SYNTHESIS AND SURFACE MODIFICATION OF LP-SBA-15 WITH POLYETHYLENEIMINE FOR CELECOXIB DELIVERY

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

ALI FARID

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
MICRO AND NANO TECHNOLOGY

SEPTEMBER 2017

Approval of the thesis:

SURFACE AND MODIFICATION OF LP-SBA-15 WITH POLYETHYLENEIMINE FOR CELECOXIB DELIVERY

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ABSTRACT

SURFACE AND MODIFICATION OF LP-SBA-15 WITH POLYETHYLENEIMINE FOR CELECOXIB DELIVERY

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September 2017, 64 pages

In this study LP-SBA-15 (LP stands for large pores) and LP-SBA-15-PEI (PEI stands for polyethyleneimine), ordered mesoporous silica support material utilized with different pore structure characteristics to investigate on their drug delivery system abilities like release and loading properties, regarding to their structural variability and implication of surface modification.

Hydrothermal synthesis method was used for synthesis of LP-SBA-15 and surface functionalization was done by polymerization of hyperbranched polyethyleneimine inside pores. The referred functional group expected to interact with surface silanols on the silica supports, and this interaction expected to be enriched by introduction of amino groups to the silica surfaces. For organic modification, necessities of larger pore mesophases is obvious. Since, accessibility of pore is highly dependent on pore size and structure. Therefore, having larger pores provides higher chance to have large empty spaces inside pores, even after modification of pores.

After functionalization, drug loading was carried out in ethanol. As a drug model, Celecoxib (CLX) was used for investigation on bioavailability and drug delivery systems characterizations which is nonsteroidal anti-inflammatory drug that shows

highly hydrophobic quality and also has very low bioavailability. Effect of particle morphology and specific pH was analyzed on release properties and release experiment was done in two different pH (pH= 5.0 and pH= 7.4).

In characterization part, LP-SBA-15 particles were characterized by using X-ray Diffraction (XRD), Small-Angle X-ray Spectrometry (SAXS), N_2 adsorption - desorption, Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM), Ultra-Violet Spectrometry (UV-VIS) and Thermogravimetric Analysis (TGA). Hence, several characterizations were applied for better understanding the bioavailability, morphology, crystallinity etc.

Consequently, an enhanced release rate on drug delivery properties were observed for LP-SBA-15-PEI in comparison to commercial drug and non-polymerized sample. More specifically, 41% of loading for LP-SBA-15-CLX and 25% for LP-SBA-15-PEI were observed. Moreover, more than 80% release in both pH= 5.0 and pH= 7.4 for LP-SBA-15-PEI-CLX and less than 50% in case of LP-SBA-15-CLX were exhibited. Which introduces LP-SBA-15-PEI as a proper choice for drug delivery systems since the release percent of Celebrex itself were less than 60% in both cases.

***Keywords*:** SBA-15 particles, surface functionalization, PEI, drug delivery systems, Celecoxib.

ÖZ

SELEKOKSIB SALINIMI İÇİN POLİETİLENİMİNLE LP-SBA-15'İN YÜZEY MODİFİKASYONU

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Eylüll 2017, 64 sayfa

Bu çalışmada, düzenli mezogözenekli silika destek malzemelerinden yararlanılarak farklı gözenek yapılarına sahip SBA-15-PEI (polietilenimin) ve büyük gözenekli LP-SBA-15 destek malzemeleri sentezlenmiştir. Bu yapısal değişikliğin ve yüzey modifikasyonlarının bu malzemelerin ilaç salınımları ve tutma özellikleri gibi ilaç taşıma kabiliyetleri üzerindeki etkileri incelenmiştir. LP-SBA-15'in sentezi için hidrotermal sentez metodu kullanılmıştır ve yüzey fonksiyonlaması ise yüksek miktarda dallanma gösteren polietilenimin yapı içindeki gözeneklerle polimerleştirilerek yapılmıştır. Fonksiyonel grubun yüzeyde silika destekli silanol grupla etkileşimi beklenmiştir, fakat bu etkileşimin yeni amino gruplarının eklenmesi ile zenginleştirilmesi muhtemeldir. Geniş gözenekli mezopor yapılarda yapılan modıfikasıyonlar gözeneklerın büyüklükleri ve yapılarını etkilemektedir. Böylece, gözeneklerin değişiminden sonra daha büyük gözeneklerin içinde daha geniş yüzey boşluklarının olma şansı artar. Yüzey fonksiyonlaması yapıldıktan sonra ilaç yüklemesi etanol içerisinde yapılmıştır. Bu araştırmada kandan dokuya geçiş hızı oldukça düşük olan ve steroid içermeyen iltihap önleyici ilaç olan Selekoksib (CLX)

model ilaç olarak kullanılmıştır. Parçacık morfolojisi ve spesifik pH'ın ilaç salınımı üzerine etkileri analiz edilmiş ve serbest salınım deneyleri iki farklı pH'da (pH= 5.0 ve pH= 7.4) gerçekleştirilmiştir. Karakterizasyon kısmında ise LP-SBA-15 parçacıkları X-ray kırınım (XRD) cihazı, küçük açı X-ray spektrometresi (SAXS), N_2 adsorpsiyon-desorpsiyon, FTIR spektrometresi, Ultra-viyole (UV-Vis) spektrometresi ve termogravimetrik metot (TGA) kullanılarak yapılmıştır. Dolasıyla, biyoyararlanım, morfoloji, kristallik gibi analizlerin daha iyi anlaşılabilmesi için birkaç karakterizasyon uygulanmıştır.

Sonuç olarak sentezlenen LP-SBA-15-PEI örneklerinin ilaç salınım özelliklerinin piyasada satılan ilaçlara göre ve polimer fonksiyonlaması yapılmamış LP-SBA-15' e göre arttığı gözlenmiş ve LP-SBA-15-PEI'nin kontrollü ilaç taşıma sistemine uygun olduğu belirlenmiştir. Daha spesifik olarak, LP-SBA-15-CLX için yükleme %41 ve LP-SBA-15-PEI için %25 iken LP-SBA-15-PEI için hem pH= 5.0 hem de pH= 7.4'de %80 den fazla serbest salınım gerçekleşmiştir. LP-SBA-15-CLX durumunda ise %50 den az olduğu görülmüştür. Celebrex'in kendi salınım yüzdesi her iki durum için %60'ın altına düştüğünden, ilaç salınım sistemleri için, LP-SBA-15-PEI'nin daha uygun bir seçim olduğu belirlenmiştir.

Anahtar Kelimeler: SBA-15, yüzey fonksiyonlama, kontrollü ilaç taşıma sistemleri, Selekoksib, PEI

To my family; my beloved parents, Habib Farid and Shayesteh Darvishi.

ACKNOWLEDGMENT

I owe my deepest gratitude to Prof. Dr. Ayşen Yılmaz for her guidance and encouragement, patience, kindness and valuable advices.

I would like to thank Prof. Dr. Leyla Tatar, Prof.Dr. Jale Hacaloğlu, Assoc. Prof. Dr. Sreeparna Banerjee and Elif Vargün for their splendid technical, moral supports and valuable comments.

I wish thank to my dear colleague and friends, Muzeffer Gencay Çelik, Ceren Özde Hızal, Metehan Severoğlu, Zeynep Seda Eren, Pelin Akman and Sera İflazoğlu for their technical and moral supports, valuable comments and great friendships.

Finally, my most heartfelt appreciation goes to my family. I would like to thank them for constant support in the pursuit of my dreams and more importantly, for always believing in me. Also, I appreciate the support of my friends; Mobin Alipour, Farzad Shams and Salar Rahimi.

It is my duty to express my gratitude to Scientific and Technical Research Council of Turkey (TUBITAK, project number: 114Z719) for the financial support during my MS thesis.

I would like to thank METU, Central Laboratory, Chemistry department and its facilities.

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LIST OF ABBREVATIONS

BET: Brunauer-Emmett-Teller

BJH: Barret-Joyner-Halenda

CLX: Celecoxib

COX: Cyclooxygenase

d: the distance between adjacent crystal planes

DDS: Drug Delivery System

FTIR: Fourier Transform Infrared Spectroscopy

GI: Gastrointestinal tract

LP: Large porous

LP-SBA-15-PEI-CLX: Large porous Santa Barbara Amorphous, PEI modified and loaded with Celecoxib

MCM: Mobil Composition Matter

MSN: Mesoporous Silica Nanoparticles

NSAID: Non-steroidal anti-inflammatory drug

PBS: Phosphate Buffer Solution

PEI: polyethyleneimine

SAXS: Small-Angle X-Ray Scattering

SBA: Santa Barbara Amorphous

SEM: Scanning Electron Microscopy

TEM: Transmission Electron Microscopy

TEOS: Tetraethylorthosilicate

UV: Ultra Violet

XRD: X-Ray Diffraction

CHAPTER 1

INTRODUCTION

Nowadays, human health care studies are one of the most priorities in science world and drug delivery systems are considering as an important field of biomedical materials. Briefly, drug delivery system (DDS) is defined as system, which is able to control some properties such as; rate of drug release, time of the release, the dosage of release and also it could be a targeted release in the desired part of the body. Advances in nanotechnology field in recent years, has made attraction for researchers to study about nanostructured materials that could be used in the field of biomedical applications. As an example, MCM-41which is mesoporous material was firstly used in DDS application in 2001 [1]. Typically, porous materials gain attention in this field in early years, which the International Union of Pure and Applied Chemistry (IUPAC), categorized them as three types; microporous, mesoporous and microporous. More specifically, macroporous have pore diameter larger than 50 nm, mesoporous has pore diameter between 2 nm and 50 nm and microporous have pore diameter smaller than 2 nm [2].

1.1. MESOPOROUS MATERIALS

Ordered mesoporous silica materials were discovered in 1990s. After this discovery, there were enormous attention on these materials due to their advantages. Generally mesoporous materials are consequents of supramolecular assemblies of particularly; surfactants, which play role as a template the inorganic component (usually silica) through synthesis [3-6]. Mesoporous materials provide some properties that utter them as a potential drug carrier. They are able to load and release the drug with more in more controlled trend or manner. Moreover, the ordered pore network with homogenous size give significant advantage to these materials. Secondly, vast surface

area which increases the possibility of drug adsorption. Additionally, surface containing silanol that could be modified to improve control loading and release of drug. Moreover, these properties, have motivated people to utilize these materials in areas such as; separation, catalysis, sensors and devices in past decade [7, 8]. Also, being biocompatible and nontoxic are two the major factors that lead this material into the field of DDS. Structure with high stability and well-defined surface properties has lead the mesoporous materials for applications of encapsulation of pharmaceutical drug, proteins and other biogenic molecules. Past investigation shows that all small or large drug molecules could be entrapped inside the mesopores by an impregnation process and later release via a diffusion-controlled mechanism [9, 10]. In general, mesoporous silica nano particles (MSNs) are synthesized by two methods; the first one is condensation of silica under a basic medium with a cationic agent. The second one is an approach to use cationic agents, and also non-ionic and anionic surfactants [11]. Mesoporous materials have different types such as; SBA, SBA-15, MCM-14, M41S and etc. which they have been under vast studies in past years. They have different characteristics such as pore size, particle size, pore wall dimensions, crystal structures and other properties that made them particular for each other. Thus, this variety in properties provides better opportunity to be chosen differently in diverse cases dependence on their characteristic and desire for the purpose of application.

One of the major reasons that raises the interest toward MSN materials is the ability of modifying their surfaces which is much easier rather than other compounds in this area. There are high amount of Si-OH groups on the surface of MSN materials. These particular materials could be easily modified with the functionalization methods [12].

1.2. TYPES OF MESOPOROUS MATERIALS

1.2.1. SBA

SBA stands for Santa Barbara Amorphous which firstly were synthesized in 1998. Depend on the synthesize method and type of polymer and reaction condition utilized in the synthesis period, the pore diameter could be obtained between 5 nm to 30 nm. SBA is silica based material, and silica is extensively used as fundamental building block of MSN materials. Since it is cheap, chemically inert, thermally stable and safe

[13] and also it is plentifully existing in nature. SBA materials have many types like; SBA, SBA-16, SBA-15, SBA-14 and SBA-11. The most synthesized and searched SBA type is SBA-15 [14].

1.2.2. SBA-15

SBA-15 provide larger pores in the world of mesoporous materials and not only pores with large size, also shows a good thermal, chemical and mechanical resistance. These properties rise the attentions toward SBA-15 as superior choice to other types of catalyst and makes it proper for drug delivery systems. Synthesis condition may change some properties of SBA-15 such as pore size which is tunable in the range of 4-30 nm. Typical synthesis of SBA-15 requires, triblock copolymers, typically non-ionic triblock copolymer as structure directing agent and Tetrapropyl Orthosilicate (TPOS), Tetramethyl Orthosilicate (TMOS) or Tetraethyl Orthosilicate (TEOS) [4] as silica source. Template removal plays very crucial part in the synthesis of SBA-15 and it characteristics too. Since SBA-15 has an absence of functionality, heteroatoms and organic functional groups are utilized by post-synthesis or direct methods to modify the functionality of SBA-15 [15]. Silanol groups on the surface of SBA-15 are able to make intermolecular hydrogen bonding with drugs. These interactions could be modified by inclusion of functional groups on the surface of SBA-15 [16]. A pore arrangement of SBA-15 is represented as follow.

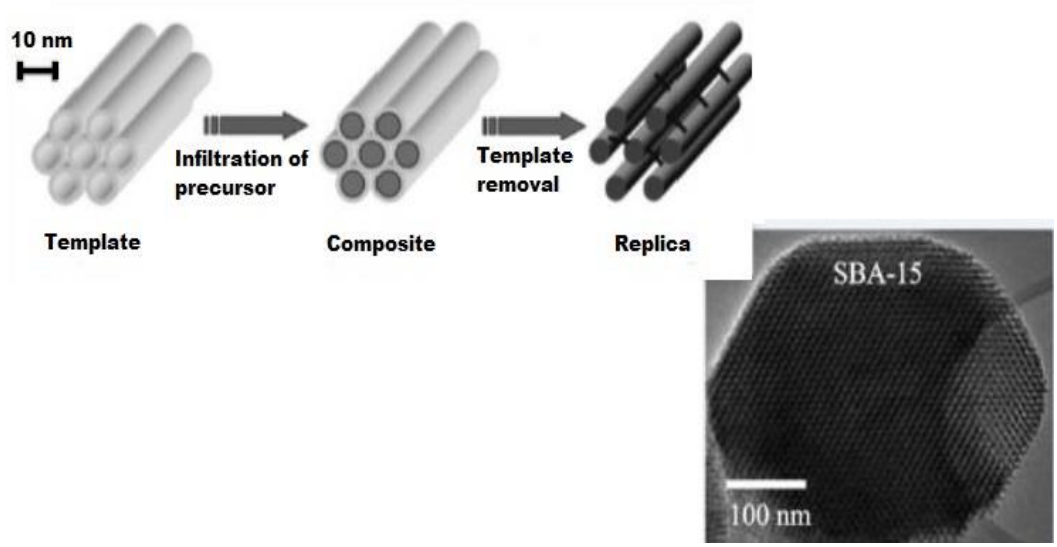


Figure 1. Depiction of arrangement of pores of ordered SBA-15 silica material [17].

In some recent studies, researchers have been trying to obtain larger pores SBA-15 to have better opportunity to modify inner of pore and obtain better interaction with pore inside. Hence, at the end it would be enough space, while existing modifier material inside pores, for other targeted material such as drug. This usually gained by adding extra material during synthesis or by usage of amphiphilic triblock copolymer in acidic media i.e., pH~1. Recently, there have been vast methods reported for manipulating the size of the pores by addition of auxiliary organic molecules, solubilized in the hydrophobic region of the templating aggregates, which leads to increase the micellar size as swelling agent [3, 18].

1.2.3. M41S

Actually, mesoporous materials were discovered accidently in 1991 by Mobil Corporation scientists. Nevertheless, they developed a procedure mixing ammonia, tetramethylammonium silicate and the surfactant cetyltrimethylammonium bromide and patented. Discovery of M41S materials also caused many advances. These materials are synthesized on alkaline conditions by utilizing cationic surfactant. Different organic materials addition and variable reaction conditions (temperature, pH, crystallization time, pressure, etc.) can control the structure of mesoporous material. There are three types of this kind, which labeled as Mobil Composition of

Matter (MCM) group; lamellar MCM-50, cubic MCM-48 and hexagonal MCM-41 [3]. Also, MCM-41 surface functionalization exhibited to regulate the material endocytosis which is a key factor for intercellular delivery. A transmission electron microscopy (TEM) is presented as follow [19]. MCM-41 has about 4 nm pore size and it has uniform mesoporous hexagonal array. One of the properties of MCM-41 is that the pore diameter is usually less than pore length. Since the pore walls of MCM-41 are made of tetrahedral silica arrangement, the interactions between pores are not energetically satisfactory [20, 21].

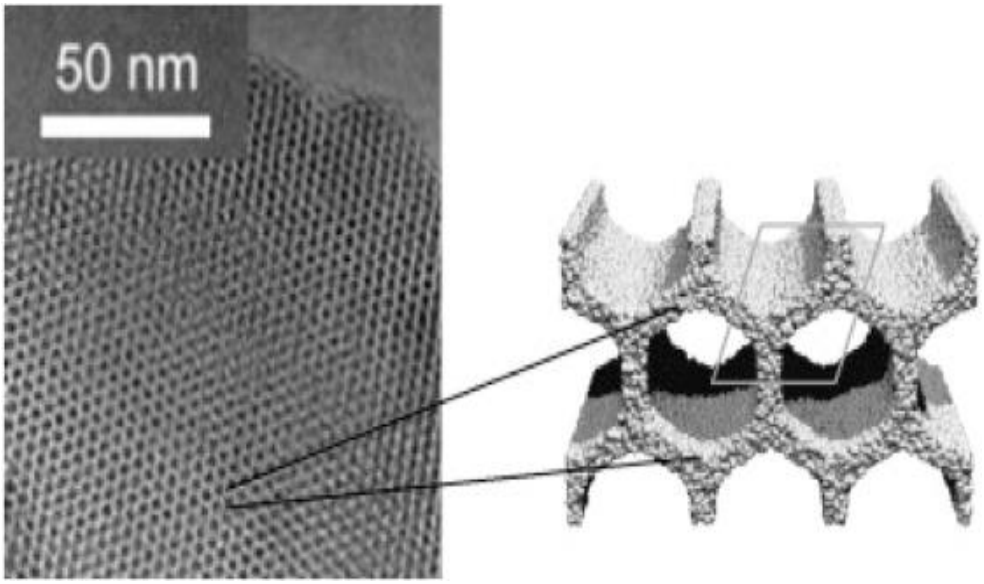


Figure 2. TEM image of the MCM-41 and a schematic representation of the hexagonal shaped one-dimensional pores.

MCM-48 sometimes uttered itself as a superior candidate for catalytic and separation technologies due to the three-dimensional interwoven structure, to be more resistance to pore blockage and higher catalytic activity than one dimensional counterpart MCM-41 [22].

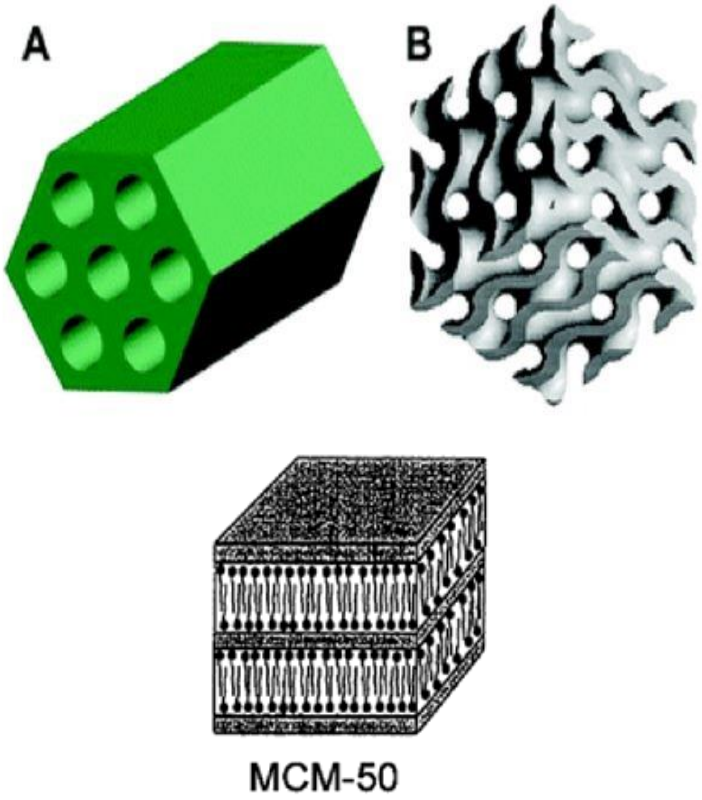


Figure 3. Illustration of a) MCM-41 b) MCM-48 c) MCM-50.

The other type as it has referred is MCM-50 which due to the hard and time-consuming synthesis steps, is less appealing for scientist to investigate on it. MCM-50 has lamellar structure and thick pore walls, though it shows low stability [23]. Also, there are many other types of mesoporous materials with different type of application and acquiring methods such as; MSU (Michigan State University), OMS (Ordered Mesoporous Silica), HMS (Hollow Mesoporous Silica), MCF (Meso Cellular Form) and etc. [20].

1.3. APPLICATION OF MESOPOROUS MATERIALS

Recently, nanoscale materials have been utilizing in many major fields, and crave of using them in other subjects by considering the probable capability enhancement of them in these subjects is rising every day. Mesoporous materials are especially under investigation for extensive applications, which they exhibit.

Mesoporous materials are widely applied in vast areas such as; biosensors, catalysis, biofuel, drug control delivery systems and membrane [24, 25].

More recently, MSN materials have uttered themselves as possible cases for optical and electronic applications, too [8]. Due to the low and non-toxicity and biocompatibility of mesoporous materials, they have seen appealing materials for the field of drug delivery systems, and the properties such as ordered mesoporous in silica materials led these materials to be hot topic for many years in this field [26]. At the end, it could be uttered that mesoporous silica materials have applications in three major areas such as engineering, therapy, and diagnostics. More particularly, for therapy applications such as targeted therapy, controlled drug release and drug solubility improvement. Also, in engineering such as; implants, adjuvants and gene transfer. Moreover, diagnostics such as; separation techniques and optical and electrochemical sensors [27].

1.4. SURFACE FUNCTIONALIZATION

Surface modification or functionalization which is one major advantage of mesoporous materials rises their ability to be utilized in advance applications. In this purpose, organic and inorganic materials have been exploited with different goals. So far, co-condensation [26, 27] and post-synthesis grafting [28, 29] are the two major methods for placing amino groups onto the silica surface.

1.4.1. POST-SYNTHESIS AND CO-CONDENSATION GRAFTING METHOD

Generally, co-condensation functionalization synthesis method, which consists of a co-condensation of silica precursor and organosilane during the self-assembly, seems to be more plausible because of less probability of providing lacks and shortage rather than post-synthesis grafting method. As an example, by post-synthesis method, there are possibilities that pore size reduction and blocking at aperture of pores might happens. Also, it would be harder to control the loading as well as distributions of the active sites [32]. This kind of porous structure can efficiently amplify the (apparent) solubility of incorporated drug with poor solubility which these properties emerges from two things. Firstly, avoidance of recrystallization upon deposition into molecular sized pores [33], and secondly, mesoporous silica itself, shows very spectacular loading capacity which is able to cargo the same weight of itself. On the other hand,

there are some specific disadvantages accompanied with both methods. Such as; inhomogeneous surface coverage in post synthesis grafting method [34]. Delectation of the degree of mesoscopic order with rising concentration of organic groups could be a disadvantage of co-condensation method. Also, large amount of functional groups are hardly accessible or inaccessible and embedded in the silica network. These drawbacks associated with the co-condensation method which are some of the other possible weaknesses of this method [35].

At the end, there are also some new methods that have been advanced to abolish the possible drawbacks go along with both methods. Such as; acid-catalyzed hyper-branching polymerization method, which provides enormous amount of reactive amino groups in the form of surface-grown polyethyleneimine (PEI) on the surface of mesoporous materials [34, 35].

1.4.2. SURFACE MODIFICATION BY PEI

In the case of polyethyleneimine (PEI) surface functionalization, aziridine is a utilized monomer. The PEI is exceedingly reactive and expressively very small which grows directly from the surface silanol groups. These properties suggest PEI as an impressive choice for effective functionalization in the range of nanometer size.

Among other methods, hyperbranching surface polymerization seems very effective for amino functionalization of mesoporous silica materials. The reason could be due to the fact that surface concentration of amino groups is much higher than post-grafting method and co-condensation method [35–38]. Another advantage of this method is that the amount of PEI layer on the surface could be readily manipulated by changing the ratio of aziridine/silica in the surface functionalization step.

The other outcome of PEI functionalization is that, as some studies demonstrated, a hydrophilized mesoporous silica material could be fully redisposed in aqueous media [41]. Besides, using PEI as surface modifier seem to be promising for capturing of numerous molecules with pharmaceutical interests owing the widespread usage of amine chemistry in bioconjugation reactions [40–44].

A disadvantage of this method is that when a large amount of organic surface groups is incorporated, there would be a high possibility that saturation of the mesoporous structure by this organic species might happen. Consequently, choosing of material

with high pore volume and large pore size is obligatory to avoid the risk of saturation [47].

Generally, surface polymerization would result to have high amount of accessible amine groups. However, sometimes, there is a possibility that first polymerization cycle leads to polymer formation in micropores in the mesopore walls, which will cause these amine groups to be inaccessible [48]. It could be mentioned that micropores would be filled with PEI and microporosity would not be available usually after surface modification by PEI.

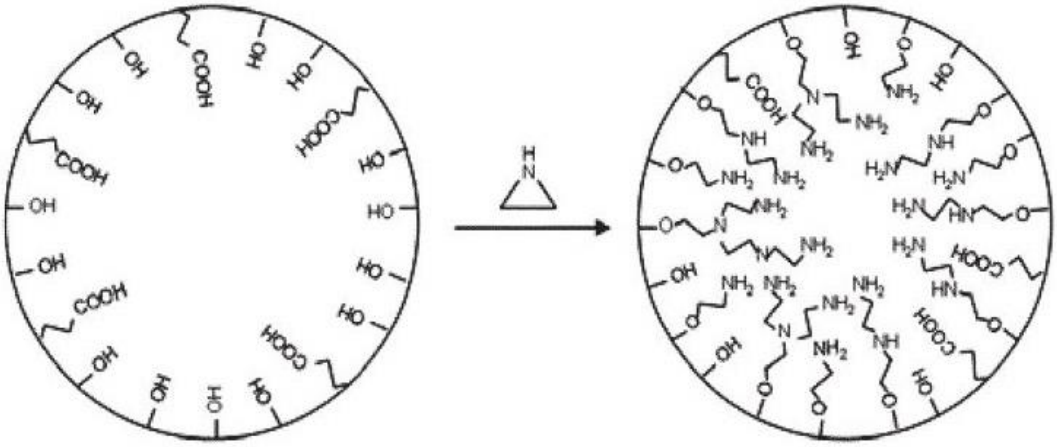


Figure 4. Schematic process of surface modification of mesopores with PEI [49].

1.5. CONTORLLED DRUG DELIVERY SYSTEMS

Controlled drug delivery system is the appropriate administration of drugs in the body through various routes. The purpose of this system is to improve health with a less possible side effects. This field is highly interdisciplinary, and it is not a young field, but it has recently evolved to take into consideration. An ideal DDS have some characteristics such as; prolonged release (the level of drug is consistent for an extended period of time), targeted release (site-specific drug delivery independent on site and route of administration), bioavailability, non-toxicity, biocompatibility, biodegradability and stability. The main difference between traditional and DDS are the period time settable and release target settable, because in some abrupt release in a specific target it might be necessary and in some other consistent dosage of drug in major area of body for long period. Also, the drug level and dosage should be below

minimal toxic concentration (MTC) and always above the minimal effective concentration (MEC), this level of range is so called as therapeutic range.

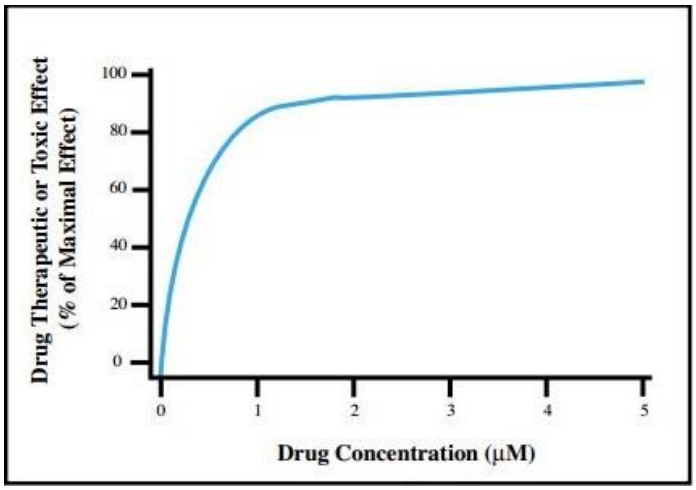


Figure 5. Relationship between drug concentration and the therapeutic and toxic effects.

The therapeutic and toxic effects of drugs are dependent by their concentration in the vicinity of drug receptors. When concentration is high, it effects plateau. Hence, the high amount of drug is not certainly the highest efficiency and it may cause toxicity. So, DDSs are actually improving the possibility to reach these goals. The main problem of DDS is drug activities before it reaches to the desire target. Drug delivery systems are approved by Food and Drug Administration (FDA). DDS are mainly having two components; particulate carrier and associated therapeutics. There are vast types and kinds of drug that could be used in DDS system such as antibiotics or chemotherapeutics [40, 48].

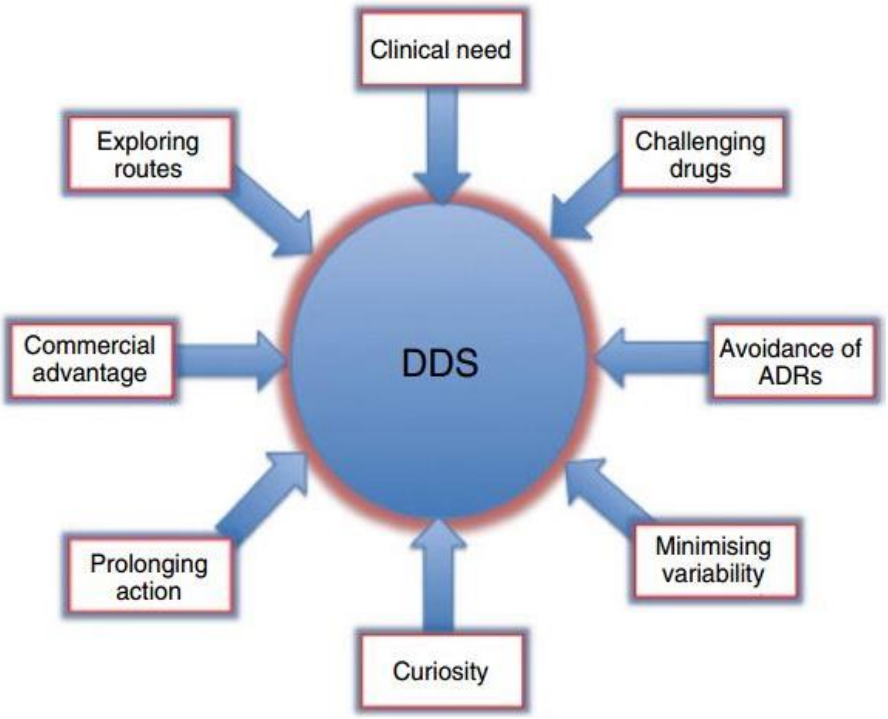


Figure 6. The driving forces for the development of optimized or controlled release systems [51].

There are diverse types of drug carriers and systems which all serve in same purpose of advancing DDS such as; usage of polymers, liposomes, nanoparticles, graphene, carbon nanotubes, quantum dots, mesoporous silica nanoparticles (MSN) and some others. Among these various types, MSN materials has been picked most efficient one due to the spectacular properties which shows such as thermal stability, pore stability, biodegradability, and more characteristics vital for DDS applications [52, 53]. There are different types of drug administration routes dependent to the target and usage such as nasal, ocular, transdermal, pulmonary and parenteral, but, the most convenient method is oral delivery [54].

There are some major factors influencing drug delivery in oral administration should be highly considered in case of studies. One of these factors is pore size compatibility, since different drugs have different size, a tunable pore size is desired. So, drug adsorption and release from the support material can be manipulated by size selectivity. For a practical example, in a case of MCM-41 used as a carrier and ibuprofen for drug model, by decreasing the pore size of MCM-41, the rate of drug

release also increases [54]. Moreover, the chemical interaction of drug and carrier and generally interactions between them should be considered, too.

For better efficiency and advance application surface modification should be applied to obtain better adsorption and release of drug molecules. Surface modification play a significant role since it can alter the hydrophobic, hydrophilic forces, electrostatic and the adhesive interactions of drug and matrix that can mainly effect the load and release behavior of drug delivery system [53, 54].

Since oral administration is most widespread method, it should be deemed that dissolution of drug molecule in the gastrointestinal (GI) milieu is a prerequisite for the absorption process. Major percent of new chemical entities (NCEs) discovered or advanced these days by pharmaceutical industry are poorly soluble or lipophilic compounds.

Hence, it could be uttered that solubility should be highly considered in these technologies. Also, low drug solubility often manifests itself; in a host of in vivo consequences, including; increased chance of food effect, diminished bioavailability, higher interpatient variability and more possibility of early or incomplete release. Scientists, active in pharmaceutical research are hardly engaged by these subjects to overcome these mentioned drawbacks, and to enable oral delivery of new chemical entities [57].

1.6. HYDROPHOBIC DRUGS

As it has been mentioned, oral administration is most common method and preferred one due to the feasibility and convenience of this method. A drug can be useful in the oral administration that is fully bioavailable. Drug should have characteristics such as good solubility, permeability and stability.

However, the problem of poor drug solubility or poor membrane penetrability still remains in traditional oral administration. Besides, it has been reported that a large portion of new drugs are poor soluble. Heterogeneous molecules which have poor solubility in water, but higher solubility in organic solvents are called as “hydrophobic drugs”. Hydrophobic drugs are divided to three major types; slightly soluble (1-10mg/ml), very slightly soluble (0.1-1 mg/ml) and insoluble (<0.1 mg/ml) [58]. Consequently, enhancement of hydrophobic drug solubilization is important subject

in the area of drug delivery. This can significantly affect the optimum adsorption of drugs. Air adsorbs to the surface of hydrophobic drugs which hinder the wettability of them. Thus, due to the high surface energies, the particles might re-aggregate to form larger particles.

Electrically induced agglomeration owing to surface charge prevents intimate contact of the drug with the dissolution media. It is not negligible that solubility and dissolution of many drugs are dependent on overcoming to this challenge of hydrophobic drugs and advancing them as drug delivery systems. It should be referred that due to the gastrointestinal tract and aqueous medium of blood, a disagreeable environment for hydrophobic drug delivery is available and could not be ignored. At the end, inadequate solubility would lead to unwanted pharmacokinetics properties and reduce the therapeutic effects of drug molecules. There are broad range of studies that endeavor to enhance the solubility and pharmacokinetic properties of these types of drugs [58].

1.6.1. MAJOR FACTORS ON HYDROPHOBIC DRUGS SOLUBILIZATION

Solubilization literally means the intermolecular and inter-ionic bonds breaking, separation of solvent or solute molecules and interaction between them. At early stage, holes of the solvents open then solid molecules breaks away from the bulk and in the last step, free solid particles are contained within the solvent.

There are some factors that majorly or slightly affect the process of solubilization of hydrophobic drugs that some could be manipulated in the process and some are untouchable. Physical form of drugs, environmental condition and composition of solvent medium, polarity of materials and molecular size of solvent, could have enormous influence on the hydrophobic drug solubilization.

It could be clarified that by decreasing the particle size of drug, the surface area is increasing, consequently, drug particles have better chance to interact with the solvent. Likewise, factors such as temperature could play role in the way that by increment of system temperature, solution will absorb energy and so, solubility of drug will rise.

Additionally, molecules with lower molecular weight could cause increase in solubility. As well it could be stated that some drugs have polar ends (positive or

negative) in the case that solvent molecules also are polar, depend on the positivity of both of them the solubility would be increased (in case of positive interacting with negative end which is known as dipole-dipole interaction enhancing the solubility of hydrophobic drugs) or decreased [59].

1.6.2. MECHANISM OF SOLUBILIZATION AND MANIPULATION METHODS FOR ENHANCEMENT OF SOLUBILIZATION

One of the effective methods to increase the solubility of hydrophobic drugs is reduction of particles size which ends to increase the surface area. Size reduction could be usually obtained by grinding or controlled crystallization. Despite of utilizing this method commonly by researchers, there are some limitation accompanied. As an example, when a surface area is large, there is high surface charge of the drug molecules which rise the agglomeration possibility. For eliminating this risk, appropriate excipients could be utilized [60].

Also, solid dispersion is a technique that increases dissolution rate and correspondingly enrich bioavailability of the drug. There are different types of solid dispersion that dependent on physical state of drug and carrier could be utilized. On the other hand, there are some drawbacks with this method, for instance; it is time consuming, generally hard, and finding a suitable solvent is a challenge. Suitable solvent should be able to dissolve both components and usually the carrier is hydrophilic and the drug is hydrophobic which make it hard to find appropriate solvent.

Moreover, reproducibility and stability of the technique is not satisfying since it is a method dependent on factors such as; heating rate, temperature, cooling method and mixing time [59, 60]. Another attempt to enhance solubility is to change the solute or solvent properties which may lead to increase the solubility of drugs. According to Yalkowsky; buffers, surfactants and complexing agents are able to shift the solubility of nonpolar drugs to higher levels [63].

The other methods that seems more promising these days is using an inorganic carrier such as silica which provides a situation that hydrophobic drug can be molecularly dispersed in the arrays of pores in inorganic carrier. Then, by introducing solvent to

the pores, drug release will occur. This method has advantages such as tunable pore size, enormous loaded capacity, ordered pore network and etc. [64].

1.6.3. CELECOXIB AND PHARMACOLOGY

Celecoxib, a non-steroidal anti-inflammatory drug (NSAID) (drug class that groups together, drugs that reduce pain, decrease fever, and, in higher doses, decrease inflammation), is the first selective cyclooxygenase-2 (COX2) inhibitor (this type, selectively and directly targets) used in the treatment of osteoarthritis and rheumatoid arthritis in adult patients.

CLX reduces hormones that cause pain and inflammation in the body, and it is used to treat pain or inflammation caused by many conditions such as menstrual pain and arthritis. Another application of CLX is for treatment of hereditary polyps in the colon [65].

Celecoxib also has a lot of usages that is not listed here. This poorly soluble drug is highly applicable for chemoprevention of colorectal cancer.

There are some obstacles for delivery of CLX to the colon, in terms of targeting the desired gastrointestinal (GI) region. These obstacles are due to the limited fluid content and the high fluid viscosity that this drug has. There has been investigations to modify by especially drug delivery systems [65].

All of these conditions would lead to a problematic situation in term of solubilization, particularly for drugs with poor water solubility. Hence, solubilization problem will strongly affect the therapeutic and clinical performance of these kinds of drugs. So, there is desire for delivering these kinds of drugs and raising their performance [66].

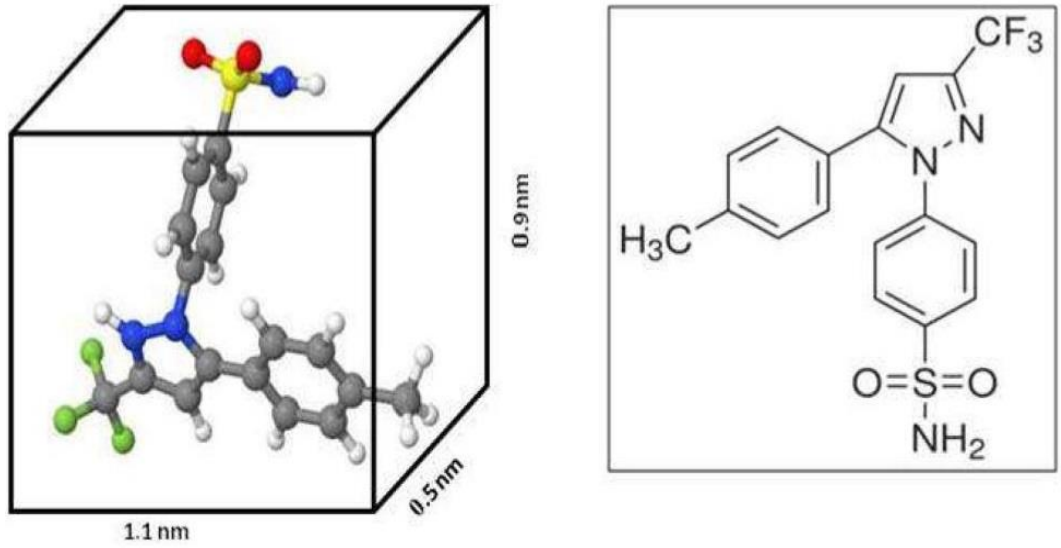


Figure 7. Structure of CLX**.**

1.7. AIM OF THIS WORK AND LITERATURE REVIEW

There have been studies about polyethyleneimine surface modification on SBA-15 majorly by J. M. Rosenholm et al. Previous studies were mostly focused on the efficiency of PEI modifying and the structure of pores and the possibility of surface modification by PEI on mesoporous materials and more specifically what characteristics should mesopore material have to be compatible for PEI modification [67]. In the mentioned research, Prednisolone was used as a drug model which is a hydrophobic and steroids type of drug. The outcome was enhancing the bioavailability of the drug by gaining more release and loading amount.

PEI modified samples are mainly focused on the area of CO_2 capturing or adsorption systems and has raised attention in past few years for this particular application. It could be uttered that usage of PEI modified SBA materials was highly promising in this field [68]. Nevertheless, there have been few investigation of them on the area of DDS. Most of research by the utilizing PEI functionalized samples, have exhibited some improvements in the bioavailability and efficiency of drug delivery system. In case of enlightening the solubility of poor soluble drug, there were very few studies that a raised in efficiency of bioavailability of the drug was observed. There was no particular study till this time, on the Celecoxib for this specific surface modification and synthesizing method for SBA [65, 67].

One of the aims of this thesis is to synthesize large pore SBA-15 particles and then functionalize the surface of the pores with polymerization of PEI, to use them as drug carriers. We intended firstly to synthesize a monomer of Aziridine and then surface modifying by polyethyleneimine. We took some characterizations to assure that polymerization from our obtained monomer have been took place.

A hydrophobic drug CLX was used as drug model to study on loading and release properties and to overcome the poor solubility of CLX and to enhance its bioavailability. In this study, for the first time, celecoxib was utilized as a drug model for PEI modified SBA samples. Since it is a poor soluble drug, is preferable candidate as a model to be studied. At the end, loading and release trend of LP-SBA-15-PEI (PEI stands for polyethyleneimine) and not functionalized form of LP-SBA-15 were compared as their drug loading and release behavior.

Various methods of characterizations have been utilized to clarify the differences among these carriers; such as pore properties, structure characters, physical and chemical, thermal properties and toxicity tests. Hence, we did some extra characterizations for better understanding of the efficiency of PEI modification such as SEM, DSC etc. Also, we did toxicity test to find out about the biocompatibility of PEI modified samples through the practical application of them.

CHAPTER 2

EXPERIMENTAL

2.1. MATERIALS

Poly (ethylene glycol)-block-poly (propylene glycol)-block-poly (ethylene glycol) (Pluronic 123, $[C_3H_6O.C_2H_4O]_x$, M_w=5800 g/mole) and tetraethyl orthosilicate (TEOS, $[C_2H_5O]_4Si$ M_w=208.33 g/mole), 1,3,5-Triisopropylbenzene (TIPB, $C_6H_3[CH(CH_3)_2]_3$, M_w= 204.35 g/mole) and 2-aminoethyl hydrogen sulfate ($NH_2CH_2CH_2OSO_3H$, M_w=114.15 g/mole) were purchased from Aldrich. 37% hydrochloric acid (HCl), celecoxib (Pfizer, Celebrex, 200 mg), NH_4F, NaOH, KOH and ethanol were purchased from Sigma-Aldrich. KH_2PO_4 was purchased from Merck, and K_2HPO_4 was obtained from Riedel-Haen. Toluene RPE ($C_6H_5CH_3$) was purchased from Carlo Erba. All of the chemical reagents were used without any purification. Deionized water was used during experiments.

2.2. SYNTHESIS OF MESOPOROUS SILICA

SBA-15 particles were synthesized according to Zhao and Huo et al.[68, 69]. The nonionic triblock copolymer (Pluronic 123) (2.4 g) and 0.027 g of NH_4F were dissolved in 84 ml 2 M HCl at room temperature under mechanical stirring. Then, the obtained solution was placed in a water bath and mechanically stirred for 1 h, after which 5.5 mL of tetraethylorthosilicate (TEOS) and 1.2 mL of 1,3,5-triisopropylbenzene (TIPB) were added gradually. The mechanical stirring was continued for 24 h at room temperature under atmospheric pressure, and formerly aged under static conditions and autogenous pressure at 90 ˚C for 24 h. At this point,

the sample was filtered, washed and the solid product was dried at 50 ˚C temperature for 24 h in an oven. This method was used to obtain large porous SBA-15 [72]. At the end, in order to eliminate the surfactant templates, calcination was carried out at 400˚C for 1 hour and then 600˚C for 12 hours [41]. The acquired SBA-15 type was labeled as LP-SBA-15.

Figure 8 represents the synthesis scheme of LP-SBA-15 and table 1 shows the precursors which were used during the experiments.

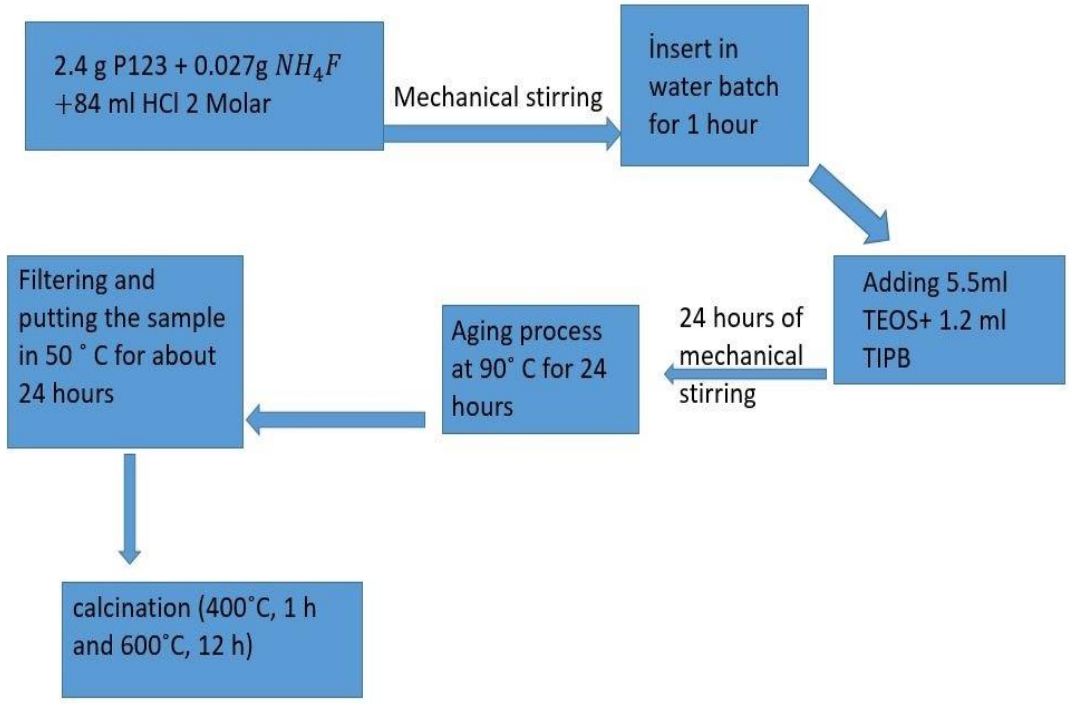


Figure 8. Schematic representation of synthesis of LP-SBA-15.

Table 1. Precursors used for LP-SBA-15 synthesis**.**

Chemicals	**m(g) or V(L)**	**Brand**	**d (g/mL)**	**MW (g/mol)**
Pluronic 123	2.4 g	Aldrich	1.01	5800
TEOS	5.5 mL	Aldrich	0.93	208.3
NH_4F	0.027 g	Sigma-Aldrich	1.01	37.04
TIPB	1.2 ml	Aldrich	0.845	204.35
HCl	84 mL	Sigma-Aldrich	1.20	36.46

2.3. SURFACE FUNCTIONALIZATION OF LP-SBA-15

2.3.1. REHYDROXYLATION PROCESS

According to the nature of this process, for obtaining a desired result, increasing the amount of amine sites is obligatory and would direct the last result to enhance one. One of the methods, which has been presented rise in surface silanol groups concentration from 2 to 5 μmol/m^2 for SBA-15 materials is rehydroxylation [37]. There was no loss of order observed for the treated samples by this method which is advantage of this specific method.

The practical sequences were; firstly, silica sample were refluxed with hydrochloric acid (18.5 % w/w) for 12 h. The amount of HCl here is dependent on the amount of silica samples and it need to get all of the powder wet enough, so it could be mechanical stirred as liquid. Latterly, the rehydroxylated silica supports were vacuum-dried at 45 ˚C for about day. The set up for this experiment shown as figure 9 and table 2 shows the precursors which were used during the experiments.

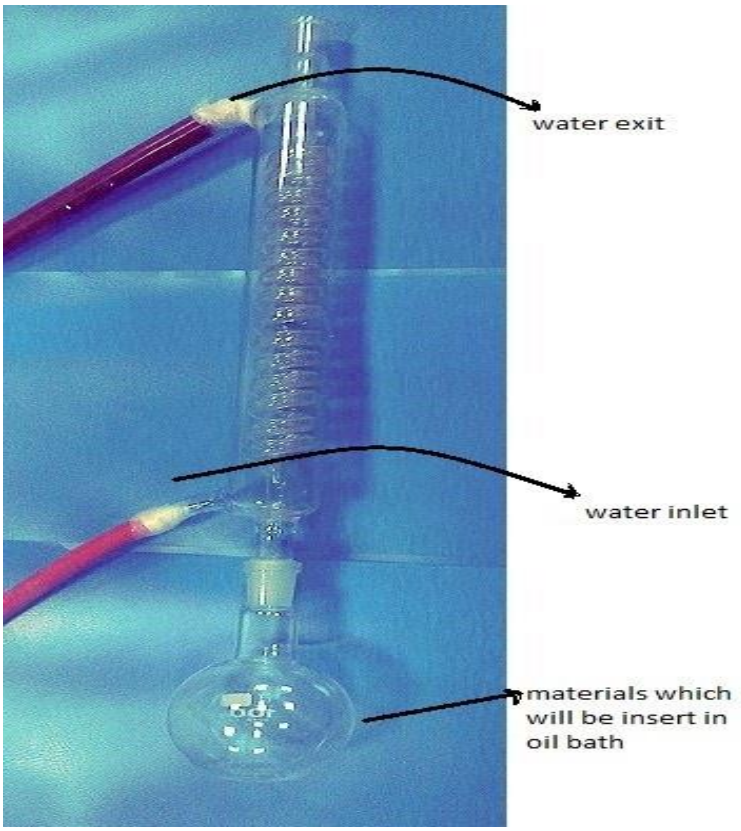


Figure 9. The set up for rehydroxylation experiment.

Table 2. Precursors used for rehydroxylation.

Chemicals	**m(g) or V(L)**	**Brand**	**d (g/mL)**	**MW (g/mol)**
HCl	100 mL	Sigma-Aldrich	1.20	36.46

2.3.2. AZIRIDINE SYNTHESIS

Before the surface functionalization of LP-SBA-15 by polyethyleneimine, aziridine monomer was synthesized. Aziridine was obtained by; primarily, gradually mixing and solving of 14.08 g NaOH and 21.12 g deionized water, then, adding 11.28 g 2-aminoethyl hydrogen sulfate. This mixture, was heated in an oil bath at boiling temperature (about 125 ˚C) in the distillation set. Distillated material was collected as quickly as possible in the well-cooled receiver. About 10 g KOH pellets was added gradually, whereupon the imine separated as an upper layer. Later, Organic layers left-over during a night in a refrigerator.

Finally, the day after the upper layer of the material was separated and taken to a different tube, the material having become the desired product and was named aziridine. The set and synthesis scheme for this experiment shown in figure 10. Table 3 shows the precursors which were used during the experiments.

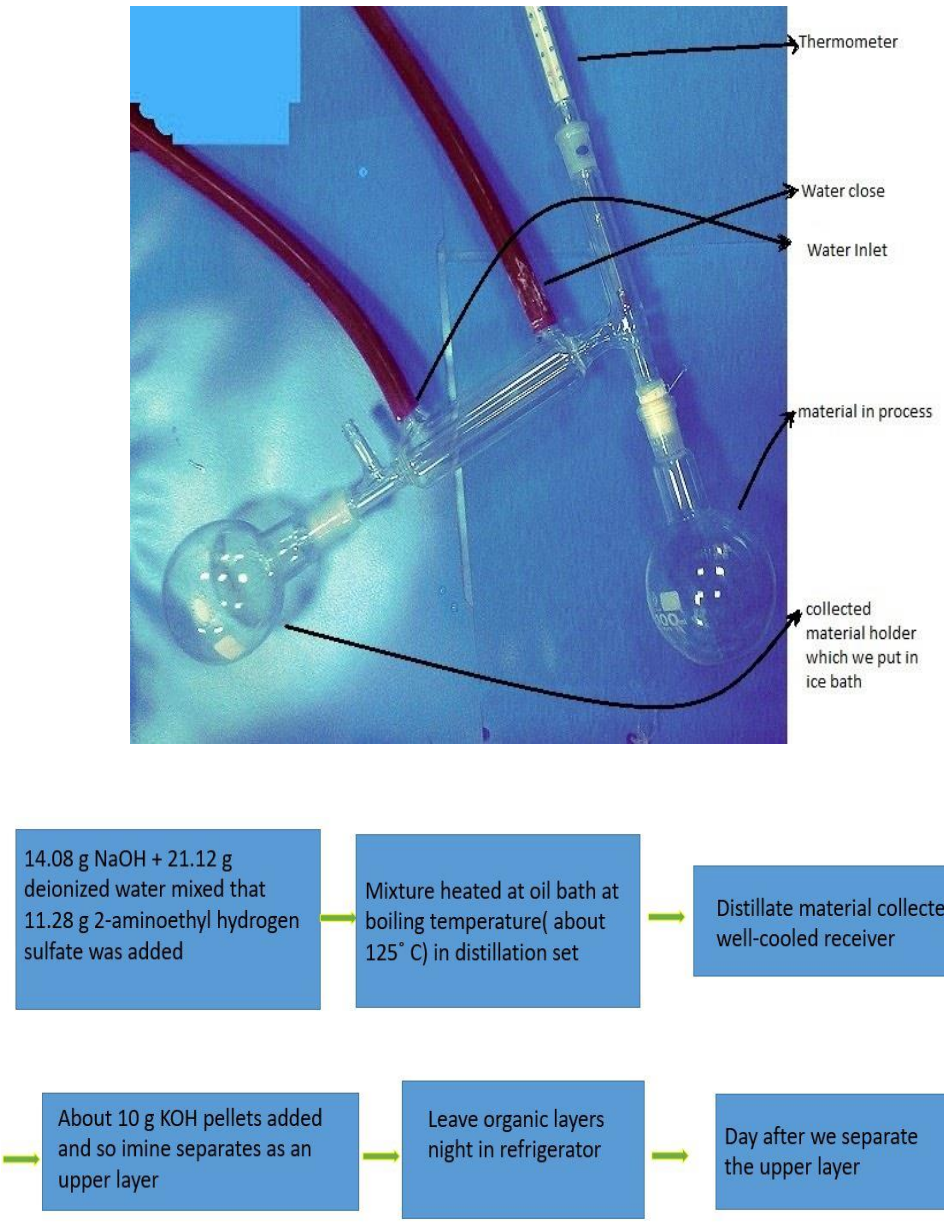


Figure 10. Set up and synthesis scheme for the aziridine synthesis.

Table 3. Precursors used for aziridine synthesis.

Chemicals	**m(g) or V(L)**	**Brand**	**d (g/mL)**	**MW (g/mol)**
NaOH	14.08 g	Sigma - Aldrich	2.13	39.99
$NH_2CH_2CH_2OSO_3H$	11.28 g	Sigma - Aldrich	———	141.15
KOH	10 g	Sigma	2.12	56.11
Deionized water	21.12 g	———	———	———

2.3.3. SURFACE POLYMERIZATION

Modification of surface from the silanol groups on the surface by polymerization was done by usage of toluene as a solvent. Firstly, 1g of silica materials were suspended in toluene in the presence of 52.8 mg of acetic acid under mechanical stirring after achieving to homogeneity, 0.5 ml of aziridine was added drop-wisely and the suspension was refluxed under nitrogen atmosphere for 2 hours at about 85˚C in an oil bath. Inert gas had been subjected to the materials for 1-2 hours and after that the condenser was removed, however, the magnetic stirring and heating continued for about 6-8 hours. Consequently, the obtained material was washed, filtered with toluene, and dried in vacuum at 45˚ C for about 24 hours.

The resultant hybrid materials were denoted as LP-SBA-15-PEI [43, 46]. The set and synthesis scheme for this experiment shown in figure 11**Figure 11**. Table 4 shows the precursors which were used during the experiments.

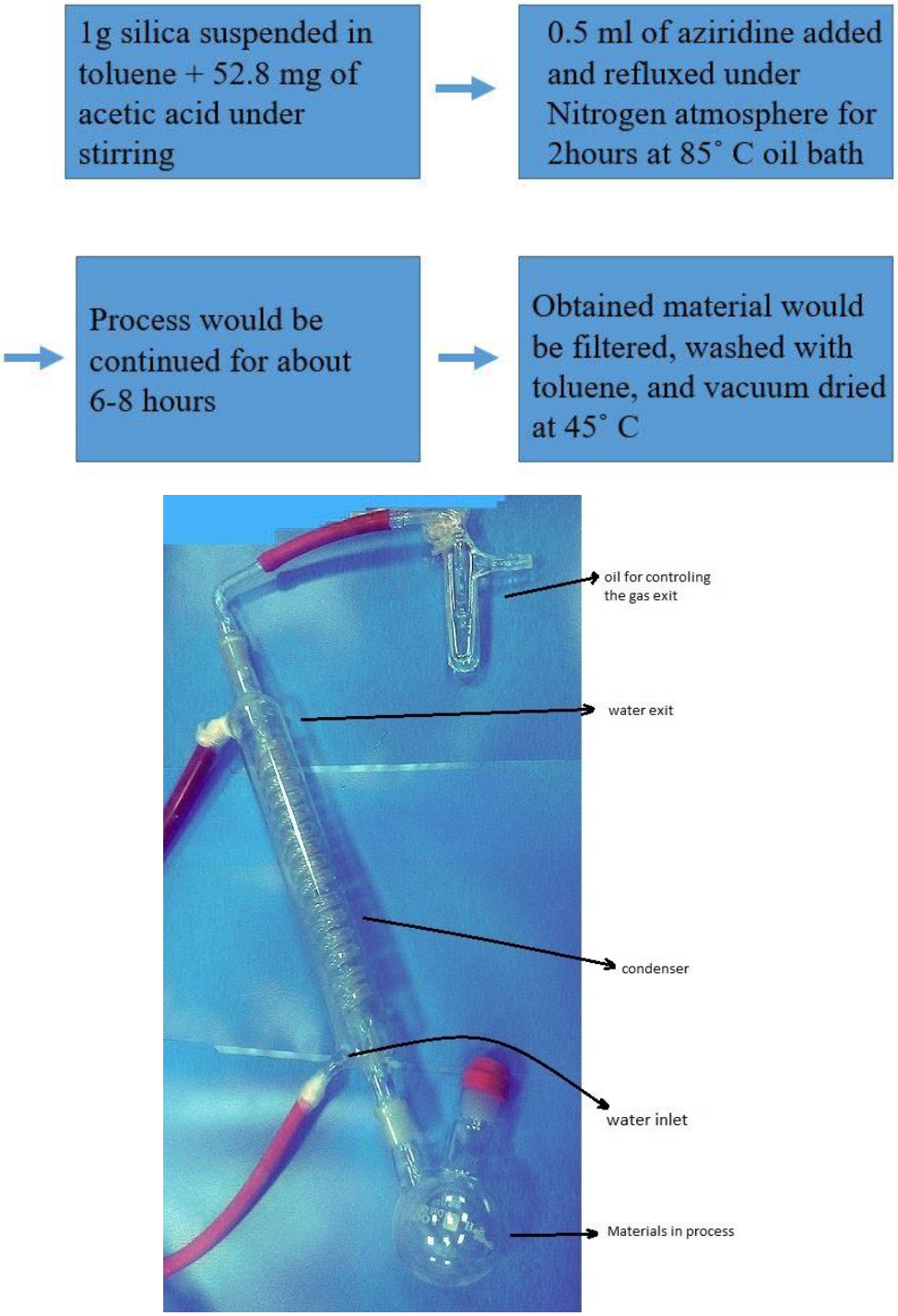


Figure 11. Set up and synthesis scheme for surface polymerization.

Table 4. Precursors used for polymerization.

Chemicals	**m(g) or V(L)**	**Brand**	**d (g/mL)**	**MW (g/mol)**
Acetic acid	52.8 mg	Sigma-aldrich	1.119	61.06

2.4. AMINE SITES QUANTIFICATION ON THE POLYETHYLENEIMINE SILICA

Assessment of amount of amine sites in silica pores or the quantification of them should be done, which for this purpose, elemental analysis was performed at METU central laboratories, and the result was compared with other publications. Mostly this compound as polyethyleneimine added to silica is used in purpose of CO_2 capturing in literature [73].

The available nitrogen indicates readiness of PEI in the sample and we are not expecting to see any nitrogen in LP-SBA-15. The availability of nitrogen percentage would assure us of present of polyethyleneimine on the sample and a higher amount of nitrogen percentage has the meaning of more PEI available inside the material in case of our sample [74].

Hence, LP-SBA-15-PEI was investigated as a sample to provide amount of nitrogen percent so it could be comparable with other literature sources. Studies that had done the identical procedures for synthesis of SBA-15-PEI with same structure obtained 6-8% amount of nitrogen as a result of elemental analysis [75]. In this procedure, we measured approximately 7.10 percent of nitrogen availability as concerned and it is reasonable. The obtained data are in good agreement with the results reported in the literature [71, 72].

2.5. CLX LOADING

For CLX loading, a liquid-phase grafting method was utilized. By considering solvent efficiency and toxicity of solvent in past studies [76], ethanol was chosen as an appropriate media for loading the drug. Since as it has been reported before, hexane shows a toxicity and methanol does not show a desired result.

To determine the effect of carrier and polymerization on drug loading; LP-SBA-15 and LP-SBA-15-PEI were taken under investigation. In the loading part Celecoxib

was examined by two different carriers. Celecoxib was loaded to the LP-SBA-15 and LP-SBA-15-PEI silica particles in ratio; 1:1 (wt: wt). For this purpose, 100 mg of carrier and 147 mg of drug was mixed in presence of 50 ml ethanol by utilizing ultrasonic for about 30 minutes and also mechanical mixing as night long.

The obtained samples were denoted as LP-SBA-15-CLX and LP-SBA-15-PEI-CLX where CLX stands for celecoxib. The CLX loaded sample, were washed with ethanol and dried at room temperature and further on at 40˚C in oven for eliminating all possible solvent left overs.

TGA was utilized to illustrate the amount of loaded drug.

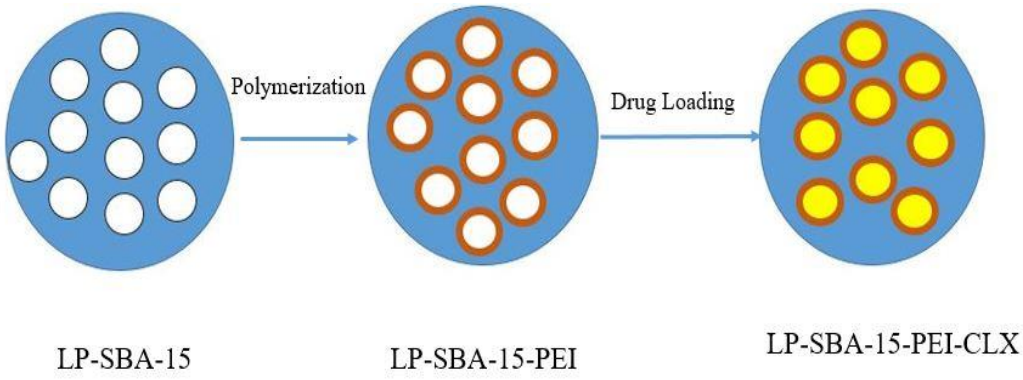


Figure 12. Schematic representation of steps toward drug loading in this research.

Table 5. Precursors used for Celecoxib loading to LP-SBA-15 and LP-SBA-15-PEI.

Chemicals	**m(g) or V(L)**	**Brand**	**d (g/mL)**	**MW (g/mol)**
LP-SBA-15 LP-SBA-15-PEI	0.1 g			60.08
Celecoxib	147 mg	Pfizer		381.7
Ethanol	50 mL	Merck	0.79	46.07

2.6. CLX RELEASE

For release part, Phosphate buffer (PBS) has been prepared of two different pH. Firstly, by mixing 13.97 g K_2HPO_4 and 2.69 g KH_2 in 1-liter deionized water, which pH= 7.4 has been obtained. Also, pH= 5.0 has been obtained by mixture of 8.96 g K_2HPO_4 and 0.5 KH_2, the amounts of these two compounds sometimes need to be manipulated to obtain the desire pH. The obtained media pH, was certified by using pH meter.

The reason of two different pHs comes from different pH of the blood in a body of human and the pH of blood around the cancer cells which is about 3-5 as the studies shows [77]. In the release experiment 0.5 g of drug loaded sample added to 50 ml of PBS and mixed by mechanical stirring at 37˚ Centigrade. Sample taking was done by time intervals of ½, 1, 2, 4, 6, 12, 24 and 72 hours after that, under study sample reaches to 37˚ C. In each step, sampling was done by taking 5 ml of total amount which represent the specific hour for release. The same procedure was performed for LP-SBA-15, LP-SBA15-PEI and commercial Celecoxib drug. Then UV characterization have been done as soon as after taking samples. By reading amount

of absorbance on 254 nm, amount of drug released could be calculated by using the Beer's law.

2.7. CHARACHTERIZATION

2.7.1 POWDER X-RAY DIFFRACTION (XRD)

Powder X-ray diffraction patterns were attained with a Rigaku X-ray Diffractometer with a Miniflex goniometer operated at 30 kV and 15 mA Cu-Kα line (α=1.54 Å) as the source of X-ray. The range of 2θ degree was arranged between 3˚ to 70˚. The scan step was 0.01 and scanning speed was adjusted to 1.0 degree per minute and the scanning mode was chosen as continuous scanning on. Also, low angel was performed in the 2θ range between 0.5-3 degrees. The speed was set up to 0.01 degree per minute for obtaining more precise result by 0.01 step size.

2.7.2. SMALL-ANGLE X-RAY SCATTERING (SAXS)

Small-angle X-ray scattering (SAXS) measurements were performed on a Kratky compact Hecus (Hecus x-ray systems, Graz, Austria) system equipped with a linear collimator and an X-ray tube having a Cu target (l = 1.54 Å), operated at a power of 2.0 kW (50 kV and 40 mA).

2.7.3. NITROGEN – SORPTION

N_2 adsorption/desorption measurements were conducted at 77 K in an Autosorb 6 (Quantachrome). Before measurement, each sample was degassed at 50 ˚C – 120 ˚C for 16 h. Pore characteristics were determined by using the BJH (Barret-Joyner-Halenda) and BET (Brunauer-Emmett-Teller) methods.

2.7.4. FOURIER TRANSFORM INFRA RED SPECTROSCOPY (FTIR)

FT-IR spectra of all samples were obtained with a Varian 1000 FT-IR (Scimitar FTS 1000) in the range of 400-4000 cm^{-1} at room temperature from KBr pellets. For preparation of pellets, firstly, KBr was dried for any possible moisture at oven for 2 hours or more at 200 ˚C. Then, about 0.01 mg of samples and 100 mg of KBr were taken and mixed homogenously. By the usage of pelletizer and presses at 5000-10000

psi on obtained mixture, the sample pellets were acquired. Both carriers were characterized before and after Celecoxib loading by FTIR.

2.7.5. THERMOGRAVIMETRIC ANALYSIS (TGA)

Thermogravimetric analysis was carried out in a Pyris 1 Perkin Elmer Thermogravimetric Analyzer under the nitrogen atmosphere at temperatures between 30 and 650˚C with a heating rate of 10˚C/min.

2.7.6. DIFFERENTIAL SCANNING ANALYSIS (DSC)

DSC (DSC N-650) analysis was carried out in the range between -120 ˚C and 250˚C at 5 ˚C/min heating rate. Under the nitrogen atmosphere to prohibit any reaction in the sample.

2.7.7. TRANSMISSION ELECTRON MICROSCOPY (TEM)

TEM images were taken with JEOL JEM 2100F STEM and a JEOL JEM 2100F Field Emission Gun at 120 kV. Before performing the analysis, samples dissolved in ethanol in the Elma S 30 H ultrasonic bath for 15 minutes. Cu grid was used in this experiment.

2.7.8. SCANNING ELECTRON MICROSCOPE (SEM)

SEM images were taken with Zeiss SUPRA 50 VP at 80 kV. For enhancement of imaging quality, the samples were coated with a thin layer of conducting material, Au/Pd alloy before characterization.

CHAPTER 3

RESULT AND DISCUSSION

3.1. X-RAY DIFFRACTION (XRD)

X-ray diffraction analysis was utilized for characterization of LP-SBA-15, LP-SBA-15-PEI and CLX loaded forms of these samples. Pure crystalline CLX, exhibited a=10.136 Å, b=16.778 Å, c=5.066 Å crystal lattice parameters and α=97.62˚, β=100.65˚, ɣ=95.95˚ values of triclinic unit cell.

Additionally, it could be uttered that pure CLX was in a crystalline state since intense peaks and typical diffraction patterns at 2θ=18.8˚ and 2θ=25.1˚ were detected [35]. Arrangement of molecules in the crystal forms with solvent effect is considered as reason for changing in the diffraction patterns of CLX loaded samples [36].

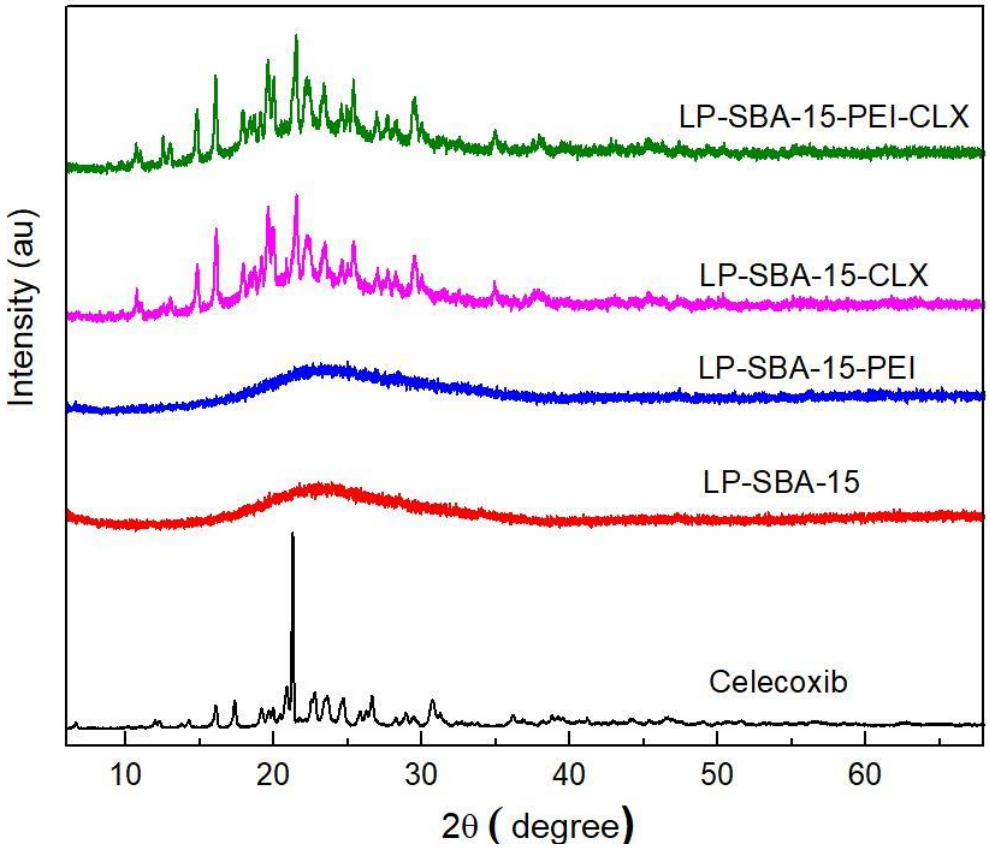


Figure 13. X-Ray diffractograms of Celecoxib, LP-SBA-15, LP-SBA-15-PEI, LP-SBA-15-CLX, and LP-SBA-15-PEI-CLX.

Figure 13 is clearly illustrates that there are few characteristic diffraction peaks. As it is shown in the figure, it could be mentioned that, by adding drug (CLX) to the mesopores of LP-SBA-15 in ethanol, characteristic peaks of Celecoxib are observed in obtained peak of drug loaded samples. Besides, settling CLX into LP-SBA-15 mesopores would impede crystallization of CLX [39].

The other point is that since there is reduction of intensity in case of LP-SBA-15, characteristic peaks of CLX commenced to notice effortlessly. At the end, as it is observed, drug loaded sample illustrate same behavior in all cases in diffraction patterns as it has been expected [48].

Furthermore, low angle XRD was performed in the 2θ range between 0.5-3 degrees. The speed and step sized considered this much low for better results. As it mentioned before, the speed was set up to 0.01 degree per minute and step size was 0.01. This method gives information about the structure. More precisely, the atoms in the crystal

state cause diffraction in the incident light, and this light would provide structure information.

As it is observed in the figure 14, there is additional proof for hexagonal structure of LP-SBA-15 [4]. LP-SBA-15-CLX, LP-SBA-15-PEI and LP-SBA-15-PEI-CLX, illustrate increase or decrease of intensity of these peaks. The appearance of these peaks in these samples could be uttered as a reason of stability of hexagonal structure of LP-SBA-15. Also the shifts of the peaks in sample are due to the increase of lattice parameters and wall thickness of our samples which is a higher amount in LP-SBA-15-PEI samples and drug loaded one, due to the presence of polyethyleneimine on the wall surfaces [78].

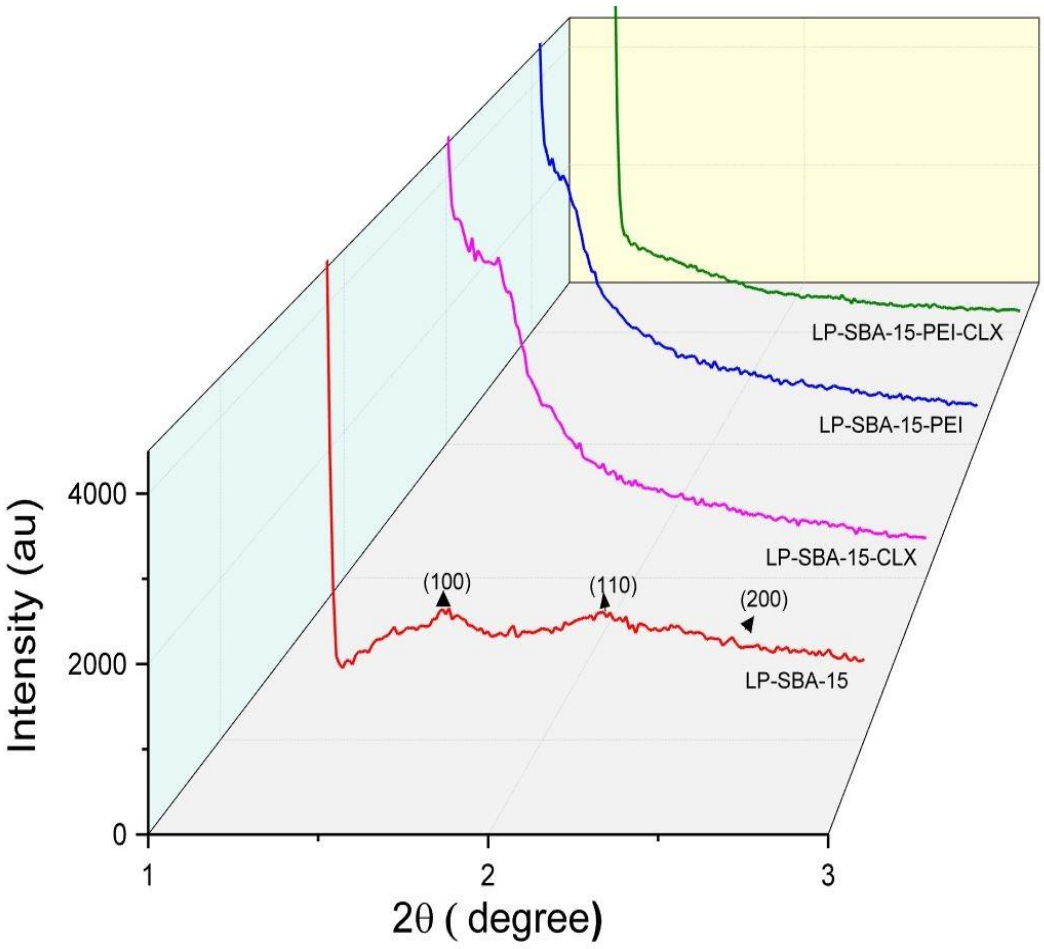


Figure 14. Low angle XRD of LP-SBA-15, LP-SBA-15-CLX, LP-SBA-15-PEI and LP-SBA-15-PEI-CLX.

3.2. SMALL ANGLE X-RAY SCATTERING ANALYSIS (SAXS)

Small angle X-ray scattering analysis was applied to characterize hexagonally ordered LP-SBA-15. As shown result in figure 15 illustrates, LP-SBA-15 displays a less ordered structure rather than SBA-15. But then, as we observed at following steps, by adding PEI, more ordered structure is developed. The most intense peak, belongs to (100). A comparison has been made to our research group previous study on SBA-15 in figure 15-c [76]. Regarding to this comparison; it could be uttered that in case of SBA material, by increasing the size of pores, structure shows a disordered assembly. This is observed in LP-SBA-15 sample.

It is noticeable that pore size increment is observed in LP-SBA-15 and also distance between planes of ordered mesopores is increased. In the case of LP-SBA-15-CLX there is very small difference for d and a value which it could be concluded that the amount of loaded drug is very low. Although when PEI added to the material we observe that pores start to become more ordered and also d_{110} planes start to notice. These clearly observed in figure 15.

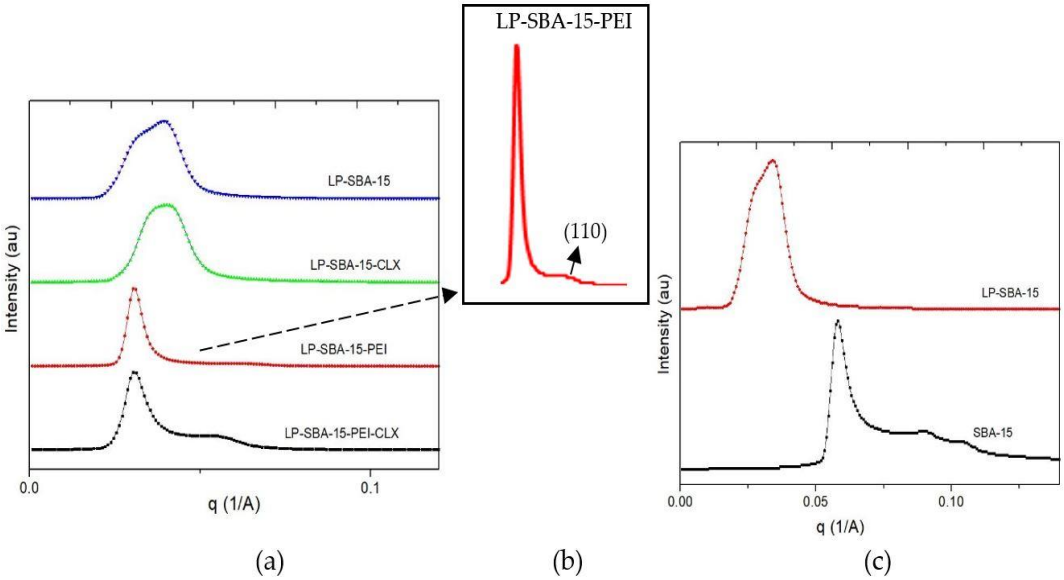


Figure 15. (a) Small angle X-ray Scattering of all samples (SAXS), (b) peaks of LP-SBA-15-PEI curve, (c) comparison of SBA-15 and LP-SBA-15.

3.3. N_2 ADSORPTION-DESORPTION ISOTHERM

N_2 adsorption and desorption analysis was used mainly to characterize the specific properties of pores, and to investigate the mesopore's structures. Also, for certifying the place of drug settlement as follow. It is expected that the obtained materials are mesoporous. By comparison of the result and the IUPAC classification, it could be strongly declared that all analyzed samples have irreversible type IV isotherms which confines their classification as a mesoporous structure materials and they also show H1 hysteresis loops [79]. It could be mentioned that the shape of the hysteresis loop reveals the underlying pore condensation-evaporation mechanism.

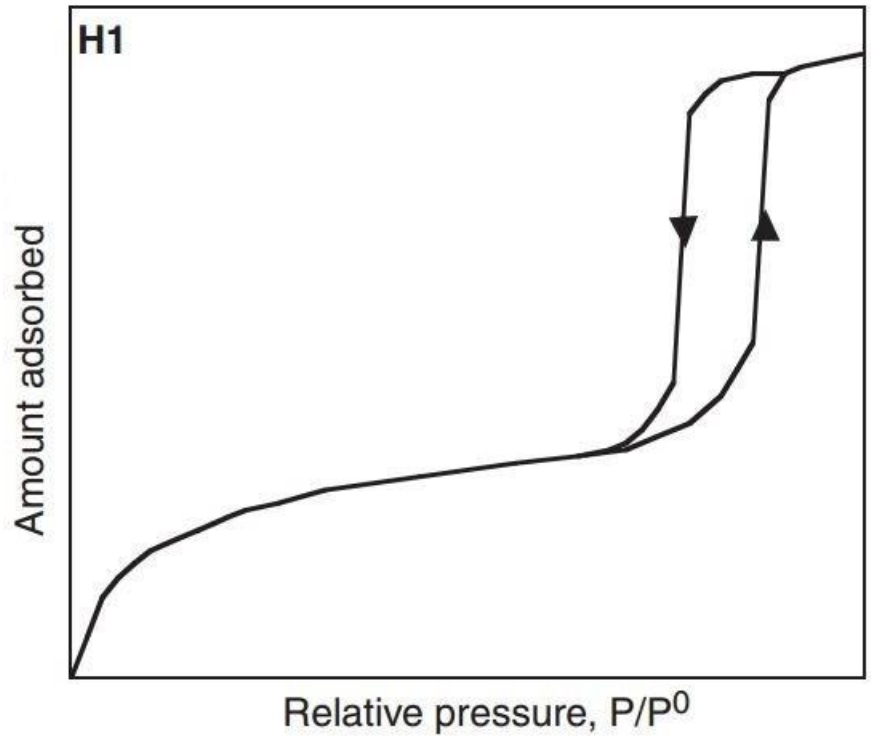


Figure 16. H1 hysteresis loops general curve [80].

Pore structures of materials with H1 hysteresis loops can be regarded as virtually rigid and, since each isotherm has a well-defined plateau at high P/P^0, it is possible to obtain an explicit assessment of the mesopore volume [81]. Besides, H1 hysteresis associated with porous materials exhibiting a narrow distribution of relatively uniform (cylindrical pores).

Table 6, summarizes the textural properties of samples. As it was stated LP-SBA-15 illustrates highest specific surface area of 453 m^2 g^{-1}, pore volume of 1.149 cm^3 g^{-1} and pore diameter of 109.3 Å. Functionalization would lead to pore volume decline due to the addition of functionalized polymer groups on the surface of the sample. This outcome also could assure us that the functional groups were located in the true place inside the pores rather than outside of surface of silica particles.

Subsequently, loaded drug would also affect a fall in specific surface area amount, in case of consideration of not loaded sample. The pore volume exhibits identical trend.

Table 6. Surface Properties of LP-SBA-15, LP-SBA-15-PEI and their CLX loaded forms.

sample	**Specific surface area (S_{BET})(m^2g^{-1})**	**Pore volume(V_p) (cm^3 g^{-1})**	**Pore Diameter (D_p) (Å)**
LP-SBA-15	453.0	1.149	109.3
LP-SBA-15-PEI	82.25	0.3544	72.34
LP-SBA-15-CLX	205.0	0.596	71.3
LP-SBA-15-PEI-CLX	14.12	0.028	17.46

3.3.1. BRUNAUER-EMMETT-TELLER METHOD (BET)

For the purpose of analyzing surface and pore characteristics of silica samples, BET (Brunauer-Emmett-Teller) theory was used. During this characterization temperature was set as a constant value and pressure was varying, and by these factors, the volume of adsorbed and desorbed gas on the silica surface was measured. Consequently, by applying BET method the value and amount of surface area, pore diameter and pore volume of silica samples are obtained.

In the figure 17 N_2 isotherms of LP-SBA-15 and LP-SBA-15-PEI and drug loaded samples of these carriers are presented.

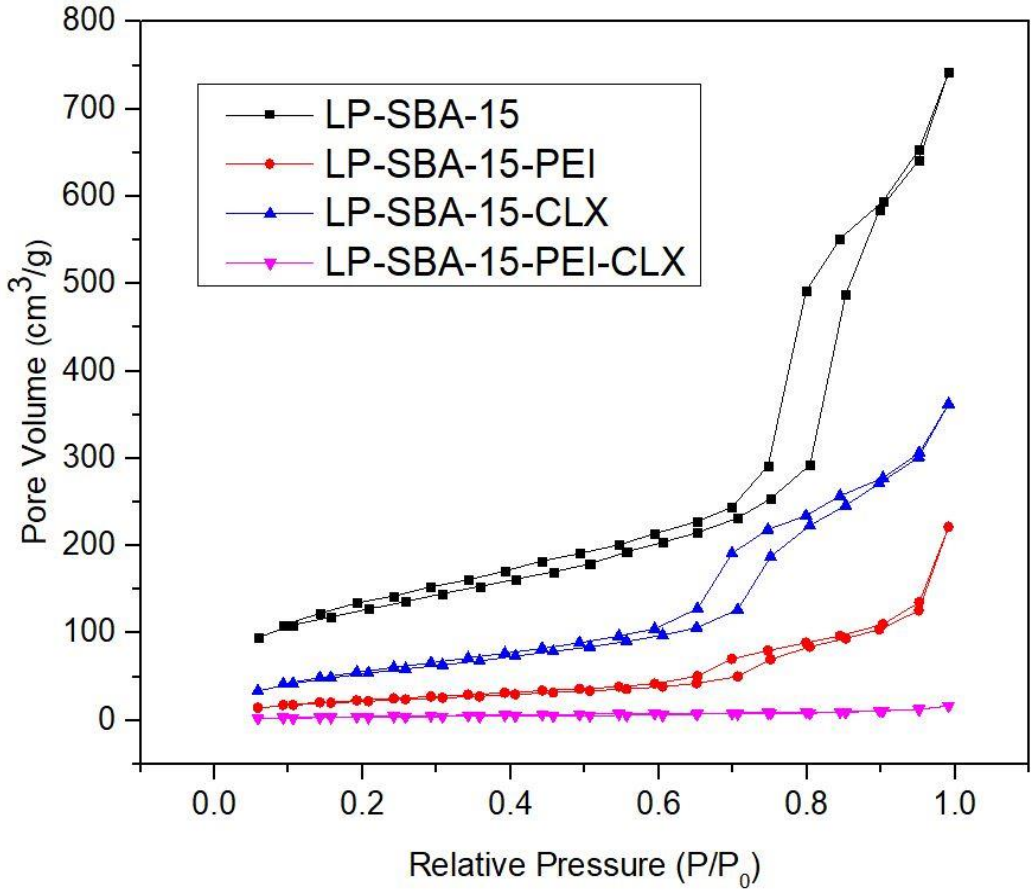


Figure 17. N_2 adsorption/desorption isotherm of LP-SBA-15, LP-SBA-15-PEI, LP-SBA-15-CLX and LP-SBA-15-PEI-CLX**.**

As it is illustrated in figure 17, a uniform pore size of LP-SBA-15 is evident. Also, a sharp increase at P/P_0=0.8 is noticeable [82]. Besides, these uptakes are related to capillary condensation in cylindrical pores [83]. Structures of large pore diameter is related with higher relative pressure of capillary condensation.

These larger pore sizes were obtained due to the particular synthesis method, which is different because of adding TIPB (1,3,5-Triisopropylbenzene) and the fact that this matter will persuade the pore diameter to enlarge in Pluronic 123 micellar solutions and acts as swelling agent [84]. In PEI samples decrease in amount of adsorbed nitrogen volume was observed, which is due to the polymer incorporation.

In ordered consequences, it is indicated that adding PEI to LP-SBA-15 and adding drug to the both carriers would result in lesser pore volume in order of relative pressure. The adsorbed nitrogen volume is very low in LP-SBA-15-PEI-CLX where

a meaningful curve as hysteresis does not provided due to the present of PEI inside pores and further adding drug, would left a very few space for nitrogen to adsorb.

3.3.2. BARRET JOYNER HALENDA ANALYSIS (BJH)

For studying about pore size distribution of the porous materials BJH (Barrett-Joyner-Halenda) method is popular way. The obtained result by this method shown in figure 18. As it is illustrated LP-SBA-15 has highest intensity peak and then by adding PEI and further adding drug would result to a lower peak till there is almost no peak spotting in LP-SBA-15-PEI-CLX case. This result is coinciding with previous result of BET as it is expected. Hence, there is no homogenous pore size distribution after loading drug into pores.

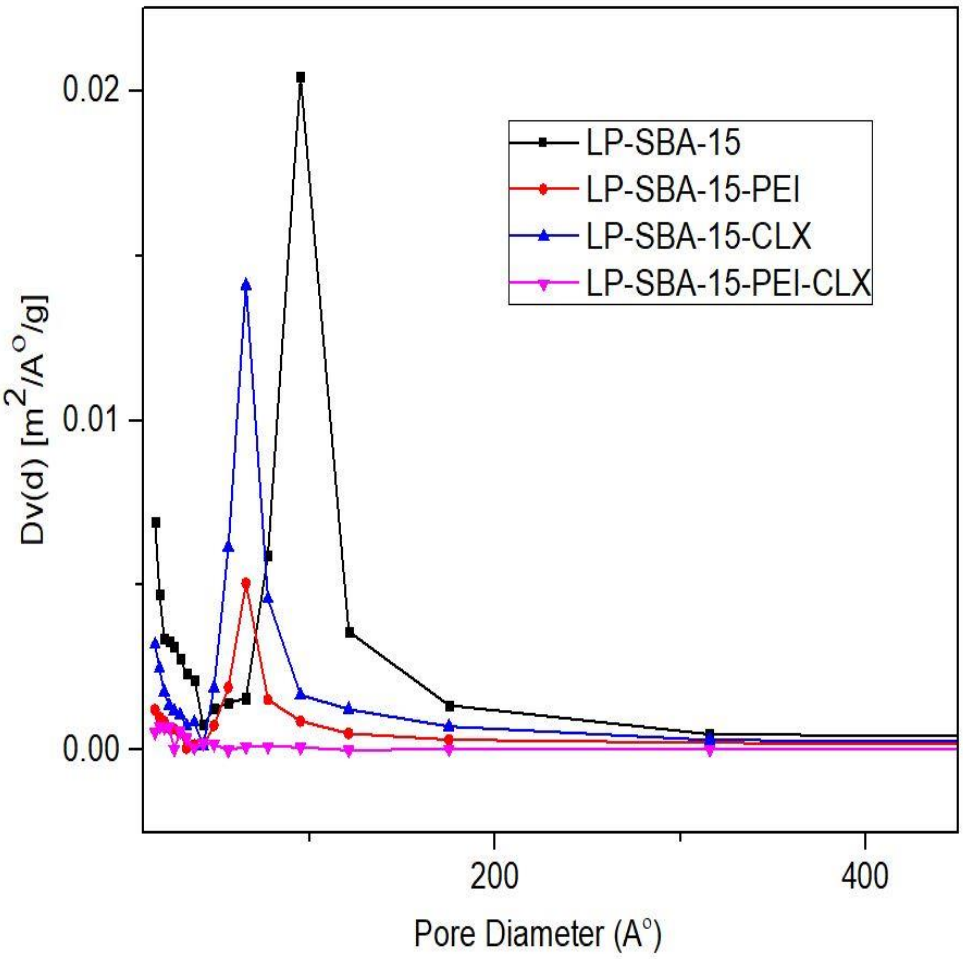


Figure 18. Pore size distributions of LP-SBA-15, LP-SBA-15-PEI, LP-SBA-15-CLX, LP-SBA-15-PEI-CLX.

3.4. FT-IR SPECTRA

All samples including LP-SBA-15 and LP-SBA-15-PEI, in both CLX loaded and not loaded situation was took under investigation by FT-IR to specify the infrared spectra of them. At the end, the result was compared with standard values and also the sample with same procedure of synthesis. The obtained data is shown in figure 19.

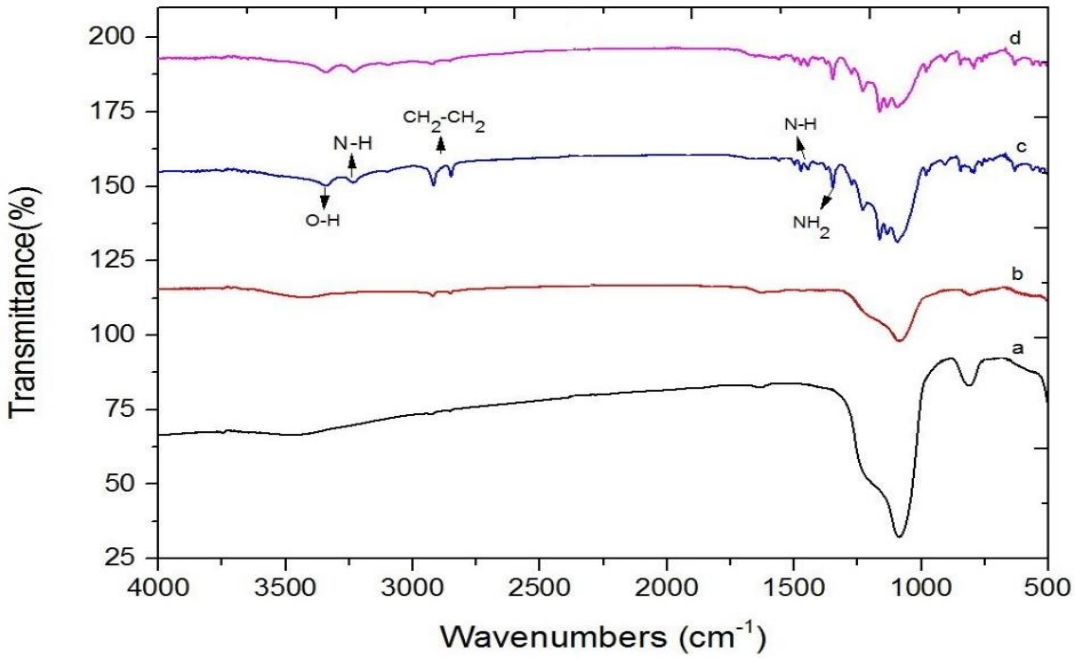


Figure 19. FT-IR spectra of (a) LP-SBA-15, (b) LP-SBA-15-PEI, (c) LP-SBA-15-CLX and (c) LP-SBA-15-PEI-CLX.

The FT-IR spectrum of LP-SBA-15, LP-SBA-15-PEI and their CLX loaded forms are shown in figure 19. All samples were shown a broad band at around 1100 cm^{-1} which is attributed to Si-O-Si asymmetric stretching vibrational mode [85].

Symmetric vibrational modes and bending vibrations of condensed silica network appeared at 800 cm^{-1} and 460 cm^{-1}, respectively. Surface silanol groups in the stretching vibrational mode appeared in the 3740-3000 cm^{-1} range. Moreover, C-H stretching arising from ethoxy groups were in the range of 2939-2908 cm^{-1} for CH stretching and 1400 cm^{-1} for hydrocarbon chain [70, 82]. Asymmetric N-H stretching bands were observed in the LP-SBA-15 samples that were surface functionalized. N-

H stretching bands were seen at 3400 cm^{-1} with an asymmetric NH_2 bending at 1475 cm^{-1}.

3.5. THERMAL METHODS

3.5.1. THERMOGRAVIMETRIC ANALYSIS (TGA)

For illustration of organic groups on the surface of silica samples Thermogravimetric analysis (TGA) was utilized [87]. The measurements were completed under nitrogen gas in temperatures between 30 and 650 ˚C with the heating rate of 10 ˚C / min. for measuring weight loss of samples, and to obtain more reliable percentage of drug loaded to carriers and to eliminate the errors of measurement, 200-650 ˚C range was considered. Because of possibilities that the obtained amount under this temperature might be come from water elimination, left over polymers and other probable unwanted materials (gases like CO_2, NH_3, NO_x, SO_y). Figure 20, illustrates the acquired result for the practical applied range in measurement.

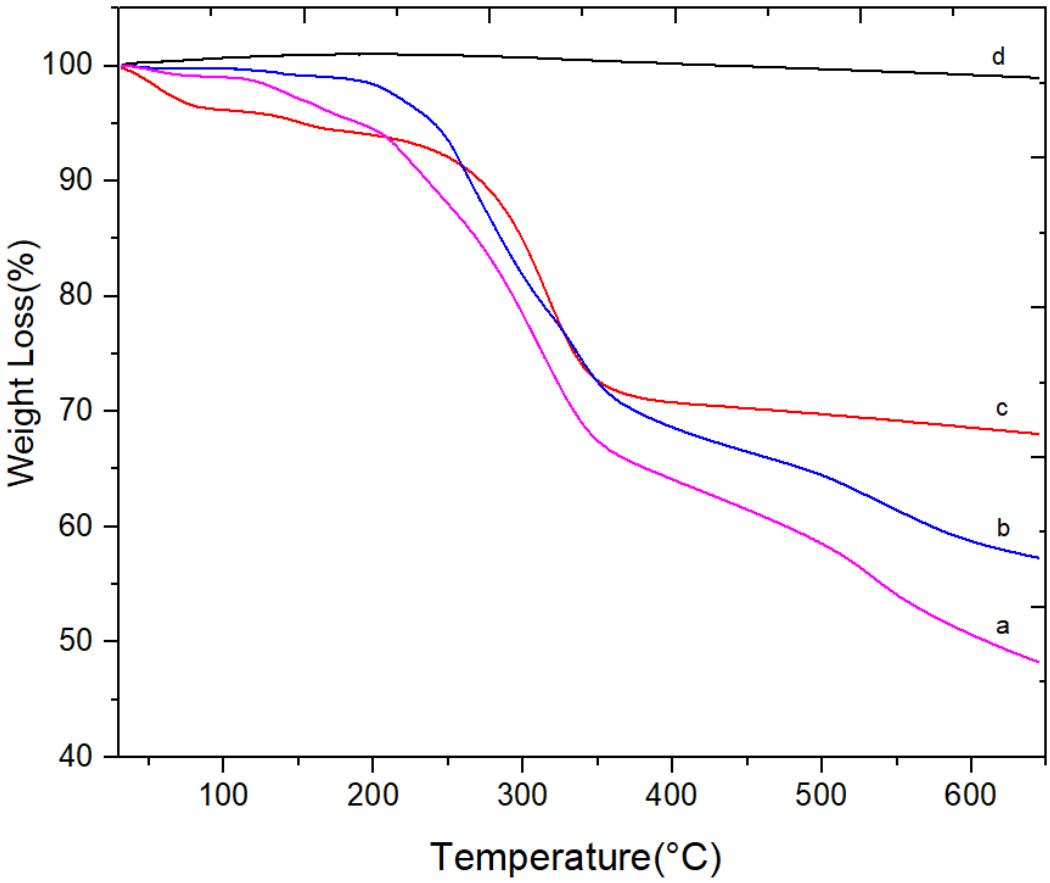


Figure 20. TGA curve of (a) LP-SBA-15-PEI-CLX, (b) LP-SBA-15-CLX, (c) LP-SBA-15-PEI and (d) LP-SBA-15.

As it is indicated in the figure 20, weight loss percent of 1%, 25%, 42% and 50% were observed respectively for LP-SBA-15, LP-SBA-15-CLX, LP-SBA-15-PEI and LP-SBA-15-PEI-CLX. This result also provided that 41% of weight loss in LP-SBA-15-CLX and 25% of LP-SBA-15-PEI-CLX belongs to the loaded drug by comparison of their not loaded samples.

Physical adsorptions like hydrogen bonding and electrostatic interactions between drug and the silica particles are important to ensure strong drug-support interactions [48]. The reason that the less amount of loaded drug is resulted for LP-SBA-15-PEI in the TGA outcome with comparison to LP-SBA-15 is probably because of the strong interaction between PEI and drug which is stronger rather than solely silica and drug interaction. This might cause the drug to be eliminated with polymers in the temperature less than 200 °C.

Although, even by considering this amount as a reliable data, and deeming the amount of release, still there would be a higher efficiency for LP-SBA-15-PEI. CLX has amine and trifluoromethyl groups that are capable of forming hydrogen bonds.

3.5.2. DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Crystallinity determination of CLX loaded silica samples, PEI itself and Celebrex was done by DSC analysis which the obtained result is shown in figure 21.

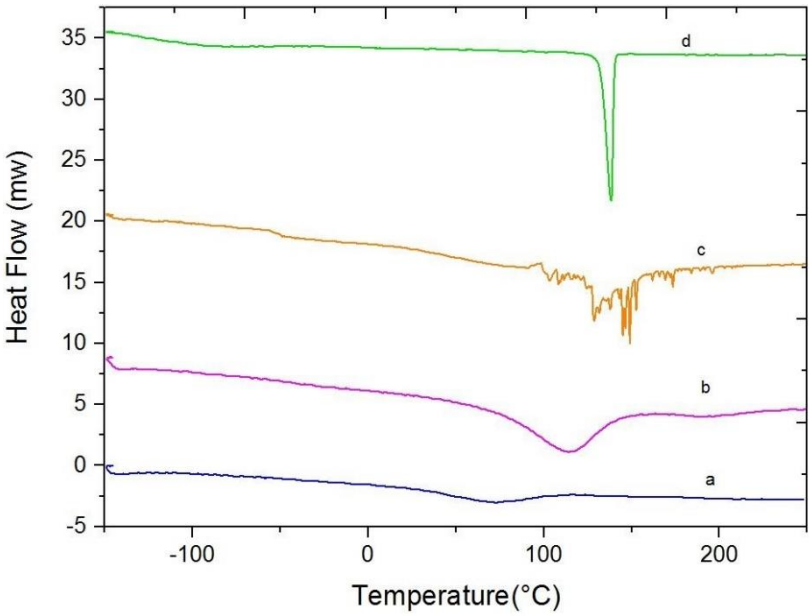


Figure 21. DSC curve of (a) LP-SBA-15-CLX, (b) LP-SBA-15-PEI-CLX, (c) PEI and (d) Celebrex.

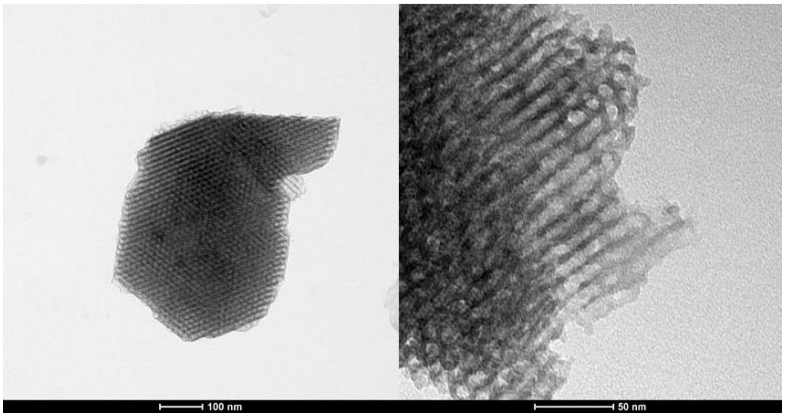
In the obtained result, CLX exhibited solely sharp endothermic melting point 161-165°C which illustrates the crystalline form of drug. Although, crystals have specific melting point, amorphous materials do not show a particular melting points, instead they illustrate range in temperature for phase changing called “glass transition temperature (Tg)”. Previous studies show that, the solubility benefits from the amorphous form [35]. This could be due to the stronger intermolecular forced in the crystals that should be broke for solvation. The amorphous form of materials characterizes as the most energetic solid state of the material thus it should delivers more solubility and bioavailability [88]. This fact could be exploited, if it can be

“stabilized” against reversion to stable crystalline form to increase the solubility. Hence, as it is exhibited, a slight shift and reducing the crystallinity peak is noticeable which leads to a better solubility when it is used with a silica carrier. The silica samples did not show any endothermic peaks since they have amorphous and non-crystalline states [48].

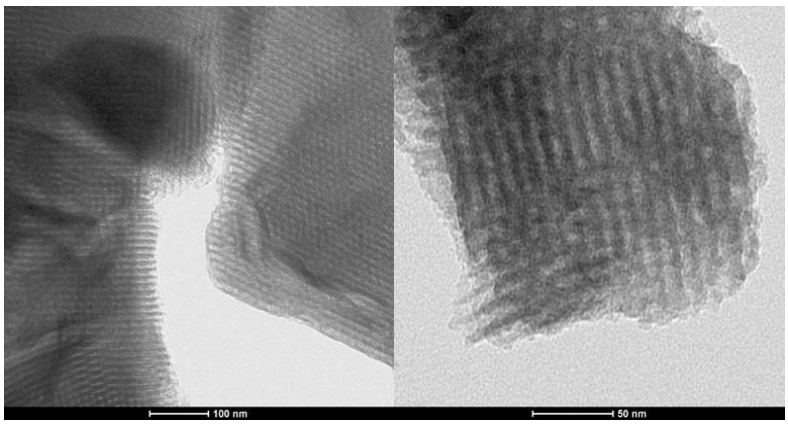
3.6. TRANSMISSION ELECTRON MICROSCOPY (TEM)

TEM used for better understanding and investigating the structure of samples. Moreover, TEM would provide information about the changes in the structure by adding polymer. Therefore, it will show us that the structure of our material is preserved or not. Transmission electron microscopy (TEM) images of samples are shown in figure 22.

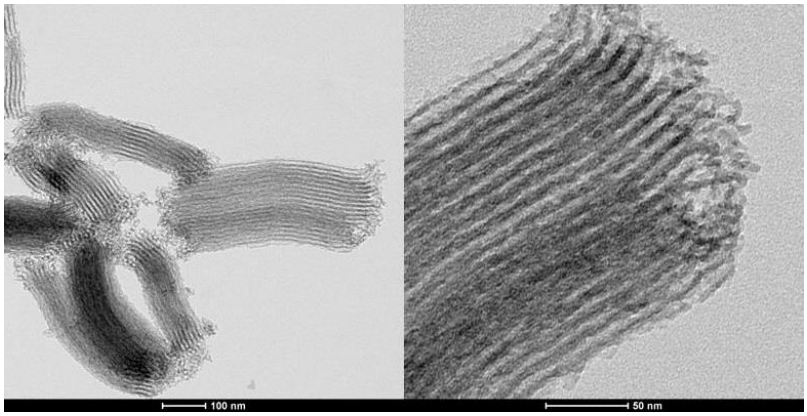
a)



b)



c)



d)

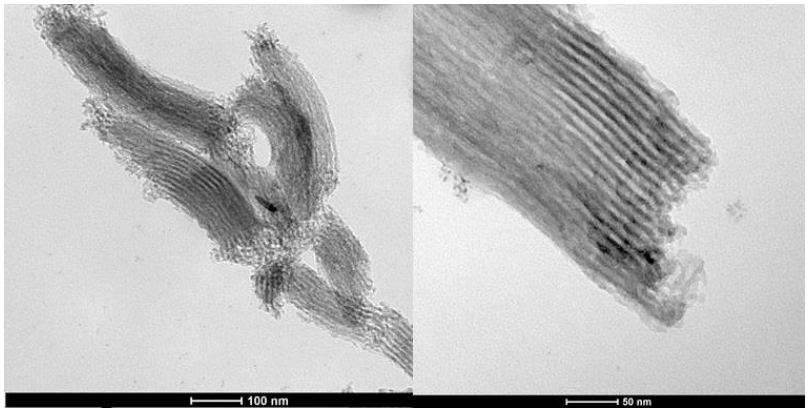


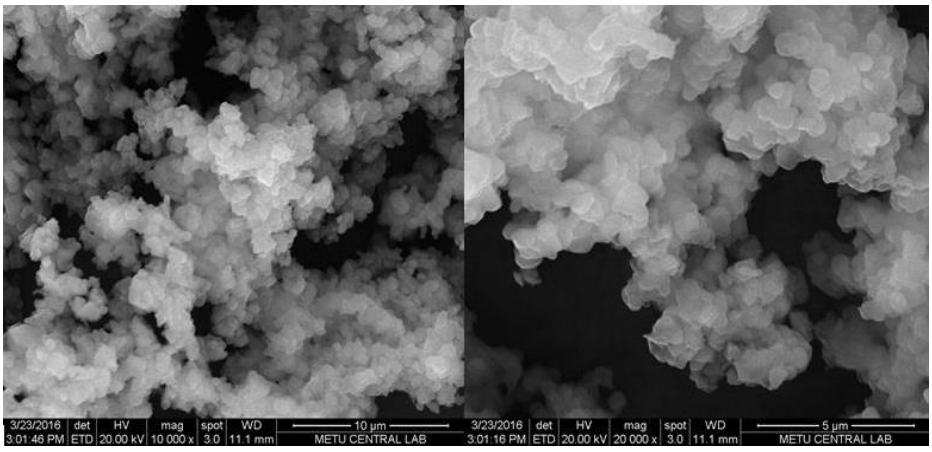
Figure 22. TEM images of (a) LP-SBA-15, (b) LP-SBA-15-CLX, (c) LP-SBA-15-PEI and (d) LP-SBA-15-PEI-CLX.

LP-SBA-15 and LP-SBA-15-PEI were exhibited well-ordered hexagonal structures, moreover, according to TEM images in the 50 nm scale; the silica samples maintained their ordered and stable structures. This probable conclusion could also be provided by TEM images that the drug was accumulated on the surface and inside the mesochannels [34]. And, they show different estimated distances between the porous layers, which coincided with the different pore sizes attained by the nitrogen adsorption–desorption isotherms. Besides, uniform pore size in all samples, polymeric aggregation is noticeable in the functionalized materials, suggesting homogeneous distribution of the organic functions.

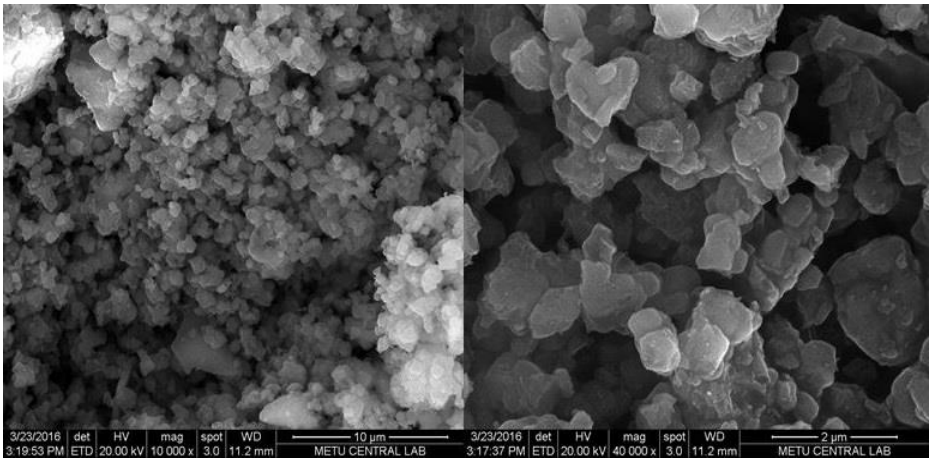
3.7. SCANNING ELECTRON MICROSCOPY (SEM)

Morphology of silica samples were probed by scanning electron microscopy (SEM) as it is provided in figure 23.

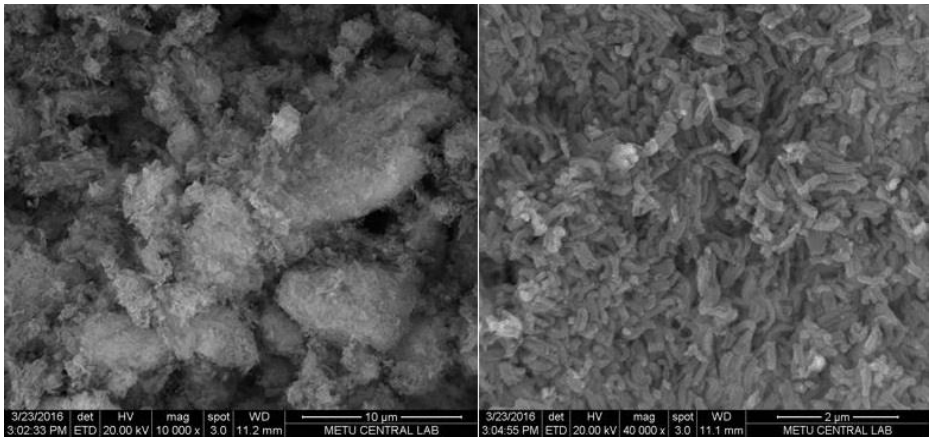
a)



b)



c)



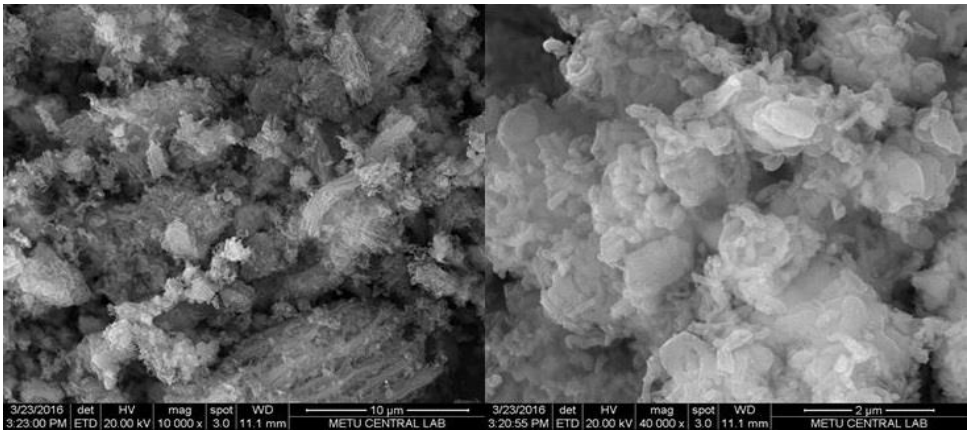
d) 

Figure 23. SEM images of (a) LP-SBA-15, (b) LP-SBA-15-CLX, (c) LP-SBA-15-PEI and (d) LP-SBA-15-PEI-CLX.

In the SEM pictures, LP-SBA-15 shows non-regular particle shapes, and CLX loaded of this sample also illustrates the same outcome.

Silica based materials usually show different type of shapes such as SBA-15 commonly has fibular shape and MCM-41 has spherical shape, more on, LP-SBA-15 used to show different types of shape in previous studies dependent on the procedure of synthesis, herein, due to the extra processes for obtaining large pores in silica during the synthesis, this particular non-regular isolated shape of particles is produced.

LP-SBA-15-PEI and LP-SBA-15-PEI-CLX showed the worm like shape, this emerges after the extra processes to obtain this specific carrier. It is observed that LP-SBA-15-PEI has more uniform particle size distribution rather than LP-SBA-15.

3.8. UV ANALYSIS

UV analysis is generally used to make quantitative determination. For this purpose, Beer- Lambert law is the common method to determine the concentration of species in solution. This law can be written as;

$$A=\varepsilon.b.c$$

(A: absorbance ε: molar absorptivity coefficient b: path length of the sample c: concentration)

Molar absorptivity is equal to the slope of calibration curve, in this equation. So, for utilizing this equation two calibration curve for PBS and ethanol was necessary which is shown in follow.

3.8.1 VITRO RELEASE STUDIES

In vitro release behaviors of samples were investigated by comparison to commercial Celebrex. The release of CLX from the carriers was studied in phosphate buffer at pH= 7.4 and pH= 5.0. The release properties of silica particles are dependent on diverse factors such as crystallinity, surface area, and hydrophilic-hydrophobic interactions between silica and the drug. Poorly water soluble drug loaded in silica particles was used to show substantially enhanced release profile and solubility [89]. Figure 24 and 26 are the calibration curve of PBS in two different pH. The amount of concentrations are the optimum amount achievable. Since Celecoxib is poorly soluble in water, attempts to increase the concentration is not possible. Besides, obtained data are in the range of the calibration curve concentrations. Slope of the curve indicated the molar absorptivity coefficient as 27.3 for PBS. In the calculation part, we also have dilution factor which is because of dilution that has been done after any sampling. Dilution factors for different hours was in order of 100%, 91%, 81%, 73% etc. These dilution factors have been calculated due to the 5ml sample taking and the fact that our samples always stays in 50ml. So, we dilute them after all sample taking steps by 5ml of PBS. For more information, the logic of dilution factors is that in first step we took 5ml from 50, so we use 100% dilution factor. Then we took 5 ml from the 45+5 ml, so it comes as 45/50 dilution factors which is 91% and in same logic the factors have been made.

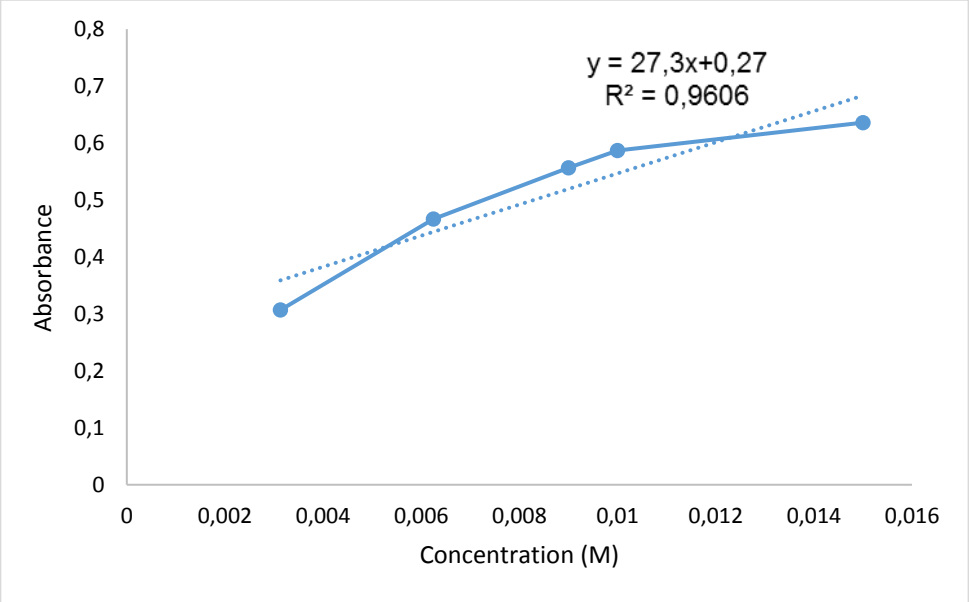


Figure 24. Calibration curve for celecoxib solution, prepared in PBS at pH= 7.4.

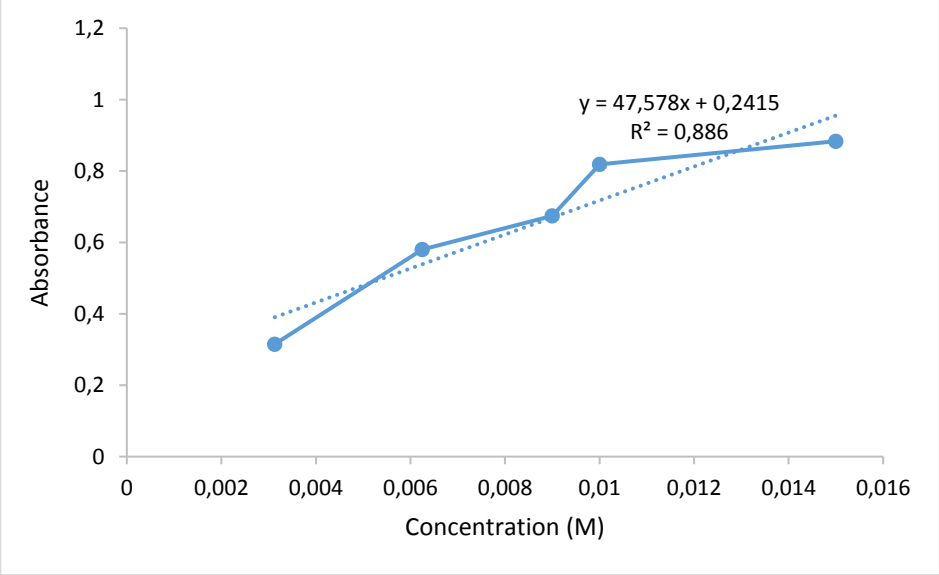


Figure 25. Calibration curve for celecoxib solution, prepared in PBS at pH= 5.0.

The release graphs are demonstrated in figure 26. According to figure 26, LP-SBA-15 shows a less than 25% of drug release while the LP-SBA-15-PEI exhibited much higher amount. Particles show a burst type of release in first hours, probably because, some of the CLX were adsorbed to the surface of the carriers which would be readily released in the process and so a burst release would be observed.

Drug release from larger sized particles generally takes longer since the drug is required to diffuse from inner pores to the release medium that is could be a reason for more controlled release of LP-SBA-15-PEI [90]. LP-SBA-15, shows a release behavior such as all SBA-15 family with a burst release at early hours and then more slow and controlled release at upcoming hours. This manner is outcome of weak intermolecular interaction of silanol groups and drugs [91]. Hence, by adding PEI here as functionalized matter, and introducing it to the surface.

Better interaction with drug has been obtained and then, more controlled release has been observed. The result in pH= 7.4 shows a faster release in case of LP-SBA-15 rather than Celebrex itself, which could be an outcome of poor water solubility of CLX. Release in pH= 5.0 shows more burst release with comparison to pH= 7.4 curves. In case of Celebrex itself, it shows a less amount of release in pH= 5.0 that could be due to the reason that Celecoxib has acidic nature [92].

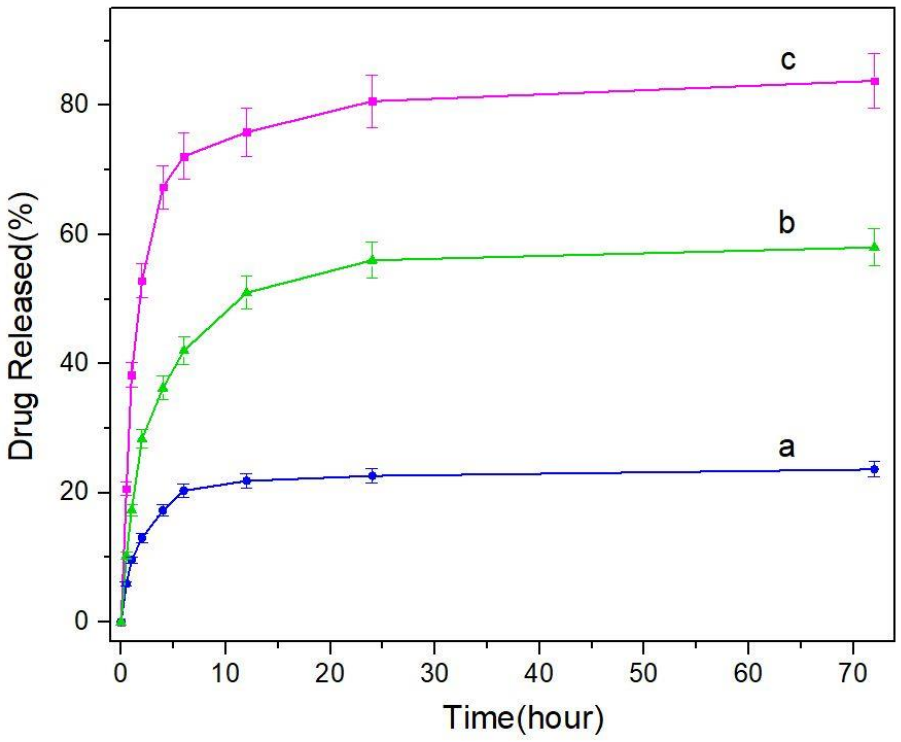


Figure 26. Release graph of (a) LP-SBA-15-CLX, (b) Celebrex and (c) LP-SBA-15-PEI-CLX at pH= 7.4.

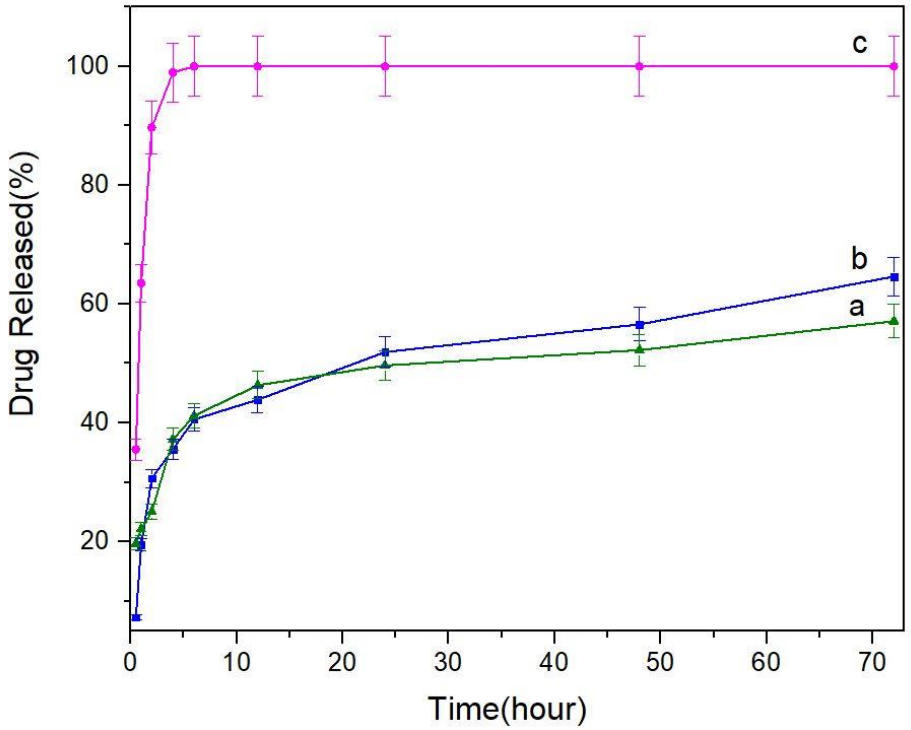


Figure 27. Release graph of (a) Celebrex, (b) LP-SBA-15 and (c) LP-SBA-15-PEI at pH=5.0.

Also, there is possibility that if the pores could be filled more uniformly the slower drug release would be observed. LP-SBA-15 indicates higher pore volume which leads it to have more burst in releasing all drugs in less time rather than LP-SBA-15-PEI.

Figure 28 is sketched for better understanding of the efficiency of LP-SBA-15-PEI carriers in pH= 7.4. In the result, we took mathematical hypothesis by considering the real data that we obtained in loaded drug.

This curve is done by assuming that the same amount of drug was loaded to our carriers in a loading step. Hence, it could be noted that if the total amount of drug was 147 mg at ethanol solution in the loading process, at the end 36.75 mg of it was loaded to LP-SBA-15-PEI and 60.2 mg was loaded to LP-SBA-15. So, at the end we could say that from 147mg initial loaded amount; there is less than 4% for LP-SBA-15 and about 5.4% for LP-SBA-15-PEI that would be released. Or it could be uttered that less than 10% of loaded amount of CLX in case of LP-SBA-15 and about 20% of loaded amount of CLX in case of LP-SBA-15-PEI would be released (since about 5.5 mg

from 60.2 mg for LP-SBA-15 and about 7.5 mg for LP-SBA-15-PEI have been released).

Moreover, at the release step, 30.57 mg drug was released from LP-SBA-15-PEI and 14.22 mg from LP-SBA-15. As a curve, it could be shown as below; hence it is easily noticeable that there is big difference in efficiency of release properties between our two carriers.

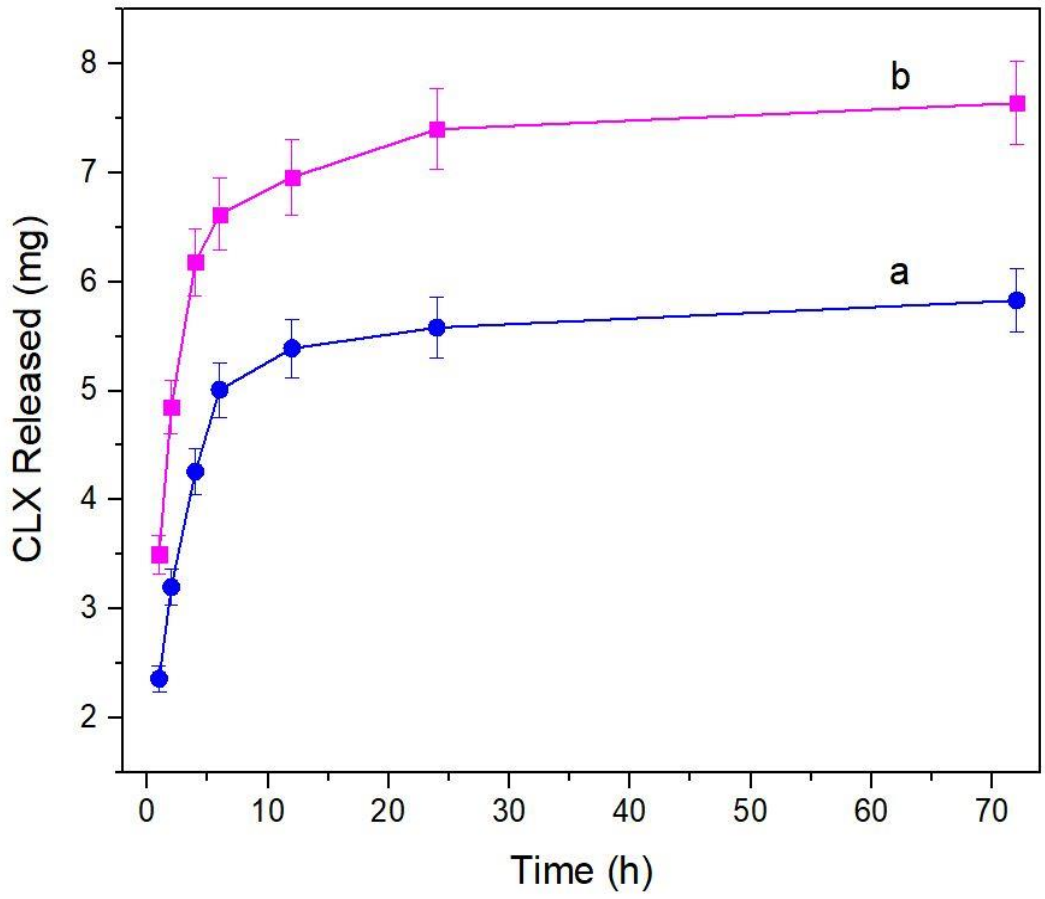


Figure 28. Release efficiency of (a) LP-SBA-15-CLX and (b) LP-SBA-15-PEI-CLX.

CHAPTER 4

CONCLUSION

In this study, there are some targeted goals such as solving the low solubility and bioavailability problem of hydrophobic drugs such as CLX and to enhance the drug delivery system characteristics of LP-SBA-15. In this manner, we investigated LP-SBA-15 mesoporous silica and surface modified this particular sample by hyperbranching polymerization of polyethyleneimine. Polymerization have been done, by usage of Aziridine monomer and introducing it to the surface of pores and more specifically inside pores not on the surface. Surface modifying was done with purpose of gaining better intermolecular interaction which would lead to a better loading and release efficiency and also would raise the bioavailability of the drug (CLX). By this surface modification, the highest probable number of accessible amine groups are presented, though, sometimes the first cycle of polymerization would lead to polymer formation in microspores in the mesopore walls, which hinders the accessibility of this amine groups. Although, as a certifier it could be cited that nitrogen sorption isotherm revealed that no partial blocking was occurred upon PEI introduction to the surface of pores and a narrow hysteresis has been obtained. All of the samples; Celebrex, silica samples and functionalized support materials have been characterized individually and precisely by different methods for their physicochemical properties. Also, CLX crystallinity is shifted and reduced as the powder XRD patterns and DSC results illustrates which is positive outcome for the purpose of the study. Also, TEM and SEM images provided data that shows applied changes in synthesis method, would result in some alters in morphology but consequently LP-SBA-15-PEI obtains worm like particle shape. However, LP-SBA-15 with a no regular shape and LP-SBA-15-PEI show an identical trend for release in

early hours, but after few hours, LP-SBA-15-PEI shows more controlled release, which is due to holding of stronger interactions between functional group and CLX rather than LP-SBA-15 which has silanol groups on surface, and silanol is able to have some weak interaction with CLX. Therefore, it could be uttered that functionalization by PEI, has lead LP-SBA-15, to be more bioavailable and to have more efficient release for poorly water-soluble Celecoxib drug. And this happens, without damaging the pore structure of LP-SBA-15 and without changing the vital properties, desired for drug delivery system from LP-SBA-15 as drug carrier. At the end, it could be declared that, this method of surface modification would enhance the properties of drug delivery system by comparison to the pure CLX and LP-SBA-15.

REFERENCES

[1] M. Vallet-Regi, A. Ramila, R. P. Del Real, and J. Perez-Pariente, “A new property of MCM-41: Drug delivery system,” Chem. Mater., vol. 13, no. 2, pp. 308–311, 2001.

[2] B. Nowack and T. D. Bucheli, “Occurrence, behavior and effects of nanoparticles in the environment,” Environmental Pollution, vol. 150, no. 1. pp. 5–22, 2007.

[3] C. T. Kresge, M. E. Leonowicz, W. J. Roth, J. C. Vartuli, and J. S. Beck, “Ordered mesoporous molecular sieves synthesized by a liquid-crystal template mechanism,” Nature, vol. 359, no. 6397, pp. 710–712, 1992.

[4] D. Zhao, J. Feng, Q. Huo, B. F. Chmelka, and G. D. Stucky, “Triblock copolymer syntheses of mesoporous silica with periodic 50 to 300 angstrom pores,” Science, vol. 279, no. 5350, pp. 548–52, 1998.

[5] Q. Huo, D. I. Margolese, and G. D. Stucky, “Surfactant Control of Phases in the Synthesis of Mesoporous Silica-Based Materials,” Chem. Mater., vol. 8, no. 5, pp. 1147–1160, 1996.

[6] Y. Sakamoto, T. W. Kim, R. Ryoo, and O. Terasaki, “Three-dimensional structure of large-pore mesoporous cubic Ia$\bar{3}$d silica with complementary pores and its carbon replica by electron crystallography,” Angew. Chemie - Int. Ed., vol. 43, no. 39, pp. 5231–5234, 2004.

[7] A. Taguchi and F. Scheth, “Ordered mesoporous materials in catalysis,” Microporous and Mesoporous Materials, vol. 77, no. 1. pp. 1–45, 2005.

[8] B. J. Scott, G. Wirnsberger, and G. D. Stucky, “Mesoporous and mesostructured materials for optical applications,” Chemistry of Materials, vol. 13, no. 10. pp. 3140–3150, 2001.

[9] M. Hartmann, “Ordered mesoporous materials for bioadsorption and biocatalysis,” Chemistry of Materials, vol. 17, no. 18. pp. 4577–4593, 2005.

[10] H. H. P. Yiu and P. A. Wright, “Enzymes supported on ordered mesoporous solids: a special case of an inorganic–organic hybrid,” J. Mater. Chem., vol. 15, no. 35–36, p. 3690, 2005.

[11] M. Colilla, B. González, and M. Vallet-Regí, “Mesoporous silica nanoparticles for the design of smart delivery nanodevices,” Biomater. Sci., pp. 114–134, 2013.

[12] Y. F. Zhu, J. L. Shi, Y. S. Li, H. R. Chen, W. H. Shen, and X. P. Dong, “Storage and release of ibuprofen drug molecules in hollow mesoporous silica spheres with modified pore surface,” Microporous Mesoporous Mater., vol. 85, no. 1–2, pp. 75–81, 2005.

[13] A. B. D. Nandiyanto, S. G. Kim, F. Iskandar, and K. Okuyama, “Synthesis of spherical mesoporous silica nanoparticles with nanometer-size controllable pores and outer diameters,” Microporous Mesoporous Mater., vol. 120, no. 3, pp. 447–453, 2009.

[14] P. Gómez-Romero and C. Sanchez, “Hybrid Materials, Functional Applications. An Introduction,” Funct. Hybrid Mater. no. June 2017, pp. 1–14, 2005.

[15] R. Huirache-Acuña, R. Nava, C. Peza-Ledesma, J. Lara-Romero, G. Alonso-Núez, B. Pawelec, and E. Rivera-Muñoz, “SBA-15 Mesoporous Silica as Catalytic Support for Hydrodesulfurization Catalysts—Review,” Materials (Basel)., vol. 6, no. 9, pp. 4139–4167, 2013.

[16] F. Sevimli and A. Yilmaz, “Surface functionalization of SBA-15 particles for amoxicillin delivery,” Microporous Mesoporous Mater., vol. 158, pp. 281–291, 2012.

[17] X.-Y. Yang, L.-H. Chen, Y. Li, J. C. Rooke, C. Sanchez, and B.-L. Su, “Hierarchically porous materials: synthesis strategies and structure design,” Chem. Soc. Rev., vol. 46, no. 2, pp. 481–558, 2017.

[18] D. Khushalani, A. Kuperman, G. A. Ozin, K. Tanaka, N. Coombs, M. M. Olken, and J. Garces, “Metamorphic materials: Restructuring siliceous mesoporous materials,” Adv. Mater., vol. 7, no. 10, pp. 842–846, 1995.

[19] I. I. Slowing, B. G. Trewyn, V. S.-Y. S.-Y. Lin, I. I. Slowing, B. G. Trewyn, B. G. Trewyn, V. S.-Y. S.-Y. Lin, and V. S.-Y. S.-Y. Lin, “Effect of surface

functionalization of MCM-41-type mesoporous silica nanoparticles on the endocytosis by human cancer cells.," J Am Chem Soc, vol. 128, no. 46, pp. 14792–14793, 2006.

[20] N. Vadia and R. Sadhana, "Mesoporous Material, MCM- 41: a New Drug Carrier," Asian J. Pharm. Clin. Res., vol. 4, no. 2, pp. 44–53, 2011.

[21] Ş. Günaydin and A. Yilmaz, "Improvement of solubility of celecoxib by inclusion in MCM-41 mesoporous silica: Drug loading and release," Turkish J. Chem., vol. 39, no. 2, pp. 317–333, 2015.

[22] A. Monnier, F. Schuth, Q. Huo, D. Kumar, D. Margolese, R. S. Maxwell, G. D. Stucky, M. Krishnamurty, P. Petroff, A. Firouzi, M. Janicke, and B. F. Chmelka, "Cooperative Formation of Inorganic-Organic Interfaces in the Synthesis of Silicate Mesostructures," Science (80-.)., vol. 261, no. 5126, pp. 1299–1303, 1993.

[23] J. Zhang, Z. Luz, and D. Goldfarb, "EPR Studies of the Formation Mechanism of the Mesoporous Materials MCM-41 and MCM-50," J. Phys. Chem. B, vol. 101, pp. 7087–7094, 1997.

[24] Y. Wang, Q. Zhao, N. Han, L. Bai, J. Li, J. Liu, E. Che, L. Hu, Q. Zhang, T. Jiang, and S. Wang, "Mesoporous silica nanoparticles in drug delivery and biomedical applications," Nanomedicine: Nanotechnology, Biology, and Medicine, vol. 11, no. 2. pp. 313–327, 2015.

[25] N. Sharma, H. Ojha, A. Bharadwaj, D. P. Pathak, and R. K. Sharma, "Preparation and catalytic applications of nanomaterials: a review," RSC Adv., vol. 5, no. 66, pp. 53381–53403, 2015.

[26] J. Lu, M. Liong, J. I. Zink, and F. Tamanoi, "Mesoporous silica nanoparticles as a delivery system for hydrophobic anticancer drugs," Small, vol. 3, no. 8, pp. 1341–1346, 2007.

[27] M. Moritz and M. Geszke-Moritz, "Mesoporous materials as multifunctional tools in biosciences: Principles and applications," Mater. Sci. Eng. C, vol. 49, no. 0, pp. 114–151, 2015.

[28] D. R. Radu, C. Y. Lai, K. Jeftinija, E. W. Rowe, S. Jeftinija, and V. S. Y. Lin, "A polyamidoamine dendrimer-capped mesoporous silica nanosphere-based gene transfection reagent," J. Am. Chem. Soc., vol. 126, no. 41, pp. 13216–13217, 2004.

[29] S. R. Hall, C. E. Fowler, S. Mann, and B. Lebeau, “Template-directed synthesis of bi-functionalized organo-MCM-41 and phenyl-MCM-48 silica mesophases,” Chem. Commun., pp. 201–202, 1999.

[30] a S. M. Chong and X. S. Zhao, “Functionalization of SBA-15 with APTES and characterization of functionalized materials,” J. Phys. Chem. B, vol. 107, no. 46, pp. 12650–12657, 2003.

[31] H. Yoshitake, T. Yokoi, and T. Tatsumi, “Adsorption of chromate and arsenate by amino-functionalized MCM-41 and SBA-1,” Chem. Mater., vol. 14, no. 11, pp. 4603–4610, 2002.

[32] A. Szegedi, M. Popova, I. Goshev, and J. Mihály, “Effect of amine functionalization of spherical MCM-41 and SBA-15 on controlled drug release,” J. Solid State Chem., vol. 184, no. 5, pp. 1201–1207, 2011.

[33] L. Mercier and T. J. Pinnavaia, “Direct synthesis of hybrid organic-inorganic nanoporous silica by a neutral amine assembly route: Structure-function control by stoichiometric incorporation of organosiloxane molecules,” Chem. Mater., vol. 12, no. 1, pp. 188–196, 2000.

[34] A. Nishiwaki, A. Watanabe, K. Higashi, Y. Tozuka, K. Moribe, and K. Yamamoto, “Molecular states of prednisolone dispersed in folded sheet mesoporous silica (FSM-16),” Int. J. Pharm., vol. 378, no. 1–2, pp. 17–22, 2009.

[35] M. H. Lim and A. Stein, “Comparative Studies of Grafting and Direct Syntheses of Inorganic−Organic Hybrid Mesoporous Materials,” Chem. Mater., vol. 11, no. 11, pp. 3285–3295, 1999.

[36] F. Hoffmann, M. Cornelius, J. Morell, and M. Fröba, “Silica-based mesoporous organic-inorganic hybrid materials,” Angewandte Chemie - International Edition, vol. 45, no. 20. pp. 3216–3251, 2006.

[37] C. O. Kim, S. J. Cho, and J. W. Park, “Hyperbranching polymerization of aziridine on silica solid substrates leading to a surface of highly dense reactive amine groups,” J. Colloid Interface Sci., vol. 260, no. 2, pp. 374–378, 2003.

[38] Sujandi, E. A. Prasetyanto, and S. E. Park, “Synthesis of short-channeled amino-functionalized SBA-15 and its beneficial applications in base-catalyzed reactions,” Appl. Catal. A Gen., vol. 350, no. 2, pp. 244–251, 2008.

[39] H. J. Kim, J. H. Moon, and J. W. Park, “A Hyperbranched Poly(ethyleneimine) Grown on Surfaces,” J. Colloid Interface Sci., vol. 227, no. 1, pp. 247–249, 2000.

[40] A. Heidari, H. Younesi, and Z. Mehraban, “Removal of Ni(II), Cd(II), and Pb(II) from a ternary aqueous solution by amino functionalized mesoporous and nano mesoporous silica,” Chem. Eng. J., vol. 153, no. 1–3, pp. 70–79, 2009.

[41] J. M. Rosenholm and M. Lindén, “Towards establishing structure-activity relationships for mesoporous silica in drug delivery applications,” J. Control. Release, vol. 128, no. 2, pp. 157–164, 2008.

[42] B. Muñoz, A. Ramila, J. Perez-Pariente, I. Díaz, and M. Vallet-Regí, “MCM-41 organic modification as drug delivery rate regulator,” Chem. Mater., vol. 15, no. 2, pp. 500–503, 2003.

[43] W. Zeng, X. F. Qian, Y. B. Zhang, J. Yin, and Z. K. Zhu, “Organic modified mesoporous MCM-41 through solvothermal process as drug delivery system,” Mater. Res. Bull., vol. 40, no. 5, pp. 766–772, 2005.

[44] F. Balas, M. Manzano, P. Horcajada, and M. Vallet-Regi, “Confinement and controlled release of bisphosphonates on ordered mesoporous silica-based materials,” J. Am. Chem. Soc., vol. 128, no. 25, pp. 8116–8117, 2006.

[45] M. Vallet-Regí, F. Balas, M. Colilla, and M. Manzano, “Bone-regenerative bioceramic implants with drug and protein controlled delivery capability,” Progress in Solid State Chemistry, vol. 36, no. 3. pp. 163–191, 2008.

[46] C. Y. Lai, B. G. Trewyn, D. M. Jeftinija, K. Jeftinija, S. Xu, S. Jeftinija, and V. S. Y. Lin, “A mesoporous silica nanosphere-based carrier system with chemically removable CdS nanoparticle caps for stimuli-responsive controlled release of neurotransmitters and drug molecules,” J. Am. Chem. Soc., vol. 125, no. 15, pp. 4451–4459, 2003.

[47] A. Martín, G. Morales, F. Martínez, R. Van Grieken, L. Cao, and M. Kruk, “Acid hybrid catalysts from poly(styrenesulfonic acid) grafted onto ultra-large-pore SBA-15 silica using atom transfer radical polymerization,” J. Mater. Chem., vol. 20, no. 37, pp. 8026–8035, 2010.

[48] J. M. Rosenholm and M. Lindén, “Wet-chemical analysis of surface concentration of accessible groups on different amino-functionalized mesoporous SBA-15 silicas,” Chem. Mater., vol. 19, no. 20, pp. 5023–5034, 2007.
[49] J. M. Rosenholm, A. Penninkangas, and M. Lindén, “Amino-functionalization of large-pore mesoscopically ordered silica by a one-step hyperbranching polymerization of a surface-grown polyethyleneimine,” Chem. Commun., no. 37, pp. 3909–3911, 2006.
[50] C. D. Delivery, “Controlling drug delivery,” Drug Deliv. Target., vol. 8, no. 3, pp. 1–24, 1999.
[51] Alexander T. Florence, A Short History of Controlled Drug Release and an Introduction. 2011.
[52] P. Yang, Z. Quan, C. Li, X. Kang, H. Lian, and J. Lin, “Bioactive, luminescent and mesoporous europium-doped hydroxyapatite as a drug carrier,” Biomaterials, vol. 29, no. 32, pp. 4341–4347, 2008.
[53] R. H. Garrett and C. . Grisham, “Biochemistry 6th edition,” in Biochemistry 6th edition, 2016, p. P450.
[54] Y. Perrie and T. Rades, “FASTtrack Pharmaceutics: Drug Delivery and Targeting,” Eur. J. Pharm. Sci., vol. 8, no. 3, pp. 1–24, 2009.
[55] M. Vallet-Regí, F. Balas, and D. Arcos, “Mesoporous materials for drug delivery,” Angew. Chemie - Int. Ed., vol. 46, no. 40, pp. 7548–7558, 2007.
[56] S. Wang, “Ordered mesoporous materials for drug delivery,” Microporous and Mesoporous Materials, vol. 117, no. 1–2. pp. 1–9, 2009.
[57] P. J. Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, “Experimental and computational approaches to estimate solubility and permeability in drug discovery and development setting,” Adv. Drug Deliv. Rev., vol. 23, pp. 3–25, 1997.
[58] C. Wischke and S. P. Schwendeman, “Principles of encapsulating hydrophobic drugs in PLA/PLGA microparticles,” International Journal of Pharmaceutics, vol. 364, no. 2. pp. 298–327, 2008.
[59] A. Chaudhary, U. Nagaich, N. Gulati, V. K. Sharma, and R. L. Khosa, “Enhancement of solubilization and bioavailability of poorly soluble drugs by physical and chemical modifications: A recent review,” J. Adv. Pharm. Educ. Res., vol. 2, no. 1, pp. 32–67, 2012.

[60] P. L. Lam and R. Gambari, “Advanced progress of microencapsulation technologies: In vivo and in vitro models for studying oral and transdermal drug deliveries,” Journal of Controlled Release, vol. 178, no. 1. pp. 25–45, 2014.
[61] K. Keshavarao, D. Mudit, P. Selvam, and A. Jain, “Preparation and Evaluation of Naproxen by Solid Dispersion Technique,” Int. Res. J. Pharm., vol. 3, no. 9, pp. 174–177, 2012.
[62] A. T. M. Serajuddln, “Solid dispersion of poorly water-soluble drugs: Early promises, subsequent problems, and recent breakthroughs,” J. Pharm. Sci., vol. 88, no. 10, pp. 1058–1066, 1999.
[63] F. A. Alvarez-Núñez and S. H. Yalkowsky, “Relationship between Polysorbate 80 solubilization descriptors and octanol-water partition coefficients of drugs,” Int. J. Pharm., vol. 200, no. 2, pp. 217–222, 2000.
[64] R. Mellaerts, C. A. Aerts, J. Van Humbeeck, P. Augustijns, G. Van den Mooter, and J. A. Martens, “Enhanced release of itraconazole from ordered mesoporous SBA-15 silica materials,” Chem. Commun., vol. d, no. 13, pp. 1375–1377, 2007.
[65] O. A. Abu-diak, D. S. Jones, and G. P. Andrews, “An Investigation into the Dissolution Properties of Celecoxib Melt Extrudates : Understanding the Role of Polymer Type and Concentration in Stabilizing Supersaturated Drug Concentrations,” pp. 1362–1371, 2011.
[66] B. Lu and Z. Zhang, “Novel colon-specific microspheres with highly dispersed hydroxycamptothecin cores: Their preparation, release behavior, and therapeutic efficiency against colonic cancer,” J. Pharm. Sci., vol. 95, no. 12, pp. 2619–2630, 2006.
[67] A. Martín, R. a. García, D. Sen Karaman, and J. M. Rosenholm, “Polyethyleneimine-functionalized large pore ordered silica materials for poorly water-soluble drug delivery,” J. Mater. Sci., vol. 49, no. 3, pp. 1437–1447, 2014.
[68] N. Gargiulo, A. Peluso, P. Aprea, F. Pepe, and D. Caputo, “CO_2 adsorption on polyethylenimine-functionalized SBA-15 mesoporous silica: Isotherms and modeling,” J. Chem. Eng. Data, vol. 59, no. 3, pp. 896–902, 2014.

[69] J. Pang, L. Zhao, L. Zhang, Z. Li, and Y. Luan, “Folate-conjugated hybrid SBA-15 particles for targeted anticancer drug delivery,” J. Colloid Interface Sci., vol. 395, no. 1, pp. 31–39, 2013.

[70] J. Thielemann, F. Girgsdies, R. Schlögl, and C. Hess, “Pore structure and surface area of silica SBA-15: influence of washing and scale-up.,” Beilstein J. Nanotechnol., vol. 2, no. 1, pp. 110–8, 2011.

[71] M. Anbia and S. Salehi, “Removal of acid dyes from aqueous media by adsorption onto amino-functionalized nanoporous silica SBA-3,” Dye. Pigment., vol. 94, no. 1, pp. 1–9, 2012.

[72] F. Shang, S. Wu, J. Guan, J. Sun, H. Liu, C. Wang, and Q. Kan, “A comparative study of aminopropyl-functionalized SBA-15 prepared by grafting in different solvents,” React. Kinet. Mech. Catal., vol. 103, no. 1, pp. 181–190, 2011.

[73] K. Li, J. Jiang, F. Yan, S. Tian, and X. Chen, “The influence of polyethyleneimine type and molecular weight on the CO_2 capture performance of PEI-nano silica adsorbents,” Appl. Energy, vol. 136, pp. 750–755, 2014.

[74] Z. Bahrami, A. Badiei, and G. M. Ziarani, “Carboxylic acid-functionalized SBA-15 nanorods for gemcitabine delivery,” J. Nanoparticle Res., vol. 17, no. 3, 2015.

[75] Y. Wang, Q. Zhao, Y. Hu, L. Sun, L. Bai, T. Jiang, and S. Wang, “Ordered nanoporous silica as carriers for improved delivery of water insoluble drugs: A comparative study between three dimensional and two dimensional macroporous silica,” Int. J. Nanomedicine, vol. 8, pp. 4015–4031, 2013.

[76] Z. S. Eren, S. Tunçer, G. Gezer, L. T. Yildirim, S. Banerjee, and A. Yilmaz, “Improved solubility of celecoxib by inclusion in SBA-15 mesoporous silica: Drug loading in different solvents and release,” Microporous Mesoporous Mater., vol. 235, no. November, pp. 211–223, 2016.

[77] I. Parolini, C. Federici, C. Raggi, L. Lugini, S. Palleschi, A. De Milito, C. Coscia, E. Iessi, M. Logozzi, A. Molinari, M. Colone, M. Tatti, M. Sargiacomo, and S. Fais, “Microenvironmental pH is a key factor for exosome traffic in tumor cells,” J. Biol. Chem., vol. 284, no. 49, pp. 34211–34222, 2009.

[78] R. Palacio, J. Gallego, Z. Gabelica, C. Batiot-Dupeyrat, J. Barrault, and S. Valange, “Decomposition of ethanol into H_2-rich gas and carbon nanotubes over Ni,

Co and Fe supported on SBA-15 and Aerosil," Appl. Catal. A Gen., vol. 504, pp. 642–653, 2015.

[79] C. K. S. Ong, P. Lirk, C. H. Tan, and R. A. Seymour, "An evidence-based update on nonsteroidal anti-inflammatory drugs," Clin. Med. Res., vol. 5, no. 1, pp. 19–34, 2007.

[80] M. Thommes, "Physical adsorption characterization of nanoporous materials," Chemie-Ingenieur-Technik, vol. 82, no. 7, pp. 1059–1073, 2010.

[81] F. Rouquerol, J. Rouquerol, K. Sing, F. Rouquerol, J. Rouquerol, and K. Sing, "CHAPTER 7 – Assessment of Mesoporosity," in Adsorption by Powders and Porous Solids, 1999, pp. 191–217.

[82] E. Ricciotti and G. A. Fitzgerald, "Prostaglandins and inflammation," Arterioscler. Thromb. Vasc. Biol., vol. 31, no. 5, pp. 986–1000, 2011.

[83] R. N. Dubois, S. B. Abramson, L. Crofford, R. A. Gupta, L. S. Simon, L. B. Van De Putte, and P. E. Lipsky, "Cyclooxygenase in biology and disease.," FASEB J., vol. 12, no. 12, pp. 1063–1073, 1998.

[84] G. Steinbach, P. M. Lynch, R. K. Phillips, M. H. Wallace, E. Hawk, G. B. Gordon, N. Wakabayashi, B. Saunders, Y. Shen, and T. Fujimura, "The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis.," N. Engl. J. Med., vol. 342, no. 26, pp. 1946–52, 2000.

[85] S. K. Paulson, M. B. Vaughn, S. M. Jessen, Y. Lawal, C. J. Gresk, B. Yan, T. J. Maziasz, C. S. Cook, and A. Karim, "Pharmacokinetics of celecoxib after oral administration in dogs and humans: effect of food and site of absorption.," J. Pharmacol. Exp. Ther., vol. 297, no. 2, pp. 638–45, 2001.

[86] A. Nieto, F. Balas, M. Colilla, M. Manzano, and M. Vallet-Regí, "Functionalization degree of SBA-15 as key factor to modulate sodium alendronate dosage," Microporous Mesoporous Mater., vol. 116, no. 1–3, pp. 4–13, 2008.

[87] A. . Chawla, G., Gupta, P., Koradadia, V., Bansal, "Gastroretention : A Means to Adress Regional Variability in Intestinal Drug Absorption, Pharmaceutical Technology," Pharm. Technol., no. July, pp. 50–60, 2003.

[88] B. C. Hancock and M. Parks, "What is the true solubility advantage for amorphous pharmaceuticals?," Pharm. Res., vol. 17, no. 4, pp. 397–404, 2000.

[89] N. H. N. Kamarudin, A. A. Jalil, S. Triwahyono, M. R. Sazegar, S. Hamdan, S. Baba, and A. Ahmad, “Elucidation of acid strength effect on ibuprofen adsorption and release by aluminated mesoporous silica nanoparticles,” RSC Adv., vol. 5, no. 38, pp. 30023–30031, 2015.

[90] P. Zhao, H. Jiang, T. Jiang, Z. Zhi, C. Wu, C. Sun, J. Zhang, and S. Wang, “Inclusion of celecoxib into fibrous ordered mesoporous carbon for enhanced oral bioavailability and reduced gastric irritancy,” Eur. J. Pharm. Sci., vol. 45, no. 5, pp. 639–647, 2012.

[91] J. C. Doadrio, A. L. Doadrio, and J. Pe, “Hexagonal ordered mesoporous material as a matrix for the controlled release of amoxicillin,” vol. 172, pp. 435–439, 2004.

[92] S. Agrawal, N. Soni, N. K. Jain, and G. P. Agrawal, “Solubility Enhancement of Poorly Water Soluble Celecoxib for Parenteral Formulations,” Int. J. Pharm. Sci. Res., vol. Shikha Agr, no. 7, pp. 2325–2336, 2012.