However, post-grafting synthesis produces properties of good surface selectivity which is helpful for surface interactions between functional group and silica surface [22].

In drug delivery systems, functionalization processes are important for tuning the properties of surface. Direct synthesis procedure seems to be non- complicated but it causes certain problems for drug delivery. The solubility of drug is affected in a bad manner due to loss of phase separation from co-condensation synthesis. Moreover, due to absence of homogenous distribution of drug reproducibility problems have to be faced. Therefore using suitable solvents along with increasing steps of synthesis by applying post grafting methods these problems can be reduced [23].

Morphology of mesoporous materials can also be changed by using functionalization process. Only silanol groups are present on the surface of pure SBA-15 and these silanol groups form weak hydrogen bond interactions with the molecules of drug so functionalization is necessary for

applications of drug delivery. Therefore, SBA-15 could be modified using effective functional groups to get efficient loading and release of drugs.

1.7 Controlled drug delivery

Drug delivery is a method of administrating a drug in such a way due to which maximum therapeutic efficiency can be achieved. Controlled drug delivery systems became the need of human health. Instead of using other techniques, these systems are preferred because of their potential properties such as toxicity reduction, increased efficiency and having better patients compliance [24]. Moreover, to achieve good medication in patients, very less amount of drug is pretty enough by using controlled release systems. Controlled drug delivery systems can be carried out by two ways

One of them is **sustained delivery**, in which specific amount of drug is released at pre- determined rate into the gastrointestinal tract (GI). This process continues for some days, weeks, months or years.

The other one is **targeted delivery** in which drug is targeted to the site of action on a one-time without affecting healthy tissues of body. The release profile for both types of delivery has been shown in figure 1.4.

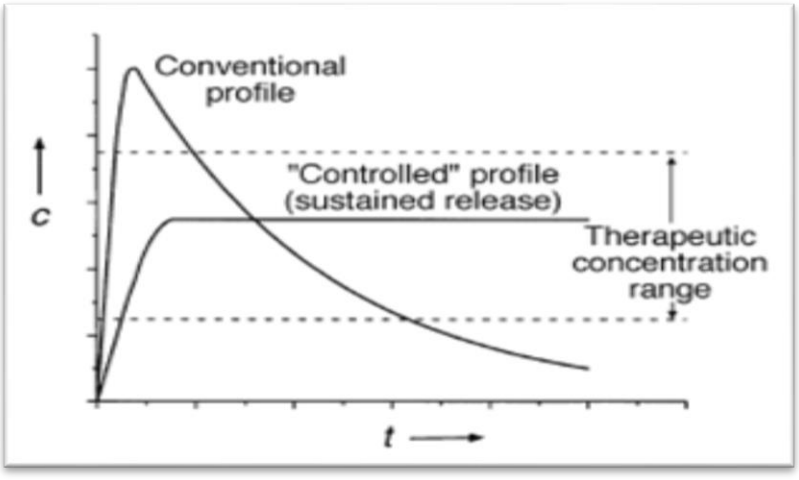

Figure 1.4: The release profile of conventional drug forms and controlled delivery system

1.7.1 Drug delivery system

Drugs are essential components for the cure of various diseases by increasing therapeutic efficiency as compared to other conventional systems. But there are few drugs which cannot be taken orally due to their hydrophobic nature, because by taking them in traditional way, activity of drug reduces before reaching the required site in the body [6]. Thus, it is not always feasible to achieve appropriate drug amount at the targeted site. Sometimes, to get good therapeutic effect, patients have to take high dose of drug because of the insolubility and non-specific nature of drug molecules [25]. In order to solve the above problems, development of active biocompatible drug carriers is needed. Drug delivery system (DDS) is a matrix or carrier which helps to deliver the drug to the targeted site. It is used for regulation the rate of drug delivery and targets the specific parts of the body to achieve maximum therapeutic effect. Hence, in this way the problems of poor solubility and less therapeutic efficiency can be solved by using nano range materials especially nanoparticles of silica are used for drug delivery systems [26].

A drug-delivery system must be ideal if it possess following characteristics

1. Less toxic and biodegradable
2. High drug loading capacity
3. Avoiding premature release and leakage of drug molecules
4. Drug carrier should have ability to minimize drugs decomposition
5. Ability to protect the drug by sealing it, so that drug can be prevented from premature release, before reaching to the target-site. It can be obtained by modifying the openings of pores with capping system, and protecting the body from harmful side effects of the drug
6. It should have ability to target the diseased cells i.e. ability to insert the small carriers inside the human body to the desired targeting site

Up to now various drug-delivery systems have been developed including polymers, micelles, polymeric nanocapsules and various nanoparticles. Mesoporous silica exhibits some promising and unique characteristics that could fulfill the desired requirements [27].

1.7.2 Drug absorption

Drug absorption is the passage of a drug from the administration site through to the plasma. The rate and efficiency of absorption is directly dependent upon the ways of administration. The main

routes of administration are orally absorption, sublingual type, rectal type, cutaneous absorption, inhalation and injection.

a. Oral administration

Most of the drugs can be taken through oral way in the liquids form, capsules, non-chewable tablets or chewable tablets. Oral route is most common way of administration because it is the most preferred and considered as safest and also not very expensive. After administration the drug first passes through the walls of intestine and moves to the liver and then transported to the targeted site via the bloodstream [28].

b. Sublingual administration

Sometimes because of instability of drug body needs a rapid response of the drug therefore by using this route oral cavity is used as absorption medium of the drug. Also, it is important to note that administered drug should not contain bad taste. In fact the drug being absorbed by sublingual method moves into the systemic circulation that lying beneath the tongue. However, drugs of high molecular weight could not be used in this method. Moreover, one main side effect is that most drugs cannot be preferred by using this method because of in complete absorption.

c. Rectal administration

This type is preferred for those drugs causing the gastric irritation and having requirement of providing a local effect. Rectum is used as absorption medium for such types of drugs and mostly passed to the liver. The drugs which show no activity at liver are given by this way.

d. Cutaneous route

For creation of local effects on skin some drugs applied to the skin and thus mostly used for the treatment of superficial skin disorders such as eczema, skin infections, viral infections of skin, continuous itching and dryness of skin.

e. Inhalation route

Administration of volatile and gaseous compounds used in anesthesia is given by using this route. The types of drugs which show activity for lungs are preferred by using inhalation route.

f. Injection (intravenous, intrathecal, intramuscular and subcutaneous)

Intravenous injection is one of the most fast and common route of administration. By using this route the uncertainties of absorption are minimized. Moreover, it is the quickest way for the delivery of certain dose of drug in a controllable manner inside the body.

Intrathecal injection is based on injecting the drug into the spinal canal. When there is need to produce rapid or local effects on the brain and spinal cord, then this method is used e.g. treatment of meningitis. Anesthetics and analgesics type drugs are mostly given by using this method.

Intramuscular or subcutaneous injections of drugs are the fastest way to cause rapid response than the oral route. The intramuscular injection is the type in which drugs is inserted by injecting the drug into the muscle tissues. In this type the drug is injected inside the tissues of skin. The intramuscular route is better than subcutaneous route for injecting the larger volumes of a drug.

1.7.3 Drug loading techniques

The aim of this research is to improve the loading of drugs on mesoporous silicon particles. Using mesoporous silicon particles as oral drug delivery vehicles is quite early stage of development. At this developmental stage, the feasibility and performance of the product can be proved by representing a model drug compound which meets all requirements. Cloxacillin is very suitable for this purpose due to its low half-life and good availability. It is the type of drug which has never been used before this for loading on mesoporous silica particles. Different methods are used for loading drugs to mesoporous silica few of them are discussed here which are employed for encapsulation of drugs in MSNs

a) **Immersion method**

In this method a solution of drug is needed to be prepared by using suitable solvent. In the next step MSNs are added to the drug solution under constantly stirring for a certain time. By using the filtration process and centrifugation technique the drug loaded MSNs are obtained. UV spectrophotometer is used to analyze the filtrate or supernatant to determine the drug loading amount. Similar approach was applied for encapsulating various drugs such as famotidine, captopril, doxorubicin, ibuprofen & celecoxib etc.

b) **Rotavap method**

In this method MSNs are added to known volume of drug solution and shaken for certain time. After shaking the solvent is slowly evaporated from the suspension using rotavapor using low pressure and increasing temperature. Drug solution is prepared in suitable solvent according to solubility and SBA-15 is added to it. Solvent was slowly evaporated from the suspension at reduced pressure using rotavapor in order to get drug loaded SBA-15. This method was used for encapsulating drug such as atazanivir [29].

c) **Fluidized bed method**

Fluidized bed drier was used to encapsulate the drug, a suspension of drug porous silica particles is sprayed. The rate of spraying and heating temperature in the fluidized bed drier helps in the loading of drug inside the pores of SBA-15 [29].

d) **Impregnation method**

This method is different from above methods because it involves soaking MSNs with drug solution with continuous stirring to obtain the dried powder resulting in impregnation of drug in pores of MSNs. Considering one such type of research in which fenofibrate was dissolved in methylene chloride at 50 mg/ml concentration and added drop wise to MCM-41. This moist mixture was continuously stirred with stirrer or spatula until dried powder was obtained. This method was used for encapsulating various drugs such as aceclofenac, carvedilol, indomethacin, and glibenclamide.

1.8 Parameters selection for drug adsorption and release

There are certain parameters which have strong effect on the loading and release behavior of drugs

a) Pore diameter

The size of mesoporous materials is determined from the diameter of pores. To confine the drug molecules inside the pores they must be smaller than the pore diameter of host. However, larger molecules would adsorb only to the external surface so pore diameter is the size selective parameter for the adsorption or loading of drug molecules. Due to larger size of bovine serum albumin (BSA) it would not be incorporated inside the porous matrix. Later, by using hydrothermal treatment pore

diameter of mesoporous SBA-15 was enlarged and BSA was successfully loaded to the porous silica and yield was increase from 15% to 27% after enlargement of pore diameter [30].

b) Surface area

Sometimes, due to lack of surface active sites drug molecules would not interact with the pore walls of silica. Hence there is direct relation of drug with the surface area. Greater the surface area, more will be retention of drug molecules and slower the release rate of drug molecules from the mesoporous matrix. In the previous study MCM-41 and SBA-15 with surface areas of 1157 and 719 m^2/g respectively were loaded with alendronate. Although SBA-15 was greater in pore diameter but less amount of alendronate was observed than MCM-41 because of the increased surface area of MCM-41.

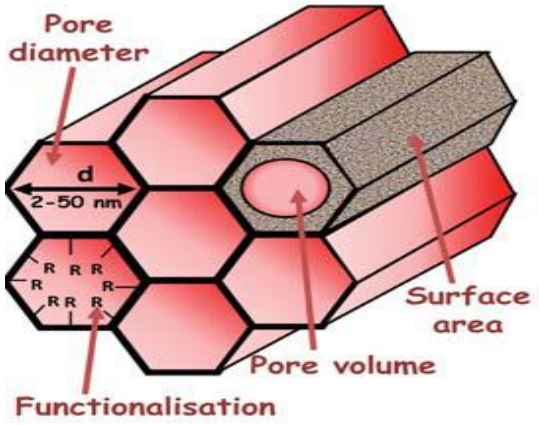

Figure 1.5 Parameters of mesoporous materials

c) Pore volume

For loading of larger molecules like proteins pore volume plays important role. Larger pore volume results in greater amount of loading. This was confirmed from the previous research experiment when BSA protein was loaded to SBA-15 and mesocellular silica foams (MSF) having pore volume 1.1 and 1.9 cm^3/g respectively. Results showed that MSF loaded 24% of the protein than 15% of SBA-15.

d) Functionalization

The silanol group present on the mesoporous walls can be easily functionalized with organic moieties. This modification result in tuning the chemical properties of the silica surface. The organic modification is selected according to the nature of drug molecules so that favorable interaction would be possible. To get stronger interaction between host and guest the surface of the host is functionalized with suitable functional group which could interact with the drug molecules. Before functionalization interaction was occurred between silanol group of host and carboxylic group of ibuprofen. After functionalizing the silanol with amine group, stronger host guest interaction was occurred which results in more loading with slow release of drug.

1.9 Antibiotics

Antibiotics also called anti-bacterial are a type of antimicrobial drugs that are used for the cure and inhibit the growth of bacteria to prevent bacterial infections. Beta lactam antibiotics are most common types of antimicrobial agents because they are usually considered safe and well tolerated and also have broad antibacterial spectrum. These antibiotics are taken in oral or parenteral forms. Working mechanism of β-lactam antibiotics includes inhibition of cell wall biosynthesis in the bacterial organism and are most commonly used group of antibiotics [31]. Study proved that most effective type of antibiotics against Gram-positive bacteria was beta lactam antibiotics but the recent studies also showed their activity against various Gram-negative organisms [32]. β - Lactam antibiotics are classified into many types including penicillin, cephalosporin, carbepenems and monobactams. In the present research the main interest is cloxacillin which is the derivative of penicillin.

1.9.1 Penicillin

Penicillin is the most common and oldest type of antimicrobial agent. It is the type of β lactam antibiotic. In 1929 Scottish scientist Alexander Fleming discovered it while its crystalline state was found in 1940. It is the first discovered natural antibiotic that was used against bacterial infections [33]. So discovery of penicillin is considered as origin of the science of antibiotics. Penicillin discovery has brought a revolution in many fields of biology, medicine and chemistry. Also it was considered as very effective chemotherapeutic agent. The half-life of penicillin is very short i.e. less than two hours which requires the increased drug dosage. These therapeutic agents are very less

absorbed having no effect on the administration route. Sensitization and allergic reactions are the only side effects of these type of antibiotics so there is need for the invention of new type of antibiotics which show resistance against all types of bacteria [34]. The first major development in the class of penicillin was the discovery of ampicillin in 1961. A broader spectrum of activity was shown by ampicillin as compared to the original penicillins. Further studies produced β-lactamase resistant penicillin, which includes three classes of flucloxacillin, dicloxacillin, and methicillin. Another development for showing the activity against gram-negative bacteria includes antipseudomonal penicillin such as carbenicillin, ticarcillin and piperacillin.

a) Natural penicillin includes

- Penicillin G
- Penicillin K
- Penicillin N
- Penicillin O
- Penicillin V

b) β-lactamase-resistant includes

- Methicillin
- Nafcillin
- Oxacillin
- Cloxacillin
- Dicloxacillin
- Flucloxacillin

c) Aminopenicillin includes

- Ampicillin
- Amoxicillin

1.9.2 Cloxacillin

Cloxacillin is an antibiotic which is effective against various bacterial infections including the inflammation in inner valves of heart, pneumonia, infections of bone and joint, and infections in skin and soft-tissues. It works by stopping the growth of bacteria. It is also used in veterinary drugs for the treatment of mastitis. It can be administered by mouth and by injection. It must be taken

orally. Dose for adults includes 250-500 mg after every six hours and maximum dosage is 6 g/day. While for children 50 mg after every 6 hours has been recommended. Over dosage may cause convulsions and paralysis. Cloxacillin is absorbed in gastro intestinal tract and well distributed in tissues [35].

Cloxacillin is found with different brand names like, cloxapen, Dry clox, and Tegopen.

Structure

Below is the structure of cloxacillin ($C_{19}H_{18}ClN_3O_5S$)

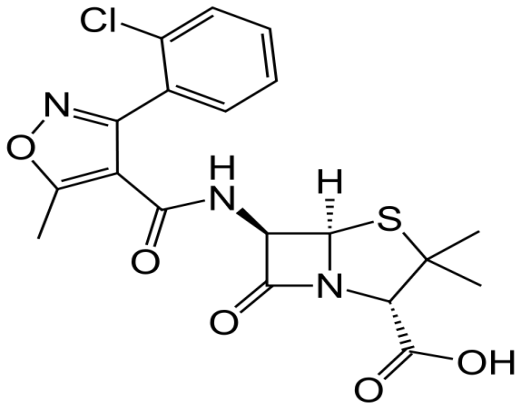

Figure 1.6. Structure of cloxacillin

Table 1.1 Cloxacillin properties

Property	Value
Molecular weight	435.88 g/mol
Bioavailability	37-90%
Half life	30 min/h
Water solubility	13.9 mg/mol
Description	Antibiotic

As can be seen cloxacillin is a small molecule and soluble in water. The solubility factor will be important for drug encapsulation and release studies.

1.9.3 Effect and mechanism

This drug has very short half-life (0.5-1.5 h) and this property forces it to be delivered in sustainable manner and for controlled drug delivery. Side effects include nausea, diarrhea, and allergic reactions [36]. It is type of penicillin which is semisynthetic in nature. Cloxacillin is effective against staphylococci producing beta-lactamase enzymes. Its larger functional group chain prevents the binding with beta lactamase. Due to weak antibacterial activity of cloxacillin than benzyl penicillin it does not cause serious toxicity except for allergic reactions. For controlling the release of drug to the target site the rate of released drug amount of the drug and the host of the drug delivery system plays important role [37]. Hence considering the physical properties, bioactivity and drug release for both inside and outside the system is important.

Chapter 2: Literature Review

Dongyuan Zhao and coworkers used triblock copolymer to synthesize highly ordered mesoporous silica structures in strong acidic media. SEM images demonstrate that mesoporous SBA-15 has a wheat like morphology with macroscopic particles that contains many rope like aggregates. Moreover, SBA-15 prepared by using triblock copolymer surfactant species at 35 ^{o}C has a pore size of 4.7 nm a pore volume of 0.56 cm^3/g and surface area of 690 m^2/g determined from BET and thickness of pore walls for hexagonal SBA-15 is about 6.4 nm. For hexagonal mesoporous SBA-15 diameter of pore can be increased up to 30 nm by the addition of organic molecules as cosolvent such as trimethylbenzene [11].

In this study **Muge sari yilmaz and coworkers** investigated that, the non-functionalized sample has capacity to absorb maximum amount of drug i.e. 27.09 wt%. The absorption causes due to higher pore volume and specific surface area. The reduction of the pore size, pore volume and specific surface area causes decreased loaded amount for organically modified sample. The release rate of drug is slower in functionalized silica samples as compared with non-functionalized samples because organic functional groups do not allow fast diffusion of fluidized drug into the cavities of mesoporous materials which results in slow and sustained release rate of the drug from the channels of mesopores [38].

Mesoporous silica materials show controlled release of drug which decreases the side effects by delivering the active agent at the targeted site as well as decrease repetitive administration of dosage. **Suman Jangra and his colleagues** prepared mesoporous silica nanoparticle SBA-15 with pore diameter (8.8 nm) and with high surface area (590 m^2/g) by the post impregnation method. When ampicillin was loaded to SBA-15 it showed that surface area decreased from 737 to 114 m^2/g and pore diameter 8.8 to 6 nm which gives the confirmation of successful loading of ampicillin to the pore channels of SBA-15. Obtained drug entrapment efficiency was 72.6% and drug loading efficiency 53%. The results of Powder XRD and electron microscopy showed the dispersion of the drug with in the channels and cavities of SBA-15 in a homogeneous manner. Moreover, the composite of SBA-15 and ampicillin appeared as highly effective for controlled released of the drug to the target site [39].

Michal Moritz and Marek studied functionalized SBA-15 which acts as carrier for anti-inflammatory drug ketoprofen. Different techniques are used for characterization like elemental

analysis, TGA, N_2 adsorption, FTIR and in vitro drug release test. After loading of drug on non-modified samples it results in decrease in BET surface area (20%) and pore volume (11%) and the micropore volume also lowered by 62%.When drug is adsorbed on modified samples there is further decrease in BET surface area (35%) pore volume (32%) and the pore size distribution is shifted towards lower value (5.7 to 5.4 nm). The results proved that all samples showed prolonged release of ketoprofen from mesoporous matrix because of slow diffusion of this drug from mesoporous and micro porous channels [40].

Mesoporous silica based materials has been considered as an attractive candidates for various applications such as bone tissue regeneration, enzymes immobilization and controlled drug delivery. **Szegedi and co researchers** did a comparative study of two types of silica materials for studying the adsorption and release behavior of selected drug ibuprofen. UV-Visible analysis showed as the non-modified SBA-15 contains high volume of pores, it exhibits higher adsorption capacity as compared to MCM-41. Modifies SBA-15 adsorbed less amount of ibuprofen and show high release as compared to MCM-41. Lower amount of adsorption is justified by blocking in the micropores in modified SBA-15 and higher release rate of SBA-15 is due to higher mesopores size as compared to MCM-41. It has been concluded that by varying the synthesis procedure and surface modification of spherical mesoporous silica releasing behavior of ibuprofen can be controlled [41].

For controlled drug delivery carriers must satisfy some properties like biocompatibility, high drug loading efficiency, zero premature release and tissue specificity. Mesoporous materials are most suitable carriers because of their good therapeutic efficiency**. Ebrahim Ahmadi and his fellows** used mesoporous SBA-15 as matrix for drug delivery in modified and non-modified forms. Synthesized SBA-15 has rope like morphology and they are modified by post grafting method. Drug loading experiments were performed by varying certain factors such as synthesis temperature, time, rate of stirring, and ratio of Ibuprofen and SBA-15. Results revealed that drug loading efficiency increased with the rise in temperature because of the increased movement of both drug molecules and silica material. While checking different ratios of drug and SBA-15 it is confirmed that maximum drug loading is achieved with drug and silica of 1:2. Comparing the modified and non-modified SBA-15 it is observed that modification not only improves the drug loading but also decreases released rate of drug [42].

In this study **Abdollah Zakeri and his colleagues** synthesized two types of mesoporous structures and then the effect of surface modification for delivery of insulin and cytotoxic behavior was

evaluated. SIF and PBS are used as media to measure the drug loading capacity and rate of release of mesoporous silica materials by using Fourier transmittance and UV–Vis spectroscopy. The results of UV Visible showed that SBA-15 particles exhibits high insulin loading capacity about 15.1% while MCM-41 and modified MCM-41 show no adsorption at all due to small pore size. High drug loading and slow release rate is due to surface modification of SBA-15. Moreover, it has been concluded that SBA-15 particles loaded with insulin showed higher cell viability while comparing to other samples. It was confirmed that modified SBA-15 show higher drug loading and sustained released of drug as well as more cell viability [43].

Doxorubicin hydrochloride (DOX) is the type of antibiotic which show high efficiency for the cure of cancer therapy. However, because of its severe side effects like inability to target the tumor cells, drug resistance and nonselective cytotoxicity, improvement of therapeutic efficiency is much needed. **Jianmei Pang and his coworkers** loaded the drug to functionalized and un-functionalized SBA-15. They evaluated in vitro cytotoxicity and cellular uptake of empty particles and drug loaded particles on two different types HeLa cells and A549 cells of cancer. Using fluorescence microscope and flow cytometry techniques it is concluded that good cellular uptake is obtained by using the drug loaded particles mediated by FA receptor. To check the vitro cytotoxicity results showed that empty FA-PEI-SBA-15 particles are effective matrices as drug carriers in drug delivery systems. Therefore, this study proved that these DOX-loaded SBA-15 particles containing FA receptors possess good therapeutic efficiency and possess great potential application for targeted cancer therapy [44].

Valeria Ambrogi and his research fellows selected furosemide for loading on mesoporous materials because of its low solubility and less bioavailability in oral administration. They used SBA-15 for the inclusion of furosemide in order to improve its solubility and release in absorptive fluids. Analytical results showed an increased rate of dissolution while comparing to both the crystalline drug and commercially available drug. Moreover it has been observed that released rate of drug loaded SBA-15 was 71% than 49% release of the commercial product. While considering the physical instability of drug, humidity and temperature are the most important factors. Physical stability studies were performed and it is analyzed that the matrix has not been reorganized in crystalline form of drug matrix [45].

The study of mesoporous materials demonstrates that to get improved drug loading and release properties surface functionalization is necessary. **Z. Bahrami and his research fellows** used thiol

functionalized SBA-15 materials for observing the adsorption and release properties of anticancer drug gemcitabine. Results of UV-Visible analysis showed that non-modified SBA-15 absorb only 75% drug while modified sample give absorption up to 13% due to the interactions of carrier and drug molecule. Moreover functionalized SBA-15 showed slow release as compared to bare SBA-15 sample. These findings describes that the functionalization of mesoporous materials provides a better platform for drug delivery due to their possibility to improve loading content and for sustained drug release [46].

Marina Martinez and coworkers demonstrated the basic analytical technique of high resolution transmission electron microscopy for studying drug loading and release mechanism from mesoporous materials. In this study by using cocondensation method SBA-15 type mesoporous silica material coated with phosphonic acid diethyl ester groups are synthesized. HRTEM studies give results of damaging of mesoporous structure due to acid treatment with HCl. This damaged structure also affects the drug release properties. The burst release of drug was up to 100% in 10 h while comparing to sustained release in 2 weeks shows the difference clearly [47].

S. W. Song, S. Kawi & K. Hidajat used post synthesis and cocondensation method for functionalizing the mesoporous SBA-15 and compared their results for drug delivery studies. It has been described that surface properties of SBA-15 type materials are important to study adsorption and release behaviors of selected drugs. Because of the ionic interaction between ibuprofen and SBA-15 synthesized by post synthesis method show sustained release of drug compared to other samples. Analytical results revealed that the most favorable candidate for the adsorption and release of bovine serum albumin are SBA-15 functionalized by one-pot synthesis. This is due to surface interactions or electrostatic and hydrophilic interaction between BSA and modified form of SBA-15 [48].

Wujun Xu and his colleagues presented a drug release system which is pH controlled by coating with hydroxypropyl methylcellulose phthalate (HPMCP) on mesoporous SBA-15 loaded with drug. In acidic pH of 1.2 drug is released in a very short time with coating of HPMCP. Similarly in stimulated gastric fluid with the increase in coating less amount of drug is released. Moreover material containing double coating of polymer show only 4% drug release in 4 hours. Whereas in stimulated intestinal fluid uncoated material have no change in release properties. This type of drug delivery system has future prospects in intestinal diseases [49].

Mesoporous materials are modified by using both direct synthesis and post grafting method. **J. Ortiz-Bustos** loaded Methylprednisolone sodium hemi succinate on modified and non-modified mesoporous materials. Results showed that organically modified material has more capacity of drug absorption than non-modified one. It was mentioned that strong electrostatic interactions between model drug and the polar amino groups are responsible for increasing the drug loading capacity. It has been also revealed that functionalization not only improves the drug loading amount but also sustained the release of drug. While checking the cell viability experimental results confirmed that organic modification do not affect it after incubation for 3 days [50].

Conventional drug delivery systems contain many drawbacks like lack of specificity on targeted tissues, large amount of doses, and adverse side effects. **Jeff Gordon and Hossein Kazemian** used microporous MIL-53(Fe) and flexible type mesoporous MIL-101 and SBA-15 materials as drug carriers for acetaminophen, progesterone and stavudine. Using incipient wetness impregnation method only 20% drugs is loaded. MIL-53(Fe) allows slow release of drug in six days in diffusion controlled process. MIL-101 show fast release due to wide pore diameter and weak interaction between drug and carrier. Due to bulk medium and mesoporous structure SBA-15 showed faster release than both materials MIL-101 and MIL-53(Fe). It has been concluded that SBA-15 has proved as useful medium for improvement of dissolution of poorly soluble drugs [51].

Development of molecular models appeared as useful technique to study the interaction between material and drug in therapeutic applications. **Antonio Doadrio and his coworkers** modified SBA-15 with APTES for the loading and release study of Chicago sky blue for the first time. Results revealed that modified SBA-15 showed sustained and slow release rate of drug than bare SBA-15 because of the strong interaction of amino group of functional group and sulfonic group of drug. However, besides the interaction of matrix and drug, molecular model describes another factor of distortion of matrix which arises because of the amino group which are responsible for the decrease of pore size and hence drug release [52].

Suman Jangra and his research fellows used azathioprine drug for loading on mesoporous SBA-15 by post impregnation method. They synthesized pure SBA-15 and loaded it with azathioprine and characterized them by using various techniques of UV–Visible spectrophotometry, TGA, SAXS, FESEM, TEM, FTIR and BET analysis to check its mesoporous nature. The decrease in

surface area (114 m^2/g) and diameter of pore (6.5 nm) confirms the successful loading of drug on mesoporous materials. UV-Vis results showed the drug entrapment efficiency was achieved up to 90.67% and loading efficiency up to 72.67%. It is concluded that controlled drug delivery system not only increases the bioavailability of drug but also decreases the administration dose as well reduces the toxicity of drug [53].

Wujun Xu and colleagues prepared mesoporous silica by using water-ethanol mixture and CTAB as structure directing agent. The amount of drug absorbed increases with the increase in surface area. For investigating the drug release properties different types of buffer media are required which includes stimulated intestinal fluid, stimulated body fluid and stimulated gastric fluid prepared at different pH. The release rate of drug is directly effects by particle size as well as dispersant of mesoporous materials. Larger and clustered structure showed the slower release of drug. Moreover, functionalization of silica surface with trimethyl silane proved to be effective for the controlled release of drug because of the hydrophobic nature of functional group [54].

By immobilizing the drug molecules within porous carriers instability problems can be avoided by interacting the drug molecules and the carrier compounds. In this work **Thanapha Numpilai and his coworkers** did a study based on comparison between the adsorption capacity and releasing behavior of poorly soluble drug ibuprofen. By increasing the size of mesopores from 5 nm to 10 nm the amount of drug loading is respectively increased from 0.74 up to 0.85 mmol/g. The dissolution rate is lowered when the particles of ibuprofen adsorbed on the outer surface of the silica based porous materials and increased dissolution is observed when the molecules of ibuprofen adsorbed inner side of mesopores [55].

Małgorzata Moritz and Michal Moritz successfully prepared 4 types of mesoporous materials including SBA-15, SBA-16, MCF and PHTS functionalized them with APTES and used them as carrier for poorly soluble drug diflunisal. Various analytical techniques including nitrogen sorption analysis, XRD, TEM, FTIR and TGA were used for the characterization of materials. It is analyzed that drug dissolution kinetics is improved for all prepared mesoporous materials at pH 4.5 while comparing to the rate of dissolution of pure diflunisal. Results revealed that modified SBA-15 and MCF showed highest adsorption for diflunisal. Obtained results confirmed that amino grafted SBA-15 and MCF are most suitable carriers for adsorption and release of diflunisal [56].

Fozia Rehman and her colleagues synthesized mesoporous SBA-15 and grafted it with hydrophobic amine bridges by stepwise synthesis. The modified material possessed porous structure in an ordered manner with a surface area of 630 m^2g^{-1}. Hydrophobic amine groups results in high loading capacity for mesoporous silica and slow and sustained release of drug in 72 hours. Results of various characterization techniques suggested that mesoporous materials act as drug delivery devices by minimizing the limitations and reduces the amount of drug dosage. Hence the porous materials and functionalizing it with suitable groups can act as a promising drug vehicle [57].

By using hydrothermal process, various types of amino modified mesoporous silica were prepared by **Yunqiang Xu and his coworkers.** Different characterization techniques were used for analysis like SAXS, SEM, TEM, FTIR, BET and X-ray photoelectron spectroscopy. Emodine was chosen as model drug for studying the loading properties and release mechanism for mesoporous materials. It is observed from the experiments that drug loading capacities are directly affected by surface areas and diameters of pores of the carrier compounds. Functionalized mesoporous compounds exhibited slow release rate than non-modified compounds. Findings suggest that modified form of SBA-15 proved to have potential to achieve sustained and prolonged release of drugs [58].

There is a need of such materials which exhibit high drug adsorption capacity and sustained release of drug in a controllable manner to avoid initial burst rate and controlled release can be achieved. **Daniel Carmona and his colleagues** synthesized two types of silica material to study the surface properties for drug release. The mesoporous surface has been functionalized using 3-mercaptopropyl trimethoxysilane (MPTS) and amino propyl dimethoxymethylsilane (APMS) to induce electrostatic charges on the surface of functional group and silica. XPS analysis revealed that drug is adsorbed inside the mesopores resulting in homogenous loading. The cubic FDU-12 proved as potential carrier with functionalized surface and sustained drug delivery properties. Such materials reduces the amount of administered drug and useful for clinical treatments [59].

The current work includes a detailed study on hard gelatin capsule for pre formulated KP-SBA-15 sample and formulated KP-SBA-15. **Ahmed Abd-Elrahman and his colleagues** loaded ketoprofen on mesoporous SBA-15 by immersion Rota vapor method. The loaded amount of sample was transformed into hard gelatin capsule. Characterization results give confirmation for successful

incorporation of drug into the porous network in an amorphous form. Moreover, mesoporous structure is not affected by loading. Release studies showed that nearly 50% of the ketoprofen was released in just first 5 min. SBA-15 mesoporous silica nanoparticle are considered as prominent drug delivery carrier to enhance the bioavailability of poorly soluble drugs [60].

This study focuses on fabrication and characterization of Mesoporous SBA-15 for the improvement in the dissolution and bioavailability of lornoxicam. It is considered as non-steroidal anti-inflammatory drug which has low solubility and high permeability. For this purpose **Dange VU and his research fellows** prepared mesoporous SBA-15 which possess large specific surface area (381.58 m^2/g) with an average particle size of about 13 nm. Results revealed that about 52% drugs have been loaded on SBA-15. Dissolution studies described that drug loaded SBA-15 exhibited 35 % higher drug release as compared to crystalline form of drug within 3 hours by using phosphate buffer medium. Hence it is concluded that hexagonal mesoporous silica can act as successful candidate for the impregnation of water insoluble drugs in drug delivery system [61].

Dae Hyun Hwang and his colleagues synthesized mesoporous SBA-15 and functionalized them with various organic groups to be used them as drug delivery carriers. Zeta potential measurements are used for confirmation of successful functionalization and it is found that the surface of the sulfonic acid-functionalized particles possessed more acidic behavior than amine and diamine functionalized particles. Moreover sulfonic acid-functionalized particles adsorbed less amount of drug than other particles. It is also found that the other organically modified particles showed slower released rate than the sulfonic acid functionalized particles. The difference in the release rate of drug is explained on the basis of different electrostatic interactions that occurs between drug and mesoporous particles surface [62].

Mesoporous materials with ordered structure possess unique features to be used as potential drug delivery carriers. **Isabel Barba and his fellow researchers** synthesized mesoporous MCM-41 and SBA-15 with different pore sizes to be considered them as drug delivery vehicles. The structural features including 3 dimensional with cubic and 2 dimensional with hexagonal pore system remains unaffected by the release kinetic profiles of drug compound. For both MCM-48 and SBA-15 drug release rate is highly affected by the pore sizes from first-order kinetic to zero-order kinetics. Moreover, to study the release mechanism of drug chemical modification with

octadecyltrimethoxysilane has also performed. By anchoring the hydrophobic chain of C-18 on silica results in weak interaction between drug and modified surface and hence results in fast released rate of drug [14].

Gang Wang and his coworkers discussed the adsorption and release properties for mesoporous materials by functionalizing with various functional groups. A comparative study for two drugs was conducted to evaluate their loading and release properties. Functionalization was carried out by three types of groups including 3-aminopropyl, 3-mercaptopropyl, vinyl and secondary amine groups to enhance release properties. UV-Vis results showed high adsorption capacity was observed for mercaptopropyl and vinyl functionalized samples for rhodamine 6-G while higher adsorption capacity for ibuprofen was shown by amine functionalized sample. As a result it is found that functionalized mesoporous materials drugs can be efficiently delivered and minimize the adverse side effects [63].

Qing-Zhou Zhai and his colleagues used hydrothermal method for preparation of mesoporous SBA-15 and then they loaded them with ramipril. The results revealed that about 90.30 mg/g of the drug have been successfully loaded. Moreover, 99.7% drug release rate is obtained from mesoporous drug composite in stimulated body fluid after 27 hours. Other results showed that in stimulated gastric fluid maximum cumulative sustained release ratio achieved 55% and in SIF ratio decreases to 34.9%.The present study suggested that SBA-15 proved as excellent carrier for the drug. It not only improves the released rate but also increases its targeted effect [64].

Material scientists are focusing on mesoporous silica preparation to be used them as carriers of drug delivery to achieve high loading capacity and sustained release of drug. **Samaneh Hashemikia and his three colleagues** synthesized amino grafted mesoporous SBA-15 and then loaded it with tetracycline hydrochloride. Drug loading was done by optimizing different techniques and using different factors like drug to silica ratio, operation time and synthesis temperature. For analysis of release mechanism of drug Higuchi equation was used and for evaluation of kinetic of drug release was done. The maximum amount of drug loading (42.3%) was achieved within 2.19 h at 33 ºC and with drug/silica ratio of 1:5. Final analysis proved that while loading of tetracycline hydrochloride on functionalized SBA-15 increases its performance by decreasing the dosing amount [65].

The development for the synthesis of mesoporous silica with full dense bulks form containing no pinholes and defects was needed which have applications in which filters and membranes are used

for gas separation and other functional materials. **Atsushi Nakahira and coworkers** prepared dense and powered form of SBA-15 by using modified form of hydrothermal method. Higher density of 1.16 to 1.19 g/cm^3 is specific for bulky materials of SBA-15 than pellet form of SBA-15 having green density of 0.68 g/cm^3. Bulk form of SBA-15 has surface area of 467 m^2/g which is considered as higher and mesopores size of 4.0 nm in diameter. The bulk form of SBA-15 showed decrease of mesopores diameter than powdered form of SBA-15 because the shrinking and densification of silicate structure occurs in SBA-15 [66].

A.L. Doadrio and other research fellows synthesized mesoporous SBA-15 to synthesize for evaluation of gentamicin delivery. The samples are loaded with gentamicin sulphate and in vitro experimental procedure was done. The release studies found release effect of 60% which is burst effect and then decreased release with slower speed was obtained. A new method containing HPLC technique was evaluated to examine the amount of gentamicin for the delivery test. The acidic or basic nature of the solution is highly affected by the drug adsorption into the pores of mesoporous compounds. The controlled release of gentamicin from SBA-15 material proved to be superior to traditional form of drug such as cream, injection and suspension having instantaneous dissolution [67].

Two types of NSAID's were incorporated on layered double hydroxide and mesoporous ordered form of silica SBA-15 by **Soledad San Roman and his colleagues**. Both types of carriers and the drugs loaded samples were characterized by using analytical techniques of XRD, SEM, TEM, FT-IR, N_2 adsorption at −196 °C and the drug release rate was also measured. Analytical results indicated that layered double hydroxide (LDH) exhibited higher drug loading than mesoporous silica. Moreover ordered mesoporous silica showed slow release rate than LDH because diffusion process takes place instead of interchanging occurred in the LDH. The release studies revealed that both materials (LDH and mesoporous silica SBA-15) can be considered for controlled drug release systems [68].

Sandra Maria and her colleagues prepared ordered form of mesoporous silicas having different pore diameters and described the adsorption of three biomolecules bovine serum albumin (BSA) cellulase enzyme and lysozyme using buffered solutions. These porous adsorbents were synthesized by using sol–gel and hydrothermal methods and characterized by X-ray diffraction, N_2 adsorption/desorption isotherms and transmission electron microscopy. Among all prepared materials the highest capacity of adsorption was observed for BSA and LYS from hydrothermally

synthesized SBA-15. Results revealed possessed larger adsorption capacity for cellulase was obtained as compared to the other materials for SBA-15 synthesized by a sol–gel process. Moreover, Cellulase enzyme have high capacity of absorption in SBA-15 synthesized by the sol–gel chemistry [69].

Sun-Young and Philip synthesized mesoporous SBA-15 for studying the controlled release properties of the allyl isothiocyanate (AITC) for the first time. Results indicated that reducing the molar ratio of silica up to 26% have no effect on cylindrical shaped pores but showed increment in the mean pore width 38% and volume in pore up to 37%. It has been observed that by decreasing the silica mole ratio higher amount of drug is adsorbed by pore filling process as a liquid phase. Release studies revealed that more than 50% of the drug has been released in first 12 hours from both silica samples. After 24 hours remaining drug has been released up to 94% for SBA-15-x and 87% for SBA-15-y. Hence a very ordered structure with large porous network appeared as an potent host for the release of drug in a controllable way [70].

The aim of research is determination of rifampin loading on silica based mesoporous particles. **Meysam Mohsenia and his colleagues** used Tetra ethyl ortho silicate and CTAB as a surfactant for the synthesis procedure. Adsorption experiments were done in order to measure the drug adsorption by using different solvents. Among all solvents Methanol has proved as most suitable for loading process and increased drug entrapment efficiency has been achieved up to 52%. Release studies showed that almost 95% drug have released in 24 hours from mesoporous particles. Hence, it has been revealed that for pulmonary drug delivery system rifampin loaded particles proved as efficient drug delivery vehicles [71].

A controlled drug-delivery system is favorable method for the controlled release of drug in which drug is released in a constant manner and steady concentration maintains in the blood stream. **Vaezeh Vavsari and her two coworkers** have given the idea for the use of SBA-15 for loading many herbal and chemical medicines to study the adsorption and release properties. Generally organic, inorganic materials and polymers are used for functionalization of mesoporous materials to make potential drug delivery systems. Results indicated that modified form of silica showed more adsorption capacity than unmodified form. This is because of the formation of strong interaction between functional group and drug molecule. It has been concluded that an efficient delivery system is developed in the form of SBA-15 for various drugs especially oral drug system [72].

Dasaa Halamovaa and other research fellows studied the release of naproxen from hexagonal periodic SBA-15 which acts as potential drug carrier. Naproxen was loaded to both modified and non-modified form of SBA-15. Loading experiments showed that modified and non-modified samples exhibited same amount of drug absorption. Comparative study revealed that drug was released up to 90% from the unfunctionalized SBA-15 in 3 days but the drug loaded modified sample showed 81% released amount. We can say that the overall rate of release for naproxen from the amine functionalized sample was lower than non-modified form because of the strong interactions of the drug with functional group. The results give the confirmation that the modification of silica surface with amino groups results in the decrease in delivery of drug compounds of from mesoporous material [73].

Study includes the determination of loading and release rate of celecoxib from porous silica particles. Celecoxib has been chosen as model drug because of its low bioavailability and hydrophobic nature. **Zeynep Seda Eren and fellow researcher** synthesized SBA-15 by hydrothermal method and functionalized them by post synthesis method. Results described very improved released rate of celecoxib than commercial drug. Released studies showed the slow release of drug from amine modified silica samples as compared to boron doped samples. Moreover the release of celecoxib is affected by pH, crystallinity of the drug and the solvent that was chosen for drug loading. It has been revealed that bioavailability of drug could be improved by the synthesized silica samples having different particles sizes, pore volumes and functionalized groups on the surface and poorly water soluble drugs can be delivered [74].

Thi Phuong Binh and coworkers synthesized SBA-15 and increased their pore size by using trimethyl benzene as a swelling agent. They also modified the silica by APTES by post synthesis method for effective separation of protein i.e. Bovine serum albumin protein abbreviated as BSA. Samples were characterized by XRD for identification of crystalline structure of sample, FTIR, SEM and TEM for analysis of morphology and chemical nature. It has been observed that adsorption capacity has been increased with the increase in temperature. Results indicated that modified form of SBA-15 adsorbed less amount of protein than APTES modified sample. Moreover, the release of protein was higher for the amine-functionalized sample than the non-functionalized one at neutral pH [75].

Yanzhuo Zhang used mesoporous silica particles for loading the poorly soluble drug, telmisartan for increasing solubility rate and increasing the drug loading amount. Pore sizes and functionalization of silica particles were examined by XRD, FTIR, TGA, SEM, TEM and differential scanning calorimeter. The total pore volume and pore diameter are responsible for drug loading amount. About 60% of the drug has been loaded on silica particles. Results showed that because of ionic interactions released rate of drug from modified samples exhibited controlled release than unmodified ones. It has been observed that the rate of dissolution for drug is highly dependent on the chemical moieties present on the surface of mesoporous particles. Study opened the new gate for increasing the dissolution behavior and sustained release of less soluble drugs [76].

Mi Mi Wan and his colleagues did an extraordinary study for loading both hydrophilic and hydrophobic types of drugs on mesoporous particles. Ibuprofen and heparin has been encapsulated to mesoporous SBA-15 in a one pot synthesis method. The advantage of in situ loading is that it reduces time as well as increasing the loading amount. Loading experiments revealed that drug has been release with a slow and sustained rate. Finding suggested that in situ drug loading procedure is environment friendly because less temperature has used and no toxic organic compounds were used for synthesis. Thus in situ drug loading and delivery system is potent for increasing the drug carrying capacity and sustained their release [77].

Research on mesoporous materials for applications in biomedical field have increased in recent years. **Maria Vallet Regi and her coworkers** described that porous materials have very promising role in bone tissue regeneration because of their use in bio ceramics. The pore size of mesoporous materials is directly related to drug adsorbed. Besides, the pore size allows the drug for entering inside the pore matrix. Similarly the higher the amount of drug adsorbed is directly dependent upon the high surface area. Moreover, the drug release can be controlled by functionalizing the surface with suitable groups to increase the drug interaction with group. It has been observed that new mesoporous carriers act as host they can protect and transfer the drug to the targeted site and could play an essential role in the new therapies [78].

In recent years due to unique properties of mesoporous materials with uniform pore size, higher surface area and more volume inside the pores proved as useful carriers for drug delivery in

controlled way. **Shaobin Wang** described as mesoporous material act as potential carrier for higher loading and release of drug with controllable manner. The release of drug is dependent upon pore size and presence of functional groups on the silica walls. Functionalization on the silica surface with various groups can change electrostatic, hydrophobic and hydrophilic forces and interactions based on adhesions are involved for both matrix and drug and causes to induce the sustained rate for drug loading and release. Moreover, mesoporous material with Biocompatible and bioactive nature would be suitable for the cellular growth and bone regeneration [79].

Chapter 3: Experimental

3.1 Materials

Table 1.1 List of materials with label and molecular weight

Chemical	Label	Molecular weight (g/mol)
P123	Sigma-Aldich	5800
HCl	Sigma-Aldrich	36.46
TEOS	Merck	208.33
Toluene	Sigma-Aldrich	92.14
Ethanol	Sigma-Aldrich	221.37
Methanol	Sigma-Aldrich	46.07
APTES	Merck	32.04
H_2O	Distilled	18.00
PBS	Merck	-

Experimental work includes the following steps

1) Synthesis of SBA-15

2) Functionalization of silica by APTES

3) Drug loading on both functionalized and non-functionalized material

4) Release of drug from both materials in phosphate buffer

3.2 SBA-15 synthesis

Synthesis was completed according to the method used by Zhao *et al.* in 1998.

For synthesis process 4 g of pluronic P123 (which is used as surfactant) was dissolved in 30 g of deionized water. After stirring for 1 hour, 120 g of HCl was added to the solution and stirred for 3

hours. Then 8.5 g of tetra ethyl orthosilcate (TEOS) has been added drop by drop. Stirring was done for 24 hours at 40 ºC for the above reaction mixture [11].

After 24 hours solution was put in Teflon autoclave and aged for 24 hours at static condition in oven. After that solution was filtered & cooled and washed with plenty of water and dried at room temperature. For the removal of surfactant, calcination was done for 6 hours by slowly increasing the temperature up to 600 ºC very slowly at the rate of 2 ºC/min. Approximately synthesis process was completed in 6 days [42]. Following figure describes the complete synthesis procedure of pure SBA-15.

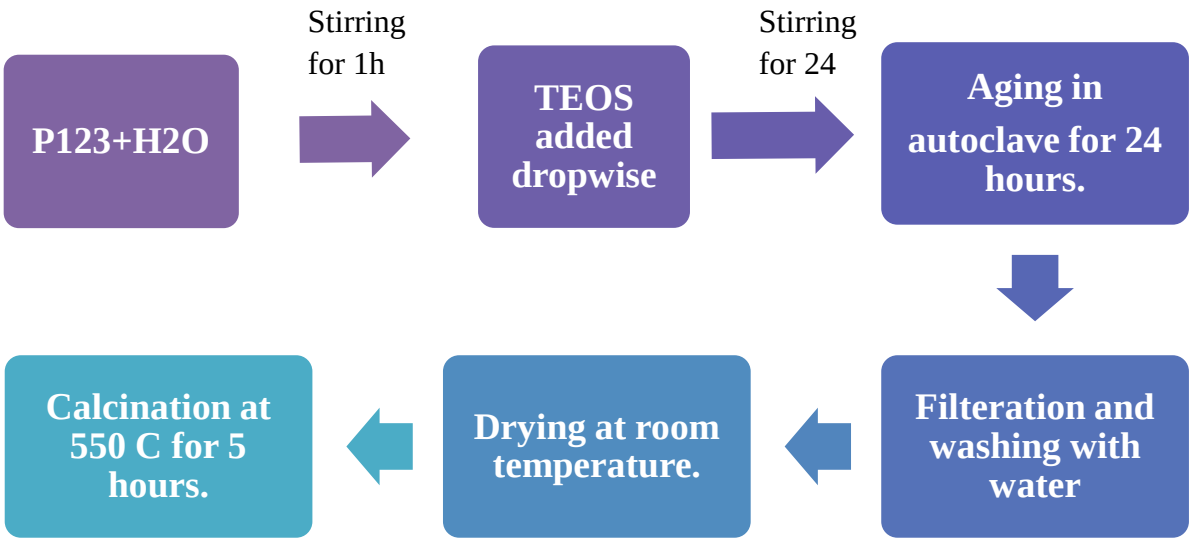

Figure 3.1 Synthesis procedure of SBA-15

However micro porosity pore size and particle morphology can be changed by changing the synthesis condition i.e. swelling agent addition use of CTAB and temperature [80].

3.3 Functionalization of SBA-15

For successful functionalization, SBA-15 was vacuum dried at 100ºC for 12 hours and sealed under vacuum to avoid the absorption of water. 2g of calcined SBA-15 was taken and dissolved in 30 mL of toluene and stirred it for 1 hour. Then 4 mmol of APTES was added to the mixture and refluxed for 24 hours at 100ºC under nitrogen environment. After cooling solution was filtered and washed with toluene, methanol and water. Finally, the product was obtained by drying it in vacuum for 24 hours at 80ºC [23].

Figure 3.2: Reaction for Functionalization of SBA-15

Actually by replacing the surface silanol groups with any functional groups by using alkoxysilanes surface functionalization is done. The silanol groups create weak interaction with the drug molecules so to improve the matrix drug interaction surface functionalization has been done [5].

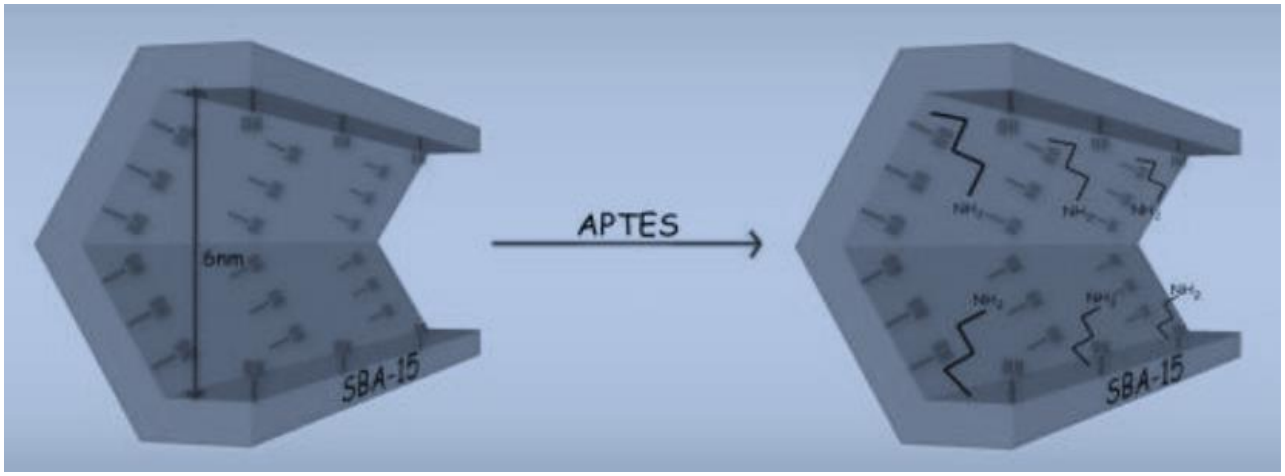

Figure 3.3 schematic representation of amine functionalization [6]

3.4 Cloxacillin Loading

Figure 3.4 Cloxacillin sodium

Cloxacillin sodium used during the experiment was kind gift of AMSON pharmaceutical company, ISLAMABD. Cloxacillin solution was prepared by taking 500 mg of cloxacillin in 100 mL of deionized water. Absorption was checked by using UV-Vis spectrophotometer. In the next step this water drug solution was mixed both in SBA-15 and modified SBA-15 and stirred for 72 hours at room temperature. After 3 days solution of cloxacillin and SBA-15 was filtered and washed with water to remove the drug adsorption on the surface. Filtrate was taken and further diluted with water and loading was checked by taking absorption through UV-Vis spectrophotometer. Wavelength was adjusted between 200-800 nm and absorption was monitored after 24 h, 48 h and 72 hours. The amount of cloxacillin was calculated by taking absorption at 275 nm. Residue left after filtration was dried at 60ºC in vacuum oven. This product is named as drug loaded SBA-15 which will be used for drug release studies. Hence, the loading of cloxacillin was completed in 6 – 7 days.

Percentage loading for both unmodified and modified samples has been calculated by the following formula

$$\textbf{Weight\%} = \boldsymbol{A12} \times \frac{\boldsymbol{100}}{\boldsymbol{A1}} \qquad (1)$$

Where A1 is the initial absorbance before drug loading and A12 is the difference in absorption before and after drug loading.

3.5 Calibration Curve for Dug Release Studies

Before doing drug release studies standard solutions of different concentrations are required for the formation of calibration curve. For this purpose nine standard solutions have been prepared by taking specific amount of drug in solvent. They include 1, 2,3,4,5,6,7,8, and 9 ppm solutions. UV analysis was done by taking the absorption of each solution at 275 nm which is the specific for Cloxacillin.

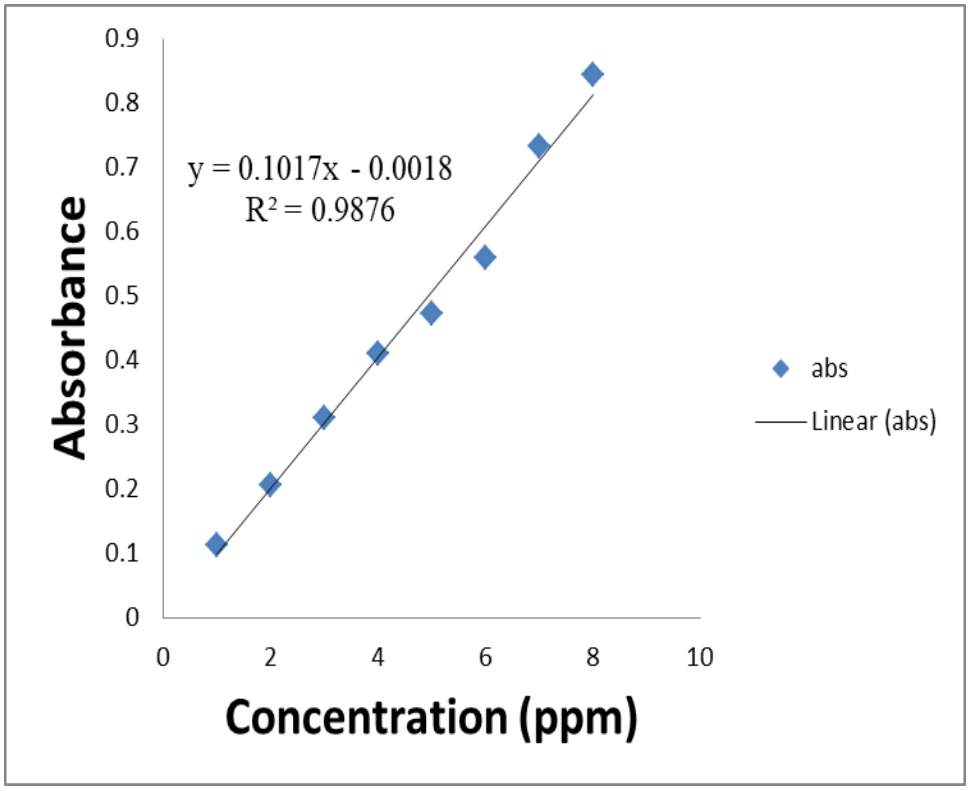

Figure 3.5 Calibration Curve for drug release

3.6 Cloxacillin Release

For released studies phosphate buffer saline (PBS) preparation is necessary. So PBS is prepared first before doing other experiments.

3.6.1 Preparation of Phosphate buffer saline (PBS)

Phosphate buffer was used as release media for loaded amount of drug. So, phosphate buffer of pH 7.4 was prepared by adding by using KH_2PO_4, K_2HPO_4, KCl, and NaCl in distilled water. The pH was adjusted to 7.4 by using HCl and NaOH [7].

Table 3.2 Required chemicals for preparation of PBS

Chemicals	Weight (g)	MW (g/mol)
KCl	8.0	74.55
NaCl	0.20	58.44
KH_2PO_4	1.44	136.02
K_2HPO_4	0.24	178.30

3.6.2 Release studies

In the next step 30 mg of drug loaded SBA-15 was taken and added in 10 mL of phosphate buffer. The mixture was kept on stirring at 37ºC in order to maintain the temperature of human body.By changing the concentration within 28 hours at 275 nm the amount of released drug was calculated. During all measurements distilled water was used as reference. The measurements were taken after 1h, 2h, 3h, 4h, 5h, 6h, 24h and 28h [8]. All the experiments were repeated three times.

Chapter 4: Results and Discussion

4.1 FTIR Results

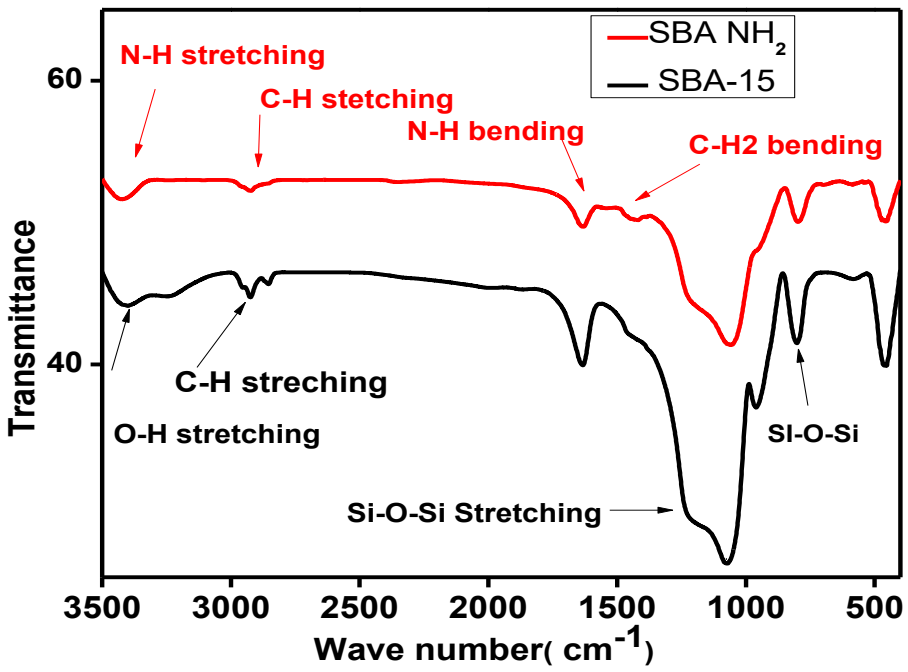

Figure 4.1 FTIR spectra of pure (SBA-15) and modified material (SBA-NH_2)

The band between 3200-3400 cm^{-1} is due to Si-OH and OH group of water. Small peaks in the range of 2800-2900 cm^{-1} is attributed to CH stretching. The broader band can be seen at around 1100 cm^{-1} which gives the indication of Si–O–Si asymmetric stretching vibration and the peak at around 800 cm^{-1} is representation of symmetric stretching vibration. The sharp peak in the range of 1500-1600 cm^{-1} was due to C-C stretching. In addition the pure and functionalized samples possess weaker peaks in the range of 400-450 cm^{-1} which occurs because of the deformation modes of the Si–O–Si. Also, the Si-OH bending are assigned for the peak at around 950 cm^{-1} [81]. In functionalized SBA-15 propyl & methyl groups show C–H stretching bands at 2895 cm^{-1} and 2980 cm^{-1}. Similarly the sharp band at 1405 cm^{-1} and 1456 cm^{-1} representing the –(CH_2) bending vibration. These bands gives indication of amino propyl groups on the silica surface [82]. In addition broad band at 3380- 3310 cm^{-1} is labelled as N-H stretching with an asymmetric NH_2

bending at 1600 cm^{-1}. Sometimes these absorption bands cannot be distinguished at this time asymmetric NH_2 bending vibrations helps in identification of presence of an amine group [62].

FTIR spectrum of cloxacillin has been shown in the following figure.

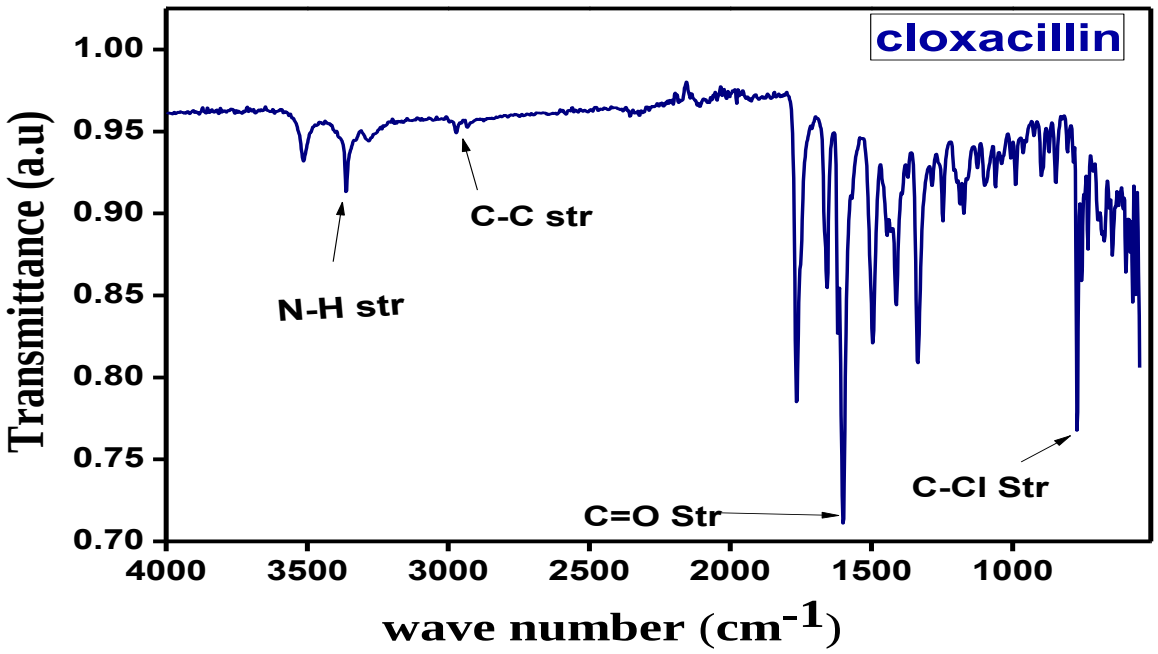

Figure 4.2 FTIR spectrum of Cloxacillin sodium

4.2 Small angle XRD Results

Small angle XRD is a useful technique used for studying the periodicity of ordered structure at atomic scale. During X-ray diffraction x-rays shows scattering phenomenon after interaction with the material. The d-spacing values can be calculated by using Bragg's law

$$\lambda = 2d_{hkl}\sin\theta \qquad (2)$$

Where λ is the wavelength, d_{hkl} is the distance between the planes and θ is the angle of incoming light.

The unit cell parameter a_o can be calculated by the following equation

$$a_o = 2d100/\sqrt{3} \qquad (3)$$

The d-spacing were calculated from the diffraction peaks and they are nearly same for both samples. Moreover, functionalized SBA-15 showed that a_o value decreased slightly than non-modified form. Following table representing the d-spacing and unit cell parameter values for both samples.

Table 4.1 d-spacing and unit cell parameters of pure and modified SBA-15

Sample	(hkl)	d-spacing (nm)	a_0 (nm)
SBA-15 pure	(100) (110) (200)	20.3 8.7 5.2	12.8
SBA-NH_2	(100) (110) (200)	20.1 8.3 5.1	11.4

From the 3 peaks of XRD results one most intense peak with 2 less intense peaks for both samples. Diffraction pattern is same for both samples which indicated that functionalization slightly affects the structural stability of pure mesoporous silica. These peaks were indexed as 100,110 and 200 respectively. These results confirmed the two dimensional hexagonal mesostructure of SBA-15 with p6mm symmetry. A very sharp and intense peak at 2θ of 0.6 was indexed as 100 and two small peaks at 1.0 and 1.9 of 2θ showing 110 and 200 respectively. The obtained results were satisfied by the previously reported results [18].

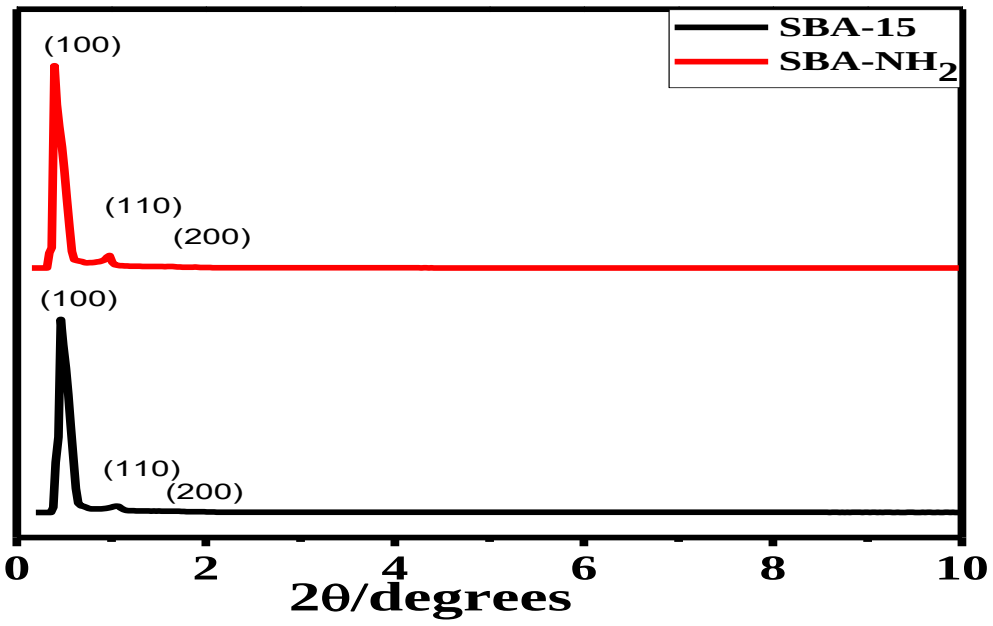

Figure 7.3 XRD spectra of pure and modified SBA-15

These values are indication of good mesoscopic structure and showing two dimensional structure of SBA-15 with hexagonal morphology [52]. According to results SBA-NH_2 showed slightly less intense peaks than unmodified form of silica. The decrease in intensity of modified silica is because of the anchoring of organic group on the silica surface. Moreover functionalized sample peaks showed slightly shifted to left side which described that the structural order affected by the functionalization on the silica surface and functionalization has been done successfully [6].

4.3 Scanning Electron Microscopy (SEM) Results

Scanning electron microscopy was used for determining the surface morphology. It was performed for studying the particles morphology, their shape and average size. The degree of magnification can be controlled because of the presence of electromagnets instead of lenses. Moreover high resolution and very clear image make it more useful technique. SEM images confirmed that SBA-15 has porous network along with hexagonal symmetry. Picture of low resolution describes that mesoporous silica present in the form of aggregates and clusters [74].

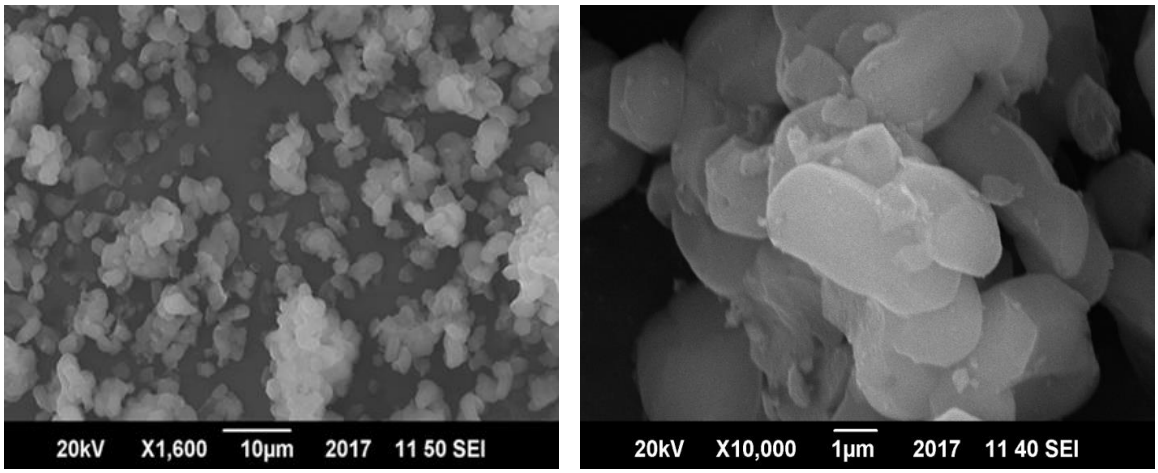

Figure 4.4 SEM images of SBA-15

The hexagonal morphology of mesoporous silica has become less ordered due to modification with amino propyl group.

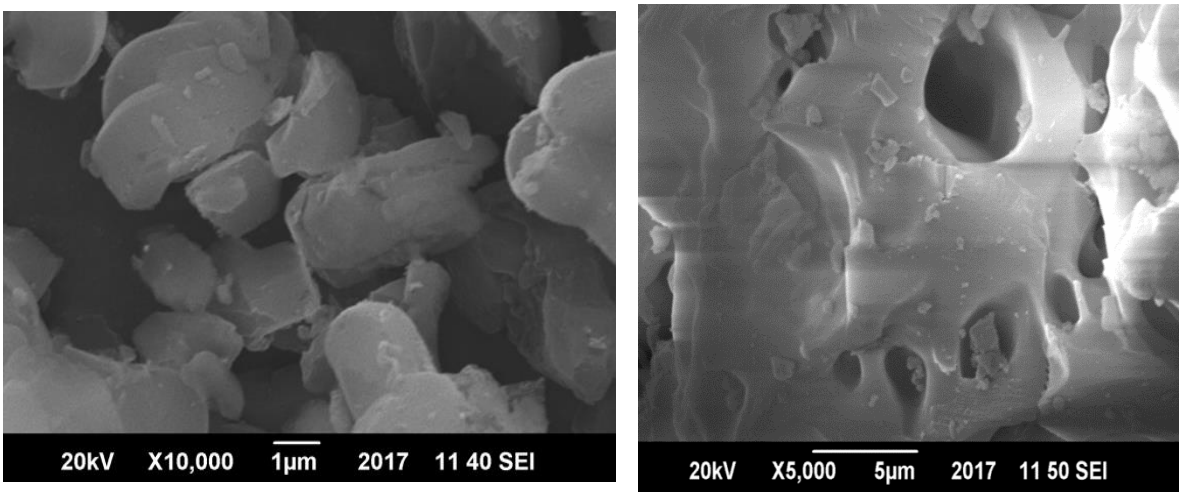

Figure 4.5 SEM images of modified SBA-15

4.4 BET Results

BET analysis is very important for the determination of porosity, pore diameter, pore volume and surface area of mesoporous silica. Sorption of gases on the surface of solid gives the direct estimation of surface area. Nitrogen with a boiling point 77K is used as common source of sorption phenomenon for determining the porosity and surface area. One advantage is that it is non-destructive technique for the analysis of micro and mesoporous materials. Mesoporous silica possess type IV isotherm which is clear identification of ordered structure of mesopores. N_2 adsorption was performed by using N_2 gas at 77K. Samples was degassed for 3 hours at 120ºC before analysis.

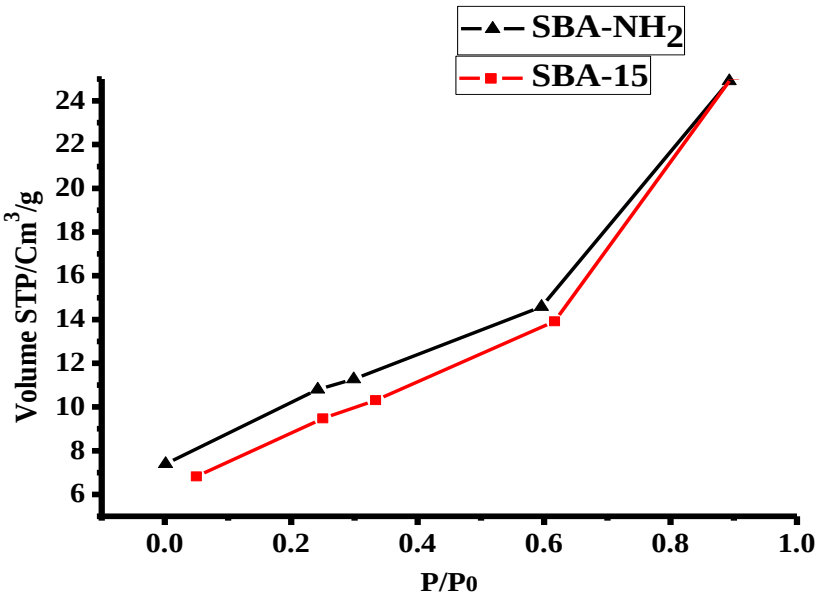

Figure 4.6 Nitrogen adsorption desorption analysis for SBA-15 and SBA-NH_2

According to the figure 4.6 a sharp isotherm obtained between 0.4-0.6 of P/P_o for pure SBA-15. While for modified SBA-15 isotherm is slightly shifted to lower value between 0.4-0.5 of P/P_o. Surface area and Pore volume decreased after functionalization. The sharp inflection point of each adsorption branch of each isotherm showed that a typical type of capillary condensation inside the uniformly arranged pores. After modification shape of hysteresis loop remains unchanged but decrease in pore size and pore volume has been observed. Moreover the surface area was reduced

from553 m^2/g to 163 m^2/g. A prominent decrease in surface area and pore size justifies the successful functionalization of amino propyl group.

Pore wall thickness can be calculated by the following equation.

$$\mathbf{Wp = a_0 - Dp} \qquad \mathbf{(4)}$$

Where Dp is the pore diameter and a_0 is unit cell parameter obtained from XRD results.

Table 4.2 Surface area obtained from BET, pore volume and pore diameter obtained from BJH method and calculated pore wall thickness.

Material	SBET (m^2/g)	Vp (cm^3/g)	Dp (nm)	Wp (nm)
SBA-15	534.46	0.036	5.1	7.7
SBA-NH_2	163.80	0.015	4.6	6.8

4.5 Cloxacillin Loading

For the loading of cloxacillin, calibration curve was plotted by preparing different ppm solution. Drug loading percentage was calculated by the equation obtained by calibration plot. 1 mL of solution was taken and diluted to 3 ml distilled water and measurements were obtained by UV analysis. Measurements were taken between 200-800 nm and absorbance was calculated at 275 nm to observe the change in concentration before and after drug loading.

4.6 UV-Visible Results

UV results described that almost 5% drug has been loaded for SBA-15 and 3% drug has been loaded by SBA-NH_2. Thus it can be stated that drug loading capacity has not been improved by functionalization process. Amine functionalized sample show slightly lower loading capacity than pure sample. The lower loading may be due to loss of amino group in drug loading process or it may be due to lower pore size of sample than pure silica. Besides micropores are sometimes blocked during functionalization process and thus the adsorption sites cannot be accessed by the drug molecules which results in lower loading capacity [83]. Another reason is that it might be possible that pH of solution and adsorption medium are not suitable for this type of adsorption. It has also been reported previously, that penicillin type drugs loading can be improved by increasing the pH up to 7.

4.7 Cloxacillin Release

Drug release includes the process which involves the migration of drug particles from the pores of material into the release medium. This process is not as simple as it seems because it is affected by various factors like nature of solute, release fluid, interaction between drug and carrier and the release environment. Release experiments for drug release were completed in 24 hours. Before observing the UV absorption, cloxacillin loaded solution was suspended in PBS of 7.2 at 37 ºC to maintain body temperature conditions. For this purpose 0.5 mL of aliquot was taken for analysis in 10 ml PBS. Later PBS was replaced by 0.5 mL of fresh PBS. In this way concentration was maintained and adequate analysis is performed. It has been stated that functionalization of APTES causes the hydrophilicity on the surface of silica so the interaction of cloxacillin with silica causes difference between loading and release capacities.

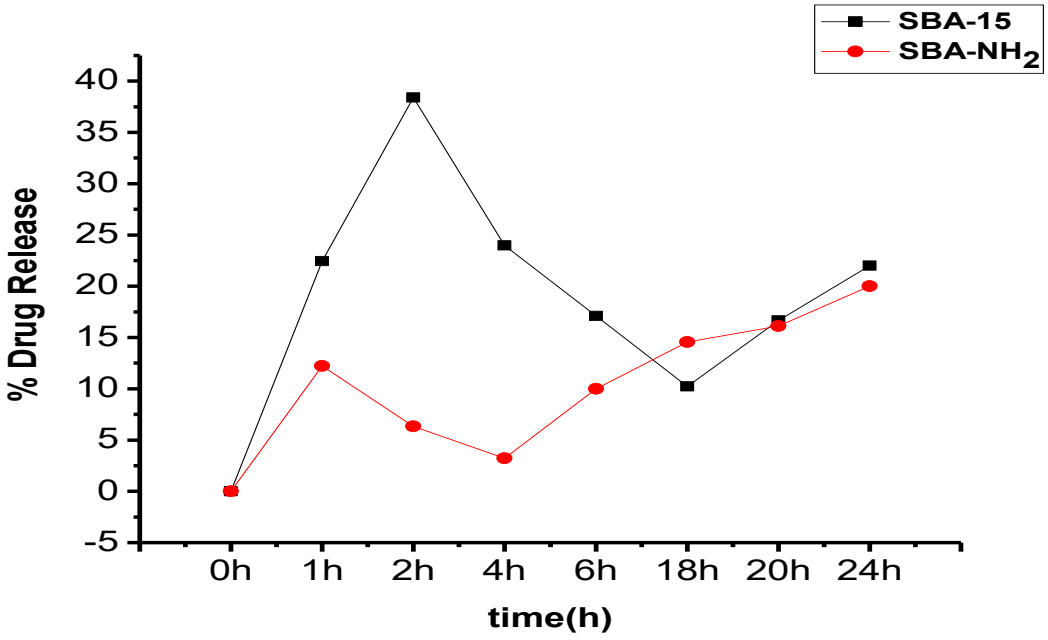

Figure 4.7 Release profiles of pure SBA-15 and SBA-NH_2

Drug release process involves two steps first step involves the diffusion of solvent in to the pores for dissolving the molecules of drug and in the second process drug molecules diffuses outside the pore and release into the fluid. In the process of drug adsorption besides absorbing inside the pores, some amount of drug has been absorbed on the outer surface of mesoporous material. Thus molecules of drug which are absorbed on the external surface releases first than the drug present

inside the pores. UV-Visible absorption results described that initial burst release rate was observed for pure SBA-15 in first 2 hours and then maintained further. Release profile showed that about 40 % of the drug has been released in first 2 hours for pure silica. In the case of modified SBA-15 burst release is very much less than pure SBA-15 and in 2 hours less than 10% of the drug has been released in basic medium. Kinetic release of Cloxacillin has completed in 24 hours and profile showed that during this period SBA-15 has maintained release up to 20% while SBA-NH_2 release rate is about 15%. It can be seen very clearly that percentage of drug release from pores of pure sample is greater than modified sample. Thus pure and functionalized silica have very promising role for the controlled release of cloxacillin. The slow and sustained release of drug from modified sample may be most suitable reason for the decrease in the size of pores and resistance in diffusion of drug from pores which occurred because of functionalization between organic moiety and silica surface. Moreover, APTES modified material holds the drug more firmly by showing slow release of drug. Strong interaction between cloxacillin and NH_2 group of functionalized silica can be a good reason for the sustained release of drug. These attractions prevents the quick and significant release [84].

Chapter 5

Summary

1. Sol gel process was used for the synthesis of mesoprous SBA-15
2. SBA-15 has been modified with amino propyl triethoxy silane (APTES)
3. Drug loading was done for both modified and non-modified materials
4. Release studies were observed in basic conditions
5. Results demonstrate the successful loading of drug on both samples
6. Modified material showed slow and sustained release than nonmodified sample

5.2 Future Prospects

Experimental results showed less amount of drug loading so drug loading can be improved by surface interactions between silica surface and functional groups. Drug release study will be done by varying temperature and pH conditions. In future work loading efficiency will be improved by surface interaction between silica and functional groups.

5.3 Conclusions

Mesoporous SBA-15 was successfully prepared by using sol-gel process and functionalized with organic group by post grafting method. Successful functionalization of SBA-15 was done by replacing the silica with amino group. For the first time cloxacillin has been loaded on modified and non-modified materials. XRD results showed that hexagonal structure remains same even after modification. According to BET evaluation, pore size, pore volume and surface area decreases after modification. UV analysis showed that about 3% drug has been loaded on modified sample and about 5% drug has been loaded by pure SBA-15. The decrease in efficiency of is due to decreased in surface area and size of pores. The release studies were performed in phosphate buffer media and concentration was observed by UV-Visible analysis. Results showed that 40% of the drug has been released from pure SBA-15 in 2h while at the same time period only 7% of the drug released for modified sample. This confirmed that modified sample exhibits slow and sustained release than pure sample. The obtained results demonstrated that SBA-15 may act as biocompatible drug carrier with excellent properties. Moreover, by changing the different functional group, pH and temperature in post synthesis method drug loading efficiency can be optimized.

Chapter 6: References

[1] A. Y. Filiz Sevimli, "Surface functionalization of SBA-15 particles for amoxicillin delivery," *Microporous and Mesoporous Materials,* vol. 158, pp. 281-291, 2012.

[2] G. Gezer, "Surface functionalizatin of SBA-15 particles for celecoxib delivery " p. 72, 2013.

[3] S. Wang, "Ordered mesoporous materials for drug delivery,," *Micropor. Mesopor. Mater.,* vol. 117, pp. 1-9, 2009.

[4] C. T. Kresge, Leonowicz, M. E., Roth, W. J., Vartuli, J. C. & Beck,, "Ordered mesoporous molecular sieves synthesized by a liquid-crystal template mechanism," *Nature,* vol. 359, pp. 710 - 712, 1992.

[5] P. E. Bagshaw SA, Pinnavaia TJ, "Templating of mesoporous molecular sieves by nonionic polyethylene oxide surfactants.," *Pinnavaia, Science.,* vol. 269, pp. 1242-1244., 1995.

[6] R. K. S. Sooyeon Kwo, Roman A Perez, Ensanya A Abou Neel, Hae-Won Kim and Wojciech Chrzanowski, "Silica-based mesoporous nanoparticles for controlled drug delivery," *Journal of Tissue Engineering,* vol. 4, p. 18, 2013.

[7] I. A. R. a. V. Padavettan, "Synthesis of Silica Nanoparticles by Sol-Gel: Size-Dependent Properties, SurfaceModification, and Applications in Silica-Polymer Nanocomposites," *Journal of Nanomaterials,* vol. 2012, p. 15, 2012.

[8] V. Meynen, Cool, P., & Vansant, E. F., "Verified synthesis of mesoporous materials.," *Microporous and Mesoporous Materials,,* vol. 125, pp. 170-223, 2009.

[9] P. C. V. Meynen, E.F. Vansant., "Verified Syntheses of Mesoporous Materials," *Microporous and Mesoporous Materials,,* vol. 125, pp. 170-223., 2009.

[10] V. Meynen, Cool, P., & Vansant, E. F., "Verified synthesis of mesoporous materials.," *Microporous and Mesoporous Materials,,* vol. 125, pp. 170 - 223., 2009.

[11] Q. H. D. Zhao, J. Feng, B.F. Chmelka, G.D. Stucky, "Nonionic Triblock and Star Diblock Copolymer and Oligomeric Surfactant Syntheses of Highly Ordered, Hydrothermally Stable, Mesoporous Silica Structures," *J. Am. Chem. Soc,* vol. 120, pp. 6024-6036., 1998.

[12] P. C. V. Meynen, E.F. Vansant, "Verified synthesis of mesoporous materials.," *Microporous and Mesoporous Materials,,* vol. 125, pp. 170-223, 2009.

[13] J. L. W.-H. Zhang, B. Han, M. Li, J. Xiu, P. Ying, C. Li., "Direct Synthesis and Characterization of Titanium-Substituted Mesoporous Molecular Sieve SBA-15," *Chem. Mater.,* vol. 14, pp. 3413-3421, 2002.

[14] I. Izquierdo-Barba, Colilla, M., Manzano, M., & Vallet-Regí, M., "In vitro stability of SBA-15 under physiological conditions.," *Microporous and Mesoporous Materials,* vol. 132, pp. 442-452, 2010.

[15] Q. He, & Shi, J., "Mesoporous silica nanoparticle based nano drug delivery systems: synthesis, controlled drug release and delivery, pharmacokinetics and biocompatibility," *J. Mater. Chem.,,* vol. 21, pp. 5845 - 5855., 2011.

[16] Q. He, Shi, J. C., Wei, C., Zhang, L., Wu, W., Bu, W., et al., "Synthesis of oxygen defiicent luminescent mesoporous silica nanoparticles for synchronous drug delivery and imaging," *Chem. Commun.,,* vol. 47, pp. 7947 - 7949., 2011.

[17] D. L. Dae Hyun Hwang, Hosub Lee, Dongcheol Choe Sang Hoon Lee, and Kangtaek Lee, "Surface functionalization of SBA-15 particles for ibuprofen delivery," *Korean J. Chem. Eng,* vol. 27(4), pp. 1087-1092, 2010.

[18] N. S. Pallavi Shah , Asmita Prabhune , Veda Ramaswamy, "Structural features of Penicillin acylase adsorption on APTES functionalized SBA-15," *Microporous and Mesoporous Materials,* vol. 116, pp. 157–165, 2008.

[19] N. Garcia, Benito, E., Guzman, J., Tiemblo, P., Morales, V., & Garcia, R. A., "Functionalization of SBA-15 by an acid-catalyzed approach: A surface characterization study," *Microporous and Mesoporous Materials,,* vol. 106, pp. 129 - 139., 2007.

[20] I. I. S. Brian G. Trewyn, Supratim Giri, Hung-Ting Chen and Victor S.-Y. Lin, "Synthesis and Functionalization of a Mesoporous Silica Nanoparticle Based on the Sol–Gel Process and Applications in Controlled Release," *Acc. Chem. Res.,* vol. 40, pp. 846-853, 2007.

[21] R. v. G. J. A. Melero, G. Morales,, "Advances in the Synthesis and Catalytic Applications of Organosulfonic-Functionalized Mesostructured Materials,," *Chem. Rev,* vol. 106, pp. 3790-3812., 2006.

[22] A. B. P. Z.-Poor, B. D. Fahlman, P. Arab, G. M. Ziarani., "One-pot synthesis of ethanolamine-modified mesoporous silica," *Ind. Eng. Chem. Res.,* vol. 50, pp. 10036-10040, 2011.

[23] L. M. Rosenholm JM, "Towards establishing structure-activity relationships for mesoporous silica in drug delivery applications.," *J Control Release.,* vol. 128, pp. 157-64, 2008.

[24] R. K. MN, "Nano and microparticles as controlled drug delivery devices.," *J Pharm Pharm Sci.,* vol. 3, pp. 234-258.

[25] U. N. Charu Bharti, Ashok Kumar Pal, Neha Gulati, "Mesoporous silica nanoparticles in target drug delivery system: A review," *International Journal of Pharmaceutical Investigation,* vol. 5, 2016.

[26] M. V.-R. Prof., "Ordered Mesoporous Materials in the Context of Drug Delivery Systems and Bone Tissue Engineering," *Chem. Eur. J.,* vol. 12, pp. 5934-5943, 2006.

[27] C. C. Muller-Goymann, "Physicochernical characterization of colloidal drug delivery systems such as reverse micelles, vesicles, liquid crystals and nanoparticles for topical administration," *European Journal of Pharmaceutics and Biopharmaceutics,* vol. 58, p. 343, 2004.

[28] B. G. Katzung, "In Basic and Clinical Pharmacology," *Norwalk, Conn: Appleton & Lange.,* pp. 69 - 71, 1995.

[29] A. p. SiddharthJambhrunkara. .ZhiQu, "Modulating in vitro release and solubility of griseofulvin using functionalized mesoporous silica nanoparticles," *Journal of Colloid and Interface Science,* vol. 434, 2014.

[30] M. M. a. M. a. Vallet-Regı´, "New developments in ordered mesoporous materials for drug delivery," *Journal of Materials Chemistry,* vol. 20, pp. 5593–5604, 2010.

[31] S. C. Piscitelli, & Rodvold, K, "In Drug interactions in infectious diseases," *Totowa: Humana Press.,* vol. 2nd edition, p. 151, 2005.

[32] S. M. B. Drawz, R. A, "Three Decades of β-Lactamase Inhibitors," *Clinical Microbiology Reviews.,* pp. 160–201, 2010.

[33] N. S. Egorov, "In Antibiotics: a scientific approach," *Moscow: Mir Publishers.,* p. 12, 1985.

[34] T. J. Franklin, & Snow, G. A., "In Biochemistry and molecular biology of antimicrobial drug action," *New York: Springer.,* p. 23, 2005.

[35] "WHO Model Prescribing Information: Drugs used in Bacterial Infections," *world health organization,* p. 179, 2001.

[36] D. Greenwood, "Antimicrobial drugs: chronicle of a twentieth century medical triumph," *Oxford University Press US,* p. 124, 2008.

[37] M. I. Joshy, Elayaraja, K., Suganthi, R. V., Veerla, S. C., & Kalkura, S. N., "In vitro sustained release of amoxicillin from lanthanum hydroxyapatite nano rods.," *Current Applied Physics,,* vol. 11, pp. 1100-1106, 2011.

[38] A. P. Muge Sari Yilmaz, Sabriye Piskin, "Release of flurbiprofen using of SBA-15 mesoporous silica: Influence of silica sources and functionalization," *Journal of Non-Crystalline Solids,* vol. 437, pp. 80-86, 2016.

[39] V. K. T. Suman Jangraa, Ashok K. Sharma, Vinod Chhokar, Rahul, Surender Duhan, "Inclusion of ampicillin trihydrate in highly ordered mesoporous silica nanoparticles," *American Journal of Biological and Pharmaceutical Research.,* vol. 2(4), pp. 153-156, 2015.

[40] M. Ł. Michał Moritz, "SBA-15 mesoporous material modified with APTES as the carrier for 2-(3-benzoylphenyl)propionic acid," *Applied Surface Science,* vol. 258, pp. 7523– 7529, 2012.

[41] M. P. A. Szegedi , I.Goshev , J.Miha ly, "Effect ofamine functionalization of spherical MCM-41and SBA-15 on controlled drug release," *Journal of Solid State Chemistry,* vol. 184, pp. 1201-1207, 2011.

[42] N. D. Ebrahim Ahmadi, Samaneh Hashemikia, "Synthesis and surface modification of mesoporous silica nanoparticles and its application as carriers for sustained drug delivery," *Informa Healthcare,* vol. 21(3), 2014.

[43] M. H. N. Abdollah Zakeri Siavashani, Fateme Fayyazbakhsh, Tayebeh Toliyat,, "Effect of amino-functionalization on insulin delivery and cell viability for two types of silica mesoporous structures," *J Mater Sci,* 2016.

[44] L. Z. Jianmei Pang, Longlong Zhang , Zhonghao Li , Yuxia Luan, "Folate-conjugated hybrid SBA-15 particles for targeted anticancer drug delivery," *Journal of Colloid and Interface Science,* vol. 395, pp. 31-39, 2013.

[45] L. P. Valeria Ambrogi , Cinzia Pagano, Fabio Marmottini , Maurizio Ricci , Anna Sagnella , Carlo Rossi, "Use of SBA-15 for furosemide oral delivery enhancement," *European Journal of Pharmaceutical Sciences,* vol. 46, pp. 43-48, 2012.

[46] Z. B. A. B. G. M. Ziarani, "Thiol-functionalized mesoporous silica as nanocarriers for anticancer drug delivery," *Int. J. Bio-Inorg. Hybr. Nanomater.,,* vol. 4(3), pp. 121-128, 2015.

[47] M. C. Marina Martínez-Carmona , M. Luisa Ruiz-Gonzalez, "High resolution transmission electron microscopy: A key tool to understand drug release from mesoporous matrices," *Microporous and Mesoporous Materials,* vol. 225, pp. 399-410, 2016.

[48] K. H. S.-W. Song, and S. Kawi, "Functionalized SBA-15 Materials as Carriers for Controlled Drug Delivery: Influence of Surface Properties on Matrix-Drug Interactions," *2005, 21, 9568-9575,* 2005.

[49] Q. G. Wujun Xu , Yao Xu, Dong Wu, Yuhan Sun, "pH-Controlled drug release from mesoporous silica tablets coated with hydroxypropyl methylcellulose phthalate," *Materials Research Bulletin,* vol. 44, pp. 606-612, 2009.

[50] A. M. J. Ortiz-Bustos, V. Morales, R. Sanz, R.A. García-Mu˜noz, "Surface-functionalization of mesoporous SBA-15 silica materials for controlled release of methylprednisolone sodium hemisuccinate: Influence of functionality type and strategies of incorporation," *Microporous and Mesoporous Materials,* vol. 240, pp. 236-245, 2017.

[51] H. K. Jeff Gordon, Sohrab Rohani, "MIL-53(Fe), MIL-101, and SBA-15 porous materials: Potential platforms for drug delivery," *Materials Science and Engineering,* vol. C47, pp. 172-179, 2015.

[52] J. M. S.-M. Antonio L. Doadrio , Juan C. Doadrio , Antonio J. Salinas ,María Vallet-Regí, "A molecular model to explain the controlled release from SBA-15 functionalized with APTES," *Microporous and Mesoporous Materials,* vol. 195, pp. 43-49, 2014.

[53] P. G. Suman Jangra, Vinod Chhokar, Vijay K. Tomer,Ashok K. Sharma• Surender Duhan, "In-vitro drug release kinetics studies of mesoporous SBA-15- azathioprine composite," *J Porous Mater,* vol. 23, pp. 679-688, 2016.

[54] Q. G. Wujun Xu , Yao Xua, Dong Wu, Yuhan Sun , Wanling Shen , Feng Deng "Controllable release of ibuprofen from size-adjustable and surface hydrophobic mesoporous silica spheres," *Powder Technology,* vol. 191, pp. 13-20, 2009.

[55] S. M. Thanapha Numpilai , Thongthai Witoon, "Impact of pore characteristics of silica materials on loading capacity and release behavior of ibuprofen," *Materials Science and Engineering,* vol. C59, pp. 43-52, 2016.

[56] M. M. Małgorzata Geszke-Moritz, "APTES-modified mesoporous silicas as the carriers for poorlywater-soluble drug. Modeling of diflunisal adsorption and release," *Applied Surface Science,* vol. 368, pp. 348–359, 2016.

[57] K. A. Fozia Rehman , Claudio Airoldi, Simon Gaisford, Asma Buanz, Abdur Rahim,Nawshad Muhammad , Pedro L.O. Volpe, "Amine bridges graftedmesoporous silica, as a prolonged/controlled drug release system for the enhanced therapeutic effect of short life drugs," *Materials Science and Engineering,* vol. C72, pp. 34-41, 2017.

[58] C. W. Yunqiang Xu, Guowei Zhou, Yue Wu, Jing Chen, "Improving the controlled release of water-insoluble emodin from amino-functionalized mesoporous silica," *Applied Surface Science,* vol. 258, pp. 6366-6372, 2012.

[59] F. B. Daniel Carmona , Jesus Santamaria, "Pore ordering and surface properties of FDU-12 and SBA-15 mesoporous materials and their relation to drug loading and release in aqueous environments," *Materials Research Bulletin,* vol. 59, pp. 311-322, 2014.

[60] M. A. E. N. Ahmed A. Abd-Elrahman, Doaa H. Hassan, and Amal A. Taha, "Ketoprofen mesoporous silica nanoparticles SBA-15 hard gelatin capsules: preparation and in vitro/in vivo characterization," *Informa Healthcare,* vol. 23(9), pp. 3387-3398, 2016.

[61] V. S. Dange VU, Kendre PN and Pande w, "Fabrication and Characterization of Hexagonal Mesoporous Silica (HMS) for Impregnating Lornoxicam as a Drug Carrier," *Austin Therapeutics,* vol. 2, 2015.

[62] D. L. Dae Hyun Hwang, Hosub Lee, Dongcheol Choe, Sang Hoon Lee and Kangtaek Lee, "Surface functionalization of SBA-15 particles for ibuprofen delivery," *Korean J. Chem. Eng,* vol. 27(4) pp. 1087-1092, 2010.

[63] A. N. O. Gang Wang, Elizabeth A. Blair, Kelley Denton, Zhimin Tao, Tewodros Asefa "Functionalized mesoporous materials for adsorption and release of different drug molecules: A comparative study," *Journal of SolidState Chemistry,* vol. 182, pp. 1649-1660, 2009.

[64] Y.-Y. W. Qing-Zhou Zhai , Xiao-Han Wang, "Synthesis, characterization and sustaining controlled release effect of mesoporous SBA-15/ramipril composite drug," *J Incl Phenom Macrocycl Chem,* vol. 77, pp. 113-120, 2013.

[65] N. H. Samaneh Hashemikia , Ebrahim Ahmadi , Majid Montazer, "Optimization of tetracycline hydrochloride adsorption on amino modified SBA-15 using response surface methodology," *Journal of Colloid and Interface Science,* vol. 443, pp. 105-114, 2015.

[66] T. H. Atsushi Nakahira , Yusuke Yamauchi, "Synthesis and properties of dense bulks for mesoporous silica SBA-15 by a modified hydrothermal method," *Materials Letters,* vol. 64, pp. 2053-2055, 2010.

[67] E. M. B. S. A.L. Doadrio, J.C. Doadrio, J. Pe´rez Pariente, I. Izquierdo-Barba, M. Vallet-Regı, "Mesoporous SBA-15 HPLC evaluation for controlled gentamicin drug delivery," *Journal of Controlled Release,* vol. 97, pp. 125-132, 2004.

[68] G. N. A. Soledad San Roman, Margarita del Arco , Cristina Martín, "Dexketoprofen and aceclofenac release from layered double hydroxide and SBA-15 ordered mesoporous material," *Applied Clay Science,* vol. 121-122, pp. 9-16, 2016.

[69] K. A. B. N. Sandra Maria Lopes dos Santos, Marlon de Souza Gama, Jeann Diniz Ferreira Lima,, "Synthesis and characterization of ordered mesoporous silica (SBA-15 and SBA-16) for adsorption of biomolecules," *Microporous and Mesoporous Materials,* vol. 180, pp. 284-292, 2013.

[70] P. P. Sun-Young Park, "Mesoporous silica SBA-15 for natural antimicrobial delivery," *Powder Technology,* vol. 223, pp. 77-82, 2012.

[71] K. G. a. S. A. M. Meysam Mohseni, "Preparation and Characterization of Rifampin Loaded Mesoporous Silica Nanoparticles as a Potential System for Pulmonary Drug Delivery," *Iranian Journal of Pharmaceutical Research,* vol. 14(1), pp. 27-34, 2015.

[72] G. M. Z. a. A. B. Vaezeh Fathi Vavsari, "The role of SBA-15 in drug delivery," *Royal society of chemistry Advances,* vol. 5, p. 91686, 2015.

[73] M. B. c. Dasaa Halamovaa, Vladimir Zelenak, Tatana Gondova, Ulla Vainio, "Naproxen drug delivery using periodic mesoporous silica SBA-15," *Applied Surface Science,* vol. 256, pp. 6489-6494, 2010.

[74] S. T. Zeynep Seda Eren, Gamze Gezer , Leyla Tatar Yildirim ,Sreeparna Banerjee , Aysen Yilmaz, "Improved solubility of celecoxib by inclusion in SBA-15 mesoporous silica: Drug loading in different solvents and release," *Microporous and Mesoporous Materials,* vol. 235, pp. 211-223, 2016.

[75] J.-W. L. Thi Phuong Binh Nguyen , Wang Geun Shim, Hee Moon, "Synthesis of functionalized SBA-15 with ordered large pore size and its adsorption properties of BSA," *Microporous and Mesoporous Materials,* vol. 110, pp. 560-569, 2008.

[76] Z. Z. Yanzhuo Zhang , Tongying Jiang, Jinghai Zhang, Zhanyou Wang, Siling Wang, "Spherical mesoporous silica nanoparticles for loading and release of the poorly water-soluble drug telmisartan," *Journal of Controlled Release,* vol. 145, pp. 257-263, 2010.

[77] Y. Y. L. Mi Mi Wan, Tian Yang,Tao Zhang,Xiao Dan Sun and Jian Hua Zhu, "In Situ Loading of Drugs into Mesoporous Silica SBA-15," *Chem. Eur. J.,* vol. 22, pp. 6294-6301, 2016.

[78] F. B. Maria Vallet-Regi, and Daniel Arcos, "Mesoporous Materials for Drug Delivery," *Angew. Chem. Internationbal,* vol. 46, pp. 7548-7558, 2007.

[79] S. Wang, "Ordered mesoporous materials for drug delivery," *Microporous and Mesoporous Materials,* vol. 117, pp. 1-9, 2009.

[80] E. M. Johansson, "Controlling the pore size and morphology of mesoporous silica," *Linköpings Universitet, Sweden :,* pp. 34-42, 2010.

[81] F. F. Sevimli, "Surface functionalization of SBA-15 for amoxacillin delivery " p. 93, September 2011,.

[82] Ghodsi Mohammadi ziarani, Alireza Badiei, Somayeh Mousavi, Negar LashariL, Afsaneh Shahbazi, "Application of Amino-Functionalized SBA-15 Type Mesoporous Silica in One-Pot Synthesis of Spirooxindoles," *Chinese Journal of Catalysis,* vol. 33 No. 11, pp. 1832–1839, 2012.

[83] M. Vallet-Regí, Doadrio, J. C., Doadrio, A. L., Izquierdo-Barba, I., & Pérez Pariente, J., "Hexagonal ordered mesoporous material as a matrix for the controlled release of amoxicillin.," *Solid State Ionics,,* vol. 172, pp. 435-439, 2004.

[84] K. S. Zhenhuan Li , Bowen Cheng , Yanchao Deng, "Organically modified MCM-type material preparation and its usage in controlled amoxicillin delivery," *Journal of Colloid and Interface Science,* vol. 342, pp. 607-613, 2010.