Sillica Nanoporous Materials Adsorption and Release Study

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ABSTRACT

The lower efficiency of the current common ways of delivering drugs to humans like oral administration and injection make it necessary to find more efficient delivery systems. In this regard, a class of nanostructured materials called mesoporous silicates such as MCM-41, SBA-15 has attracted the attention of many scientists as drug delivery vehicles for their outstanding features like high surface area, high porosity, well-ordered, tunable nanometer pores and “non-cytotoxic” properties.

In this dissertation, comparative investigations of the adsorption capacity and drug release properties of these materials whose surfaces were functionalized with judiciously chosen organic groups via either post-grafting or co-condensation synthesis were conducted. Furthermore, two model drugs of different hydrophobicity and hydrophilicity were used in the study. The hydrophobicity of the ordered mesoporous silica can also be altered by modification of the surface with organic groups and thus, the hydrophobic interaction with the drug molecules can be improved. Here a comparative study between the two material synthesis method, the use of different functional groups and the two model drug molecules, Rhodamine 6G and ibuprofen, were performed. The results indicated that functional groups really make an obvious difference on the adsorption capacity and release profile depending on the type of functional group and drug molecules. Furthermore, differences in adsorption capacity and drug release properties between the materials synthesized via co-condensation and those synthesized via post-grafting were also observed. The results of the study may give further insight into rational synthetic approaches to functionalized mesoporous materials with improved adsorption capacity and release properties for a variety of hydrophobic and hydrophilic drugs. In addition, pH responsive release study was conducted. Ibuprofen and R6G were used as model drug molecules. For the ibuprofen loading, amine functionalized MCM (NH₂-MCM) was chosen as carrier while amine and thiol bi-functionalized MCM
(NH$_2$-MCM-SH) was chosen as carrier for the adsorption R6G. Poly (acrylic acid) PAA was encapsulated onto the silica matrix as pH stimuli because it is a well-know bioadhesive hydrogel and often used in drug formulation.

Among mesoporous materials, SBA-15 has large surface area, large pore size, and thick walls. It possesses a hexagonal array of mesopores 6.0-20 nm in diameter, which is much larger than the 3.0-nm pores characteristic of the MCM-41 and MCM-48. Their wall thickness is an important parameter for providing a high degree of hydrothermal stability and improved mechanical stability compared to mesoporous MCM-41 and related silicas, and thus a sufficiently long lifetime in biological environments. Therefore, SBA-15 mesoporous materials could be modified with various surface functional groups and still preserve the large pore channels and surface areas for drug adsorption and desorption after modification. So, drug adsorption and release study over SBA-15 and comparative study between SBA-15 and MCM-41 was also investigated. Results show that SBA-15 has larger release amount than MCM-41.

In this dissertation, the oxidation of epinephrine in the presence of SBA-15 and MCM-41 particles was also carried out. Dense silica particle was test as a control. It was reported that caffeine (1,3,7-trimethylxanthine) can work as a scavenger of the hydroxyl radical at millimolar concentrations. Here we investigated the epinephrine oxidation in the presence of caffeine. Antioxidant effects of caffeine and its metabolites were also evaluated by the epinephrine oxidation rate. Same experiment was also performed with its isomer, isocaffeine. It was found that influence of mesoporous silica (MCM-41 and SBA-15) nanoparticles and dense silica nanoparticles on epinephrine oxidation is pH-dependent. Aslo, in the presence of either caffeine or isocaffeine, the oxidation rate of epinephrine by MSN will be slowed down and with the increase of caffeine or isocaffeine concentration, the oxidation rate decrease accordingly.
SILICA NANOPOROUS MATERIALS ADSORPTION AND RELEASE STUDY

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DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry in the Graduate School of Syracuse University

August 2012
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ACKNOWLEDGEMENTS

I would especially like to express my deepest gratitude to my advisor, Professor Jerry Goodisman, for his generous time and guidance in numerous ways throughout the PhD thesis preparation. Without his support and guidance, this dissertation would not become a reality.

I am also very grateful to my dissertation committee members, Dr. James Dabrowiak, Dr. James T. Spencer, Dr. Yan-Yeung Luk, Dr. Karin Ruhlandt-Senge, and Dr. Eric Schiff for their supervision and valuable advice on the dissertation.

I would like to thank my all my lab mates, graduates and undergraduates, who worked with me and make me not lonely in this journey to my PhD degree. I also would like to express gratitude to Zhimin Tao for his great help in my research work.

I would like to express my gratitude to my parents for giving me constant support and encouragement throughout my education. I am also very grateful to my wife and my daughters for their support, understanding and patience throughout this dissertation work.
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CHAPTER 1 INTRODUCTION

1.1 Background

Nanoparticles presence in commercial products has been more and more common these days. According to the ASTM standard definition, nanoparticles refer to particles with a lengths ranging from 1 to 100 nanometers in two or three dimensions.\(^1\) It is predicted that production of nanoparticles will increase from the about 2300 tons to 58000 tons by 2020.\(^2\) Nanoparticles have been attracting great interest of many scientists because they act as a bridge connecting bulk materials and atomic or molecular structures. While bulk materials have constant physical properties no matter what the sizes they have, it is very different when it comes to nanomaterials. Size-dependent properties like quantum confinement in semiconductor particles are observed. When the size of the materials is decreased to the nanoscale, the materials can show very different properties compared to the properties they have on a macroscale and thus result in many unique applications. For instance, opaque substances such as copper become transparent; inert materials such as platinum attain catalytic properties; stable materials such as aluminum turn combustible; solids like gold turn into liquids at room temperature and insulators like silicon become conductors when it come to nanoscale.\(^3\)

Among these nanoparticle materials, nanoporous material is one of the most studied fields, especially mesoporous materials. Generally, nanoporous materials can be divided into three categories based on their size according to IUPAC notation: \(^4\) (1) microporous materials, (2) mesoporous materials, (3) macroporous materials

Microporous materials have pore diameters of less than 2 nm, mesoporous materials have pore diameters between 2 nm and 50 nm and macroporous materials have pore diameters of
greater than 50 nm. One example of microporous materials is Zeolites which are aluminosilicate minerals commonly used as commercial adsorbents. Zeolites are widely used in industry for water purification, as catalysts, and in nuclear reprocessing. Their biggest use is in the production of laundry detergents. They are also used in medicine and in agriculture. But one drawback of this kind of materials is its small size especially in the applications involved in guest and host molecule interaction. For example in catalyst application, their small size limits the transportation of molecule while fast mass transfer of the reactants and products to and from the active sites is required for catalysts. Also it doesn’t work when it comes to bigger molecules and reactants which sizes are too big to go into and come out of the small pores. Same situation happens in the drug delivery application for big drug molecules.

The main research work in this thesis is drug delivery and mesoporous materials were used in all the studies.

1. 2 Mesoporous silica materials

The zeolite has small pore size of about 1.5nm and was therefore limited in its application while mesoporous materials have larger pore sizes than zeolite molecular sieves. Besides their larger sizes, the mesoporous materials also have very large specific surface areas, ordered pore systems, and well-defined pore radius distributions like zeolites. Compared to the dimensions of the zeolite micropores (<2nm), mesopores (2-50 nm) make faster migration of guest molecules a reality in the host frameworks. The first mesoporous material of a long range order was first reported in 1990 by T. Yanagisawa et al. Later, it was also developed by the group of the former Mobil Oil Company in 1991. Since then, the development of porous materials with large specific surface areas is currently an area of extensive research, particularly with regard to potential applications in areas such as adsorption, chromatography, catalysis, sensor technology,
gas storage, drug delivery, gas sensing, optics, and photovoltaics. The most studied M41S materials include the silica solids MCM-41 (with a hexagonal arrangement of the mesopores), MCM-48 (with a cubic arrangement of the mesopores), and MCM-50 (with a laminar structure). Among these MCM materials, MCM-41 is chosen and investigated in this thesis. Besides MCM materials, another most commonly used mesoporous silicas have been SBA-type (Santa Barbara Amorphous) mesoporous materials. SBA materials were first synthesized in Galen Stucky’s group at the University of California at Santa Barbara. SBA-type mesoporous materials can be synthesized in the presence of triblock poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) copolymers, such as EO20PO70EO20 (Pluronic P123). A series of ordered SBA-type mesoporous silica materials with different d spacings and pore sizes was prepared by using poly (alkylene oxide) triblock copolymers with different EO to PO ratios. Among these SBA materials, SBA-15 is one of the most investigated one from SBA family with hexagonal mesoporous silica structure. Upon removal of the pluronic templates, a highly ordered mesoporous SBA-15 with large pores and thicker silica wall can be obtained. It has BET surface areas of 690-1040 m²/g, large pore sizes of 46-300 Å, and unusually large pore volumes up to 2.5 cm³/g, with silica wall thicknesses ranging from 31 to 64 Å. SBA-15 mesoporous materials could be modified with various surface functional groups and still preserve the large pore channels and surface areas for drug adsorption and desorption. The pH of the solution is an important factor for the successful arrangement and assembly of the alkyl ammonium or the tri-block copolymer templates. Besides, the diameter of SBA is much larger than the pore size of the MCM-41 and MCM-48, there is also difference from M41S type materials in the preparation process. The pH value of the medium at which M41S and SBA-type
materials are synthesized is also completely different. SBA-type materials are synthesized under acidic solution (pH 2.0) while M41S ones are conducted in basic solutions, i.e. at a pH above 12.

The large pore size, high surface area and large pore volume make the mesoporous materials suitable as a potential carrier for various chemical compounds such as molecules with biological activity. However, in order to achieve an effective control of adsorption and release of the guest molecules, it would be necessary to modify the chemical nature of the wall of mesoporous pore with specific functional group base on the hydrophobicity and hydrophilicity of adsorbed molecules. In this context, the presence of a high concentration of silanol groups on the surface inside and outside the pore can be functionalized to control pore size and surface properties, and thus the drug delivery kinetics would be affected by the interactions between the drug and matrix. MCM-41, MCM-48, and SBA-15 silica materials have been functionalized with, for example, amino, diamino, triamino, ethylenediamine, malonamide, carboxy, 21, 26 Carboxylic Groups thiol, sulfonic acid groups, dithiocarbamate, and imidazole groups, alkyl, 1-allyl, can be incorporated into the pore walls of the silica network.

Generally speaking, surface functionalization of mesoporous silica materials via covalent bonding of organic groups can be achieved by three methods:

1) Post-grafting of the surface of extracted mesoporous silica material (grafting),

2) Condensation of silica precursor (TEOS or TMOS) and organosilica precursor mixtures (co-condensation),

3) Incorporation of organic groups directly into the materials by the use of bridging organosilica precursors (periodic mesoporous organosilicas).

1.2. 1 Post-synthetic method (Post-grafting)
Grafting procedures are based on modification of the silica surface with organic groups through silylation reactions occurring on isolated (=Si-OH) and geminal (=Si(OH)₂) silanol groups. Organosilanes of the (RO)₃SiR type like (CH₃O)₃Si(CH₂)₃NH₂, (CH₃O)₃Si(CH₂)₃SH is chosen and react with the free silanol group on the surface. Different organic group can be functionalized on the surfaces by the changing of organic residue R. On the one hand, post-grafting method can retain starting silica materials and results in well-ordered functionalized mesostructured materials, on the other hand, it often produces non-uniformly distributed organic groups because the organic moieties can congregate more on channel opening and on the exterior surfaces and the further diffusion of molecules into the center of the pores can thus be affected.

Generally, after surfactant forms a micellar structure, the mixture of tetraalkoxysilanes (TEOS or TMOS) and terminal trialkoxyorganosilanes are added to it and then hydrolyzed and condense on the walls of surfactant micellar template via electrostatic attraction. Since the organic functionalities are directly composed into the silica matrix, the congregation of organic moieties at pore entrance will be not a problem via the co-condensation method. Furthermore, Co-condensation synthetic method of mesoporous materials involves a one-step procedure and allows better control of the loading and distribution of the organic groups although it often produces materials with less ordered mesoporous structures. Generally speaking, low degree of structural integrity and long-range periodicity as well as lower surface area would be produced when the organosilane concentration in the synthesis exceeds ~25%.

In addition, an increased loading of the incorporated organic moieties will result in the decrease of the pore diameter, pore volume, and surface areas. In order not to destroy the organic
functionality during removal of the surfactant, extractive methods are usually used, instead of calcination.

1.2. 2 Co-Condensation

Besides post-grafting method, another alternative method to synthesize organically functionalized mesoporous silica materials is the co-condensation method or one-pot synthesis. It involves a one-step co-condensation of tetraalkoxysilanes such as tetraethyl orthosilicate (TEOS) with organosilanes such as MPTS, VTS, and offers a higher and more uniform surface coverage of functional groups and thus a better control over the surface properties of the resulting functionalized materials. Since the pioneering work in the research groups of Stein,74 Mann,75,76 Macquarrie77 and Stucky,78 co-condensation reactions have been used to prepare hybrid mesoporous silicates under a wide range of reaction conditions. A number of organically modified silica phases have been synthesized by co-condensation. Through the use of the respective organosilane, organic functionalities such as carboxylic acid group34 alkyl,51,52 vinyl/allyl,51,53-59 thiol,51,58,60,61 aromatic groups52, 58, 62-66 and amino.58,59,67-73

1.2.3 Preparation of Periodic Mesoporous Organosilicas (PMOs)

PMOs were first synthesized in 1999 by three different research groups.81-83 Since then, a number PMOs were synthesized in the same way84-93. They applied the same procedure for the synthesis of ordered pure mesoporous silica by structuring with ionic surfactants to organosilica hybrid after replacing organosilica precursors with bridged organosilica precursors (RO)3Si-R-Si(OR)3. Hydrolysis and condensation reactions of bridged organosilica precursors result in the materials in which the organic units are incorporated in the three-dimensional network structure
of the silica matrix through two covalent bonds. So, the organic units are distributed homogeneously in the silica matrix. It is very different from the materials obtained by grafting and co-condensation method. These materials have large inner surface areas of up to 1800 m$^2$/g as well as high thermal stability. However, the pores are completely disordered of a wider size distribution. Generally speaking, PMO materials have significant advantages over pure mesoporous silica and organic functionalized mesoporous silica synthesized by postgrafting and co-condensation method. They are considered as promising candidates for a number of applications, such as in catalysis, adsorption, chromatography, nanoelectronics and active compound release systems.

1.2. 4 Application in drug delivery

Drug delivery system (DDS) is a carrier which can regulate the drug delivery rate and targets specific areas of the body by modifying the materials with specific groups. The method by which a drug is delivered can have a significant effect on its therapeutic efficacy. Because with traditional therapy, the drug usually lose effect before it reach the desired site in the body. So, DDSs are designed to keep therapeutic effect during the treatment period till it reach the desired site.$^{143}$ An ideal drug-delivery system need meet the following characters:

(1) Non-toxic and biodegradable;

(2) High loading capacity;

(3) Ability to protect the drugs from decomposition;

(4) Ability to seal the drug in order to prevent the drug from being released prior to reaching the target-site by modified pore openings with caps, and protect the body from possible side-effects of the drug;
(5) Ability to direct the small carrier structure throughout the human organism to the desired target-site;

(6) The caps should open reliably in order to release the drug efficiently after reaching the target-site.

Considering these demanding requirements, various drug-delivery systems (polymers, micelles, polymeric nanocapsules, and various nanoparticles etc.) have been studied so far. It is not easy to meet all the criteria for the delivery systems and the drug delivery systems mentioned above all have some disadvantages. For example, drug is released instantaneously or is decomposed before reaching the desired site in some system. The controlled drug release process is attractive and has been widely and intensively studied. Much of this work involves polymers which enable the drug to be delivered at relative constant rate by diffusion control from polymer or polymer composites over time. However, in the polymer delivery system, the drug is usually deposited via coating, wet granulation or direct compression, even by mechanical mixing both matrix and drug which have the disadvantage of non-uniform distribution and thus can influence the release rate between different drug compositions. More importantly, cell-targeting remains a great challenge for many classes of drug-delivery systems. Therefore, much improvement in this field and a smart drug delivery alternative are badly needed and other material and method avoiding these disadvantages was explored. Nanomaterials has been used by many researchers for drug delivery and the mesoporous material MCM-41 was first used as a DDS in 2001.\textsuperscript{144} The reason to choose MCM-41 as DDSs is its non-toxic, biodegradable and biocompatible characters;\textsuperscript{145-148} it has an ordered and tunable pore network of homogeneous size which allows fine control of the drug load and release kinetics; The high pore volume and high surface area of the MCM-41 allows to host the required amount of pharmaceutical and implies high potential for drug
adsorption; porous network that can be capped; possibility to be modified with specific receptors on the surface. The last but not the least, it has free silanol on the surface which can be functionalized by desired functionalized organic group to allow better control over drug loading and release. The characters of mesoporous silica materials make it a perfect candidate for many applications, like catalysis, adsorbents, drug delivery and sensor. Among these applications, application in drug delivery is a promising research field and research presented in this thesis is focused on this.

1.3 Overview of Dissertation

In Chapter 2, surfaces of mesoporous materials are functionalized with judiciously chosen organic groups via post-grafting or co-condensation of various organosilanes, and then comparative and systematic investigations of the adsorption capacity and drug release properties of mesoporous materials were conducted. Furthermore, two different molecules of different hydrophobicity and hydrophilicity were used as model drugs in the study. In Chapter 3, R6G and ibuprofen adsorption and release study over different functionalized SBA-15, i.e., Ex-SBA, SH-SBA, COOH-SBA was studied, and then COOH-MCM was also studied to compare the different adsorption and release properties from SBA materials. In Chapter 4, influence of mesoporous silica (MCM-41 and SBA-15) nanoparticles and dense silica nanoparticles on epinephrine oxidation was investigated. Antioxidant effects of caffeine and its metabolites were also evaluated by the epinephrine oxidation rate. Same experiment was also investigated with its isomer, isocaffeine. In Chapter 5, pH responsive release study of R6G over amine functionalized MCM (NH2-MCM) and ibuprofen release study over amine and thiol functionalized MCM (NH2-MCM-SH) were conducted. Poly (acrylic acid) PAA was encapsulated onto the silica matrix as
pH stimuli and the release study was carried out at pH 1.0 and pH 7.4. Finally, the conclusion and future study are addressed in chapter 6.

1.4 References


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CHAPTER 2 FUNCTIONALIZED MESOPOROUS MATERIALS FOR ADSORPTION AND RELEASE OF DRUG MOLECULES

2.1 Introduction and Background

At present, the most common ways of delivering drugs to humans are oral administration and injection. However, these methods have lower efficiency for some therapies. Some therapeutic agents are unstable or are poorly soluble; therefore, new delivery systems for them are required. Functionalized nanostructured materials are increasingly considered as great candidates to make drug delivery vehicles and controlled drug release systems. This is because they have suitable platforms that can help minimize adverse reactions and unwanted side effects, that many conventional drugs used today often pose. 1,2 With some drug administration methods, the drugs have to often pass through various physiological obstacles before they reach to their desired target, thus decreasing the amount of drug that gets to the targeted site. The inability to deliver controlled therapeutic concentration of drugs to the desired location can result in a decrease in the efficacy of the drug. 1,2 Increasing the concentrations of drugs to be delivered by using nanomaterial based drug delivery vehicles with improved capacity of adsorption and controlled drug release properties can enhance the efficiency of the drugs.

Since their discovery in the early 1990’s, 3-6 a class of nanostructured materials called mesoporous silicates such as MCM-41 have attracted the attention of many scientists as drug delivery vehicles 7-12 because of their outstanding features such as high surface area (typically 1000 m²/g), high porosity (typical pore volumes of 0.5 – 1.5 cm³/g), well-ordered, tunable nanometer pores (typically 2-15 nm pore diameter) 13-19 and “non-cytotoxic” properties. 20-23 Figure 2.1 shows the procedure of the synthesis.
Figure 2. Assembly of surfactant and followed by condensation of silica precursor and thus result in hexagonal arrangement of mesoporous material via calcination or extraction.

In fact, different types of mesoporous silica nanomaterials were already proved to be capable of carrying high dosages of a variety of drugs in their mesopores. Additional benefits of mesoporous silica materials for drug delivery include the simplicity of tuning their pore sizes by changing their templates in order to better accommodate drug molecules of different sizes as demonstrated by extensive works by Vallet-Regí and co-workers as well as other researchers. While smaller drug molecules and biomolecules can be accommodated in mesoporous materials with smaller as well as bigger pore sizes, larger drug molecules require materials with bigger pore diameters. Furthermore, mesoporous silica materials contain residual silanol groups that can further be functionalized by different organic groups in order to modify their surface properties. This creates favorable surface – drug interactions, which in turn result in improved adsorption capacity of the materials for drug molecules. Lin and co-workers have shown that the organic functionalization of mesoporous materials can also influence their biocompatibility. In addition to surface functional groups, the morphology and size of the mesoporous materials also have an important influence on drug release characteristics.

Generally, surface functionalization of mesoporous silica materials via covalent bonding of organic groups can be achieved by two methods: i.e., post-grafting synthesis and co-
Although post-grafting method results in well-ordered functionalized mesostructured materials, it often produces non-uniformly distributed organic groups because the organic moieties can congregate more on the channel pore mouth and on the exterior surfaces.  

Co-condensation synthetic method of mesoporous materials involves a one-step procedure and allows better control of the loading and distribution of the organic groups although it often produces materials with less ordered mesoporous structures. In particular, low degree of structural integrity and long-range periodicity as well as lower surface area would be produced when the organosilane concentration in the synthesis exceeds ~25%.

Herein comparative investigations of the adsorption capacity and drug release properties of mesoporous materials whose surfaces are functionalized with judiciously chosen organic groups via post-grafting or co-condensation of various organosilanes were conducted. Furthermore, two different hydrophobic and hydrophilic molecules were used as model drugs in the study. The approach of organic functionalization of mesoporous materials for drug delivery has been considered previously. The most studied system is ibuprofen adsorption on functionalised matrices. When the MCM-41 or SBA-15 surface is functionalised with amino groups, there is an ionic interaction between the carboxylate groups of ibuprofen and the ammonium groups on the matrix surface. The hydrophobicity of the ordered mesoporous silica can also be altered by modification of the surface with alkyl chains. As a result, the hydrophobic interaction with hydrophobic drugs can be improved. For instance, erythromycin release from functionalized matrices was much slower compared to unmodified material. Here a comparative study between grafting and co-condensation as well as the use of different functional groups and two different (model) drug molecules have been performed. Functional groups including 3-aminopropyl, 3-mercaptopropyl, vinyl, and secondary amine groups were used to functionalize
the mesoporous materials while rhodamine 6G (Figure 2.2) and ibuprofen were used as probe molecules to investigate the materials’ adsorption and release properties. Our comparative study is intended to obtain the relative effect of functional groups as well as type of synthetic method to the functionalized materials on the adsorption and release properties of the materials for different molecules. The self-assembly of the mesoporous materials was carried out with cetyltrimethylammonium bromide (CTAB) surfactant producing MCM-41 type materials with pore diameters of ~2.7-3.3 nm and moderate to high surface areas up to ~1000 m²/g. By changing the organic groups, the properties of the mesoporous materials from hydrophobic to hydrophilic were tuned and their adsorption and release properties for different (model) drug molecules such as rhodamine 6G and ibuprofen were varied.

Rhodamine 6G and ibuprofen were chosen as probe molecules in our study because of their differences in hydrophilicity (or hydrophobicity), which allows the investigation of interaction of different molecules with functionalized mesoporous materials. 61-67 Furthermore they are easy to probe by UV-Vis spectroscopy. The solubility of rhodamine 6G and ibuprofen is dependent on solvent and pH of solution. For instance, the solubility of rhodamine 6G is 20 g/L in water; 40 g/L in butanol, 80 g/L in ethanol, 15 g/L in propanol and 100 g/L in diethylene glycol. Rhodamine 6G has increased solubility at when pH increases. 73 Ibuprofen, which is a relatively weak acid with pKa value of 4.4, has low solubility in water and at acidic pH. Ibuprofen has an intrinsic solubility of ~0.06 mg /mL⁻¹ in water. Ibuprofen is sparingly soluble in hexane and freely soluble in ethanol, octanol and dimethyl sulfoxide and chloroform with values of >10 g/L in acetone, >10 g/L in ethanol, 33 g/L in octanol, and 3.3 g/L in hexane. The solubility of ibuprofen increases sharply with pH, i.e. the drug being largely insoluble at low pH, but readily soluble at alkaline pH. For example in water, its solubility is ~0.5 x 10⁻¹ g/L at pH < 2.00 but
~1x10^2 g/L at pH = 7.5.\textsuperscript{74} Based on these properties or because rhodamine 6G is quite soluble in water while ibuprofen is rather soluble in solvents such as hexane and ethanol, we have considered rhodamine 6G to be hydrophilic probe molecule while ibuprofen to be hydrophobic in this study. They, therefore, are expected to show different adsorption and release properties in organic functionalized mesoporous materials.

Our studies indicated that while the samples functionalized with mercaptopropyl and vinyl groups resulted in high adsorption capacity for rhodamine 6G, those functionalized with amine groups showed higher adsorption capacity for ibuprofen. Similarly, the drug release properties also varied from sample to sample, depending on the type of functional groups they contained. Furthermore, differences in adsorption capacity and drug release properties between the materials synthesized via co-condensation and those synthesized via post-grafting were also observed. The results of our study may give further insight into rational synthetic approaches to functionalized mesoporous materials with improved adsorption capacity and release properties for a variety of hydrophobic and hydrophilic drugs.

![Figure 2. 2 Structure of Rhodamine 6G molecule](image)

2.2 Results and Discussion

2.2.1. Characterization of Functionalized Mesoporous Materials

2.2.1.1 Powder X-ray Deffraction and Transmission Electron Microscopy (TEM)
The X-ray diffraction (XRD) patterns of the functionalized mesoporous materials and representative TEM images of samples synthesized by post-grafting and co-condensation are shown in Figure 2.3, Figure 2.4, and Figure 2.5 respectively. The X-ray diffraction (XRD) patterns of all the samples synthesized by postgrafting exhibited hexagonally ordered mesoporous structure that is characteristic of MCM-41type materials (Figure 2.3). The X-ray diffraction (XRD) patterns (A and C in Figure 2.4) of the samples synthesized by co-condensation from VTS and MPTS (Co-VTS and Co-MPTS) also showed ordered mesostructures; however, those synthesized from APTS (Co-APTS) and BTSPA (Co-BTSPA) revealed less ordered mesostructures. The weakly ordered mesostructure in the latter results probably because hydrophilic organoamine groups in APTS and BTSPA cause some perturbation in the center or the hydrophobic core of the surfactant micelles during co-condensation synthesis of mesostructured materials from these organosilanes. From the (100) Bragg peak, the \( d \)-spacing values of both Co-VTS and Co-MPTS samples as well as those synthesized by post-grafting were calculated to be ~4.3-4.5 nm. Furthermore, except for a slight decrease in their (100) Bragg reflection, the samples remained mesostructured with the same unit cell size after adsorption and release of rhodamine 6G (R6G) and ibuprofen guest molecules (B and D in Figure 2.4). The decrease in the intensity of Bragg reflection is probably a result of a slight decrease in electron contrast between the mesopores and the mesopore channel walls due to the confinement of the R6G and ibuprofen guest molecules inside the mesopores. Although the unit cells remained the same, the pore diameters and surface areas of the materials decreased after adsorption of the drug or guest molecules (See below).

The morphology and mesopores of the materials are shown in Figure 2.5. Although the
morphology and size of the mesoporous materials are known to have an important influence on drug release characteristics, the materials we used for our comparative studies are made from the same batch of parent material, and consequently had similar morphologies. This is, therefore, expected to cause little difference on the adsorption and release properties of the materials in our studies.

(A)

(B)
Figure 2. 3 Powder X-ray diffraction (XRD) patterns of functionalized mesoporous samples synthesized by post-grafting of organosilanes (A) APTS, (B) BTSPA, (C) MPTS and (D) VTS onto mesoporous silica
Figure 2.4 Powder X-ray diffraction (XRD) patterns of functionalized mesoporous samples synthesized by co-codensation of organosilanes (A) MPTS and (C) VTS with the corresponding XRD patterns of the samples after R6G adsorption shown in (B) and (D), respectively.

Figure 2.5 Low resolution and high resolution TEM Images of mesoporous samples synthesized by post-grafting (A, B) and (C, D) co-condensation.
2.2.1.2 Nitrogen Gas Adsorption (BET)

The structures of the functionalized samples were also characterized by nitrogen gas adsorption. The gas adsorption (Figure 2.6) showed a type IV isotherm with sharp capillary condensation steps for all the samples synthesized by post-grafting as well as for samples Co-MPTS and Co-VTS that were synthesized by co-condensation. This is indicative of the presence of mesoporous structure in these materials. This result is also consistent with their XRD patterns shown in Fig. 2.3 and Fig. 2.4. The specific surface area and pore sizes of the materials are reported in Table 2.1. It is worth noting that all the samples synthesized by post-grafting and the samples synthesized by co-condensation from VTS and MPTS showed high surface areas. The samples synthesized from APTS and BTSPA by co-condensation showed significantly lower surface areas.

(A)
Figure 2.6 Nitrogen adsorption isotherms samples synthesized via (A) post-grafting and (B) co-condensation of different organosilanes

The pore size distributions of the materials are shown in Figure 2.7 and their average mesoporous diameters are listed in Table 2.1. It is important to note here that the absolute values of the pore diameters have to be treated with caution as the BJH method, in general, underestimates the pore diameter of mesoporous materials.\textsuperscript{78-80} We also calculated corrected values of pore diameters by using the equation developed by Kruk and Jaroniec.\textsuperscript{78, 80} The results reveal that the materials synthesized by post-grafting have average mesopore diameters between 2.7-3.2 nm while the samples synthesized by co-condensation have average mesopore diameters of 3.0-3.3 nm (Table 2.1). The average pore diameters of samples Co-VTS and Co-MPTS were slightly lower than the corresponding samples synthesized by post-grafting. This is consistent with previously reported materials from co-condensation of hydrophobic organosilanes, which showed smaller pore sizes and whose pore sizes depend on the relative concentration of the organonosilane in the solution.\textsuperscript{81, 82} Furthermore, upon immobilization of the guest or drug
molecules, the surface areas of the materials decreased. For instance, the surface area of the Co-MPTS sample decreased from 885 m²/g to 352 m²/g after getting saturated with rhodamine 6G (see below)

(A)

(B)

Figure 2. 7 Pore size distribution of functionalized samples synthesized by (A) post-grafting APTS, BTSPA, MPTS, and VTS, onto MCM-41 and (B) co-condensation of APTS, BTSPA, MPTS, and VTS with TEOS
Table 2.1 Structural data of the organic functionalized mesoporous materials

<table>
<thead>
<tr>
<th>Samples</th>
<th>Surface Area (m²/g)</th>
<th>Average BJH Mesopore Diameter (Å) (^a)</th>
<th>Average Mesopore Diameter by KJS Method (Å) (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co-APTS</td>
<td>189.0</td>
<td>2.4</td>
<td>3.1</td>
</tr>
<tr>
<td>Co-BTSPA</td>
<td>238.1</td>
<td>2.3</td>
<td>3.2</td>
</tr>
<tr>
<td>Co-MPTS</td>
<td>885.1</td>
<td>2.2</td>
<td>2.7</td>
</tr>
<tr>
<td>Co-VTS</td>
<td>1183.4</td>
<td>2.4</td>
<td>3.0</td>
</tr>
<tr>
<td>Grafting-APTS</td>
<td>887.3</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Grafting-BTSPA</td>
<td>852.5</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Grafting-MPTS</td>
<td>952.1</td>
<td>2.1</td>
<td>3.2</td>
</tr>
<tr>
<td>Grafting-VTS</td>
<td>812.0</td>
<td>2.2</td>
<td>3.3</td>
</tr>
</tbody>
</table>

\(^a\) The pore diameters were obtained by the BJH method, which is known to underestimate the pore diameter of mesoporous materials.\(^{78-80}\) It is, therefore, worth noting that the absolute values of these pore diameters have to be treated with caution. \(^b\) The pore diameters were calculated by an equation developed by Kruk and Jaroniec.\(^{78, 80}\)

2.2.1.3 Solid-state NMR spectra of various functionalized mesoporous samples

Typical \(^{29}\)Si CP-MAS NMR spectra for the functionalized samples are shown in Supplementary Materials (Figure 2.13). The spectra showed peaks at around \(-100\) and \(-110\) ppm which correspond to hydroxyl containing silicon sites (\(Q^3\) or \(SiO_{1.5}OH\)) and cross-linked silicon (\(Q^4\) or \(SiO_2\)), respectively. The spectra also exhibited peaks between \(-50\) and \(-80\) ppm that were assigned to the Si atoms covalently bonded to organic groups \{or \(T^2 [(SiO)_2Si(OH)(CH_2)]\) and \(T^3 [(SiO)_3Si(CH_2)]\) sites\}.\(^{68-72}\) The \(^{13}\)C CP-MAS NMR spectra (Figure 2.14) of APTS and MPTS functionalized samples showed a signal at \(-10\) ppm, which was assigned to methylene, -CH₂-, groups that are directly bonded to silicon atom. The resonances between 20-30 ppm were
attributed to the other methylene and the terminal methyl groups of the propyl chain. Furthermore, Figure 2.14, B, Figure 2.14, C, and Figure 2.14, D showed additional minor peaks at ~30 ppm that were assigned to carbon atoms of residual CTAB surfactant in the pores of the samples. These peaks were observed in the spectra for samples grafted with MPTS, VTS and BTSPA while they were not visible on the spectrum for the sample grafted with APTS (Figure 2.13, A) though all these four samples were prepared from the same batch of surfactant-extracted MCM-41. This is because the density of organic groups grafted in the former three samples was significantly lower than that in the APTS-grafted sample. Thus, the residual surfactant peaks appeared more visible with respect to the peaks corresponding to the organic functional groups in the former than in the latter.

2.2.1.4 Thermogravimetric (TGA) traces of various functionalized mesoporous materials

The mesoporous materials were further characterized by thermogravimetric analysis under nitrogen (Table 2.6 and Figure 2.15) and elemental analysis (Table 2.2). These results further corroborated the results obtained from solid-state NMR. Thermogravimetric analysis of organic functionalized mesoporous materials under nitrogen or air were previously used by many groups for characterization of organic functionalized mesoporous materials.84-87 The TGA traces of the functionalized samples (Figure 2.15,) showed a weight loss below 100 °C due to physisorbed water88 and, most importantly, a weight reduction between ~200-600°C due to the loss of organic functional groups. Furthermore, a slight weight reduction after 600°C due to loss of water molecules from condensation of residual silanols was observed. Elemental analysis revealed that the mmol organic/g of sample synthesized by post-grafting of APTS, BTSPA, MPTS, and VTS were 2.17, 0.94, 0.97, and 1.53 mmol/g, respectively (Table 2.2). This indicated that the surface density of functional groups that were grafted decreased in the order of APTS >
VTS > MPTS ≈ BTSPA. On the other hand, the mmol organic/g sample in the samples synthesized by co condensation, Co-MPTS and Co-VTS, were 2.18 and 2.57 mmol/g, respectively (Table 2.2). This indicates that the mmol of functional groups in the samples is higher for sample prepared from VTS than that from MPTS (or Co-VTS > Co-MPTS). We can also conclude from Table 2.2 that the sample synthesized from MPTS and VTS by co-condensation had more functional groups compared to the corresponding sample synthesized by post-grafting.

**Table 2.2** Density of grafted organic groups (mmol/g) of functionalized mesoporous materials determined from elemental analysis and adsorption capacity of the samples for rhodamine 6G.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Grafting APTS mmol R6G adsorbed/g sample</th>
<th>Grafting BTSPA mmol organic groups/g sample</th>
<th>Grafting MPTS</th>
<th>Grafting VTS a</th>
<th>Co-MPTS</th>
<th>Co-VTS a</th>
</tr>
</thead>
<tbody>
<tr>
<td>mmol R6G adsorbed/g sample</td>
<td>1 x 10^{-4}</td>
<td>2 x 10^{-4}</td>
<td>0.14</td>
<td>0.04</td>
<td>0.29</td>
<td>0.08</td>
</tr>
<tr>
<td>mmol organic groups/g sample b</td>
<td>2.17</td>
<td>0.94</td>
<td>0.97</td>
<td>1.53</td>
<td>2.18</td>
<td>2.57</td>
</tr>
<tr>
<td>Average number of adsorbed molecules per one surface ligand</td>
<td>4.6 x 10^{-5}</td>
<td>2.1 x 10^{-4}</td>
<td>1.4 x 10^{-1}</td>
<td>2.6 x 10^{-2}</td>
<td>1.3 x 10^{-2}</td>
<td>3.1 x 10^{-4}</td>
</tr>
<tr>
<td>Normalized with Surface Area (mmol R6G adsorbed/Surface area, mmol/m²)</td>
<td>1.1 x 10^{-7}</td>
<td>2.3 x 10^{-7}</td>
<td>1.5 x 10^{-3}</td>
<td>4.9 x 10^{-3}</td>
<td>3.3 x 10^{-4}</td>
<td>6.8 x 10^{-5}</td>
</tr>
</tbody>
</table>

a mmol/g the samples functionalized with VTS could be slightly off as they were calculated based on the wt. % of C and as the samples have some additional carbon from the residual surfactant. b Determined from elemental analysis.
2.2.2 Adsorption and release study

By using the Beer-Lambert law, the amount of molecules adsorbed in the mesoporous samples or released by the samples was calculated. Although there was no significant difference in surface area and pore size (Table 2.1) between the four samples synthesized by post-grafting, the samples functionalized with vinyl and mercaptopropyl groups adsorbed significantly more rhodamine 6G than the samples functionalized with primary and secondary amines (Table 2.2 and Figure 2.8). This trend was true also for samples synthesized by co-condensation. Upon comparing samples synthesized under similar conditions, the samples containing mercaptopropyl groups were found to adsorb more rhodamine 6G than the sample containing vinyl groups, which in turn adsorbed more than the amine-functionalized sample (Table 2.2). Generally, the adsorption capacity values for rhodamine 6G appeared to be the highest in the sample containing mercaptopropyl groups than vinyl groups and in samples synthesized by co-condensation than by post-grafting (Table 2.2). The adsorption capacity of the amine functionalized samples for rhodamine 6G was significantly lower. Careful analysis of the adsorption capacities by normalizing them with respect to the density of functional groups in the materials as well as their surface areas were conducted (Table 2.2). It indicated that the samples containing mercaptopropyl groups with grafting and co-condensation synthesis gave values of $1.5 \times 10^{-4}$ and $3.3 \times 10^{-4}$ mmol/m$^2$, respectively, while those containing vinyl groups with grafting and co-condensation gave $4.9 \times 10^{-5}$ mmol/m$^2$ and $6.8 \times 10^{-5}$, respectively. The samples synthesized from APTS and BTSPA by grafting gave values of $1.1 \times 10^{-7}$ and $2.3 \times 10^{-7}$ mmol/m$^2$, respectively. This indicates that the mercaptopropyl groups results in an order of magnitude higher adsorption capacity than those containing vinyl groups, which in turn showed two orders of magnitude higher than the amine functionalized samples. The rate of adsorption seems to be very similar in
all the samples in the initial period (for 0-11 h). The samples functionalized with VTS by post-grafting as well as by co-condensation were almost saturated within 11 h. However, the samples functionalized with MPTS continued to adsorb rhodamine 6G for more than 11 h.

The adsorption profiles plotted by measuring adsorption versus concentration are shown in Figure 2.8 (A). All the lines were fit to \( Y = A \left(1 - e^{-bX}\right) \), where \( A \) and \( b \) are constants (Table 2.3). The term \( A \) represents the maximum drug adsorption by the materials while the value of \( (bA) \) represents for the initial adsorption rate of the drug by the nanoparticles. Analysis of these terms \( A \) and \( b \) indicates that MPTS-functionalized samples have higher adsorption capacity than their corresponding VTS-functionalized samples in both cases, i.e. samples synthesized by either grafting or co-condensation. Furthermore, the data shows that the initial adsorption rates of the samples decrease in the order of Co-MPTS > Grafting-MPTS > Co-VTS = Grafting-VTS.

### Table 2.3 Values of terms “A” and “b” in the graphs for adsorption versus concentration of rhodamine 6G that are fit into a function: \( Y = A \left(1 - e^{-bX}\right) \), where \( A \) and \( b \) are constants for each specific adsorption.

<table>
<thead>
<tr>
<th>Particles</th>
<th>A, ( % )^a</th>
<th>b, ( \mu\text{mol}^{-1}\text{L}^{-1} )^b</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co-MPTS</td>
<td>43</td>
<td>5 \times 10^{-4}</td>
<td>0.98</td>
</tr>
<tr>
<td>Co-VTS</td>
<td>40</td>
<td>3 \times 10^{-4}</td>
<td>0.99</td>
</tr>
<tr>
<td>Grafting-MPTS</td>
<td>55</td>
<td>2 \times 10^{-4}</td>
<td>0.98</td>
</tr>
<tr>
<td>Grafting-VTS</td>
<td>30</td>
<td>4 \times 10^{-4}</td>
<td>0.99</td>
</tr>
</tbody>
</table>

^a The term \( A \) represents the maximum drug adsorption by the materials. ^b The term \( (bA) \) indicates the initial adsorption rate of the drug by the nanoparticles.
Figure 2.8 Adsorption properties of rhodamine 6G in various functionalized mesoporous materials. (A) Adsorption versus concentration of R6G for samples synthesized by post-grafting and co-condensation. (B) Percent of R6G adsorbed versus time profile of different samples synthesized by post-grafting and co-condensation using aqueous 50 µM rhodamine 6G solution. (C) Digital images showing colors of the functionalized mesoporous samples synthesized by post-grafting after adsorption of R6G.

The adsorption results shown in Figure 2.8 were further complemented by comparing the weight losses between ~150-700 °C on thermogravimetric analysis (TGA) of the samples before and adsorption of R6G. The weight loss between ~150-700 °C is correlated or can correspond with decomposition of R6G or drug molecules. Figure 2.9 shows the TGA traces of the samples after incubation in 50 µM R6G solutions (or the samples shown in Figure 2.8 B). The TGA traces of sample Co-MPTS exhibited a 15.2% weight loss before adsorption of R6G adsorption and a 23.4% weight loss after adsorption of R6G (Figure 2.9 I). This corresponds to adsorbed R6G weight of 8.2 % and consistent with the adsorption capacity obtained in the adsorption
profile in Figure 2.8 B. The TGA traces of sample Co-VTS exhibited a 5.1% weight loss before rhodamine adsorption and a 10.0% weight loss after R6G adsorption (Figure 2.9 II). This corresponds to R6G weight of 4.9%, which is also consistent with the adsorption capacity of the sample obtained in Figure 2.8 B. The increase in the values of weight losses after adsorption of R6G clearly suggests that R6G was adsorbed in the samples and the difference between the two values corresponds to the amount of R6G adsorbed. These data indicate again that Co-MPTS adsorbed more R6G than Co-VTS and is consistent with the data obtained from the UV-Vis spectra analysis.

(I)
Figure 2.9 Thermogravimetric (TGA) traces of before surfactant extraction (A), after surfactant extraction (B), and after R6G adsorption (C) for (I) samples synthesized by co-condensation of MPTS and (II) samples synthesized by co-condensation of VTS.

The difference in the degree of adsorption of R6G by the samples can also be clearly seen by simply looking at the color of the mesoporous materials after treatment with R6G solution (Figure 2.8 C). After mixing the same mass mesoporous materials with the same volume and concentration of R6G solution for several hours and then separating the solid from the supernatant, samples with colors ranging from white to dark pink were obtained. While the samples functionalized with APTS and BTSPA still appear white, those functionalized with MPTS and VTS are deep pink in color. This indicates that the samples functionalized with APTS and BTSPA adsorbed little rhodamine 6G while the samples functionalized with MPTS and VTS by both methods adsorbed greater amount of R6G.

Comparison of the density of functional groups in the materials with respect to their adsorption properties to R6G reveals an interesting trend. Although the density of grafted groups in the APTS functionalized sample is higher than that in the MPTS functionalized
sample, the former has a lower degree of adsorption of R6G. The adsorption capacity of the samples was normalized with the density of organic functional groups in the materials and their surface areas. It reveals that the differences in adsorption properties of the samples to rhodamine 6G could be partially attributed to the differences in the type of the functional groups and possible degree of interaction between the functional groups and R6G molecules. Recently Suh et al. 54 have made extensive studies in this area, which indicated that rhodamine based dyes with positive charges such as rhodamine 6G and rhodamine 123 show faster uptake than those with negative charges such as rhodamine 101. Their results suggested that the molecular charge of the drug has an important effect for the uptake rate of the drugs in the materials. This molecular-charge effect is the result of negative charges that are developed in the pore surfaces by the ionization of silanol groups in aqueous solution and their interactions with the charges of the incoming dye molecules. Among the positively charged dyes, both the molecular weight and the magnitude of the dipole moment seem to contribute to the diffusion kinetics of the dyes. Their study and that of Sekine and Nakatani 59 have also reported uptake half-lives of 10-30 min at room temperature depending on the solution pH, the ionic strength, the type of material (powder versus thin film) as well as the channel length and pore structures. We observed that the uptake half-times are slightly longer than what was observed by others, with the MPTS-functionalized samples reaching to uptake half-lives more slowly than the VTS-functionalized samples. However, the former gave higher overall uptake of rhodamine 6G than the corresponding VTS-functionalized sample. Generally, the MPTS-and VTS-functionalized samples gave higher adsorption capacity than MCM-41 and amine-functionalized sample. The functional groups such as mercaptopropyl and vinyl, which are hydrophobic, favorably interact with the hydrophobic parts of
rhodamine 6G, producing higher adsorption capacity to R6G compared to MCM-41 and the materials functionalized with organoamines. It appears that the hydrophilic organoamines, -NH$_2$ and -NH groups, in the APTS and BTSPA functionalized materials, respectively, produce unfavorable interaction with the aromatic and alkyl chains of rhodamine 6G molecule and thus, producing lower adsorption capacity to R6G. In fact, the amine functional groups would likely to be protonated by abstracting a proton from the silanol groups. This then leads to samples that have a positively charged surface which is not conducive for adsorption of positively charged R6G molecules. The amount of rhodamine 6G on the external area was calculated to be 2.0-4.5% by subtracting the amount of rhodamine 6G removed in the washing steps from the total amount adsorbed initially. The total loading of rhodamine 6G in the mesoporous channels is calculated to be 30-55 mg for 100 mg material.

The trend in the degree of R6G release (Figure 2.10) by the functionalized samples was found to be the reverse of the one observed for adsorption. The studies of R6G release were conducted in a simulated body fluid (SBF) solution of pH of 7.40 at 37 ºC. SBF solution was chosen as it has similar composition as what is in our body and as it helps understanding how drug release properties of the materials would be in our body. The samples functionalized with VTS by post-grafting method released R6G molecules faster than the corresponding sample synthesized by co-condensation. On the other hand, the sample synthesized by post-grafting with MPTS released R6G molecules faster than the corresponding sample synthesized by co-condensation, and the samples functionalized with VTS released R6G faster than the corresponding samples functionalized with MPTS.
Figure 2.10 Comparative release profiles percent of R6G released for different samples synthesized by post-grafting and co-condensation of MPTS and VTS. The graphs were fitted as function \( Y = at^n \), where \( Y \) is % Release and \( t \) is time. This indicated that for samples grafted with MPTS, \( a = 0.0011, n = 2.6, r^2 = 0.991 \); grafted with VTS, \( a = 0.16, n = 1.7, r^2 = 0.991 \); sample Co-MPTS, \( a = 1.14 \times 10^{-5}, n = 3.2, r^2 = 0.990 \); and sample Co-VTS, \( a = 9.1 \times 10^{-4}, n = 2.9, r^2 = 0.987 \).

The diffusional release data for R6G was fitted to a non-Fickian or a Super Case-II transport model\(^{37, 38}\) and followed diffusional release process that follow Equation (1) below with \( n > 1 \):

\[
Y = at^n \tag{1}
\]

Where \( Y \) is % Release and \( t \) is time. Further analysis of the data and the graph in Fig. 6 gave values of \( a = 0.0011, n = 2.6, r^2 = 0.991 \) for sample grafted with MPTS; \( a = 0.16, n = 1.7, r^2 = 0.991 \) for sample grafted with VTS; \( a = 1.14 \times 10^{-5}, n = 3.2, r^2 = 0.990 \) for sample Co-MPTS synthesized by co-condensation; and \( a = 9.1 \times 10^{-4}, n = 2.9, r^2 = 0.987 \) for sample Co-VTS synthesized by co-condensation.

However, the adsorption experiments with ibuprofen (Figure 2.11 A) exhibited a totally different trend compared to the results obtained for adsorption of R6G for the same series of samples. The UV-Vis data showed that the samples functionalized with APTS and BTSPA by post-grafting had much more adsorption capacity for ibuprofen than for R6G. Furthermore, the sample grafted with APTS showed higher adsorption capacity of ibuprofen than the
corresponding sample grafted with BTSPA (Figure 2.11 A). On the other hand, the samples synthesized from MPTS and VTS, either by co-condensation or grafting, and the parent MCM-41 showed almost no adsorption of ibuprofen (Figures not shown). The UV-Vis absorption results were corroborated by TGA data (Table 2.4 and Figure 2.11 B). These results are interesting considering the fact that elemental analysis (Table 2.2) shows the BTSPA-grafted sample has almost same number of grafted groups as the MPTS-grafted sample, but fewer than the VTS-grafted sample. This further indicates that the type of functional groups (or the degree of their interaction with ibuprofen) and not their density in the materials is mainly responsible for the differences in adsorption capacity of materials. Since ibuprofen contains a carboxylic acid group, these carboxylic acid groups could interact favorably with the -NH$_2$ and -NH-groups in the amine-functionalized samples via hydrogen bonding, producing higher adsorption capacity of these samples for ibuprofen. On the other hand, the hydrophobic mercaptopropyl and vinyl groups in VTS and MPTS functionalized samples, respectively, have unfavorable interaction with ibuprofen, producing less adsorption capacity for ibuprofen. These results are consistent with those reported by others for similar functional groups.\textsuperscript{90}

\textbf{Table 2.4} Percent weight loss between 200-600°C for samples grafted with APTS and BTSPA, before and after adsorption of ibuprofen.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Before adsorption</th>
<th>After ibuprofen adsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grafting APTS</td>
<td>11.8%</td>
<td>18.0%</td>
</tr>
<tr>
<td>Grafting BTSPA</td>
<td>11.0%</td>
<td>16.2%</td>
</tr>
</tbody>
</table>
Adsorption properties of ibuprofen by various amine functionalized mesoporous materials. (A) Percent of ibuprofen adsorbed versus time profile of samples synthesized by postgrafting. (B) Thermogravimetric analysis of mesoporous materials before and after adsorption of ibuprofen.

The percent of adsorption of ibuprofen on the outer surface of the materials was obtained by soaking the samples in ibuprofen solution, then filtering and quickly washing the materials once with 20 mL ethanol. The value was found to be 3.6 and 9.8% for the APTS and BTSPA functionalized samples, respectively. The corresponding total load of ibuprofen in 50 mg samples were 30.2 and 33.2 mg, respectively.

The rate of drug release of ibuprofen by the samples functionalized with APTS and BTSPA were found to be essentially similar (Figure 2.12). The diffusional release data for ibuprofen
followed a Fickian diffusion process and it was fitted on the Higuchi equation\(^91\text{--}93\) \(Y = at^n\), Where \(Y\) is % release, \(t\) is time, and \(k\) stands for the rate at which ibuprofen gets released by the particles. After peak fitting the data, we obtained \(n = 1\) for \(t < t^*\), where \(t^*\) is a time constant when the release reaches equilibrium (\(A\), i.e., the maximum release), and has values of 2.9 and 3.3 h for APTS and BTSPA grafted samples, respectively (Equation 2) (Table 2.5):

\[
Y = k \times t 
\]

(2)

And \(n = 0\) for \(t > t^*\) (Equation 3):

\[
Y = 100
\]

(3)

As can be seen above, the diffusional release of the two molecules, R6G and ibuprofen, from the functionalized materials follows different mechanisms. This may have to do with the fact that the interaction of R6G with the organic functional groups is dominated by hydrophobic interaction while the interaction between ibuprofen and the organoamine and residual silanol groups of the functionalized mesoporous materials is dominated by hydrogen bonding. Suh et al \(^55\) reported that the control of the inclusion and release characteristics of drug molecules in mesoporous materials by manipulating the molecular interactions such as hydrogen bonding between the host and guest molecules is possible.

Table 2.5 Data of ibuprofen release shown in Figure 2.12 for APTS and BTSPA grafted samples after the graphs are fitted to \(Y = k \times t\) (if \(t < t^*\)) or \(Y = A\) (if \(t > t^*\)), where \(Y\) represents percentage of ibuprofen released, and \(t\) is time. \(t^*\) is a time constant when the release reaches equilibrium (\(A\), i.e., the maximum release), whereas \(k\) stands for the rate at which ibuprofen gets released by the materials.

<table>
<thead>
<tr>
<th>Samples</th>
<th>(k) (h(^{-1}))</th>
<th>(t^*) (h)</th>
<th>(A) (%)</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grafting-APTS</td>
<td>34.2</td>
<td>2.9</td>
<td>100.3</td>
<td>0.95</td>
</tr>
</tbody>
</table>
Grafting-BTSPA | 32.9 | 3.3 | 108.6 | 0.94

Figure 2.12 (A) Release of ibuprofen in SBF solution by different samples functionalized with APTS and BTSPA. The graph for sample Grafting APTS was fitted as $Y = kt^{1/2}$ ($t < 2.6$), $Y = 100$ ($t > 2.6$), $r^2 = 0.996$ and the graph for sample Grafting BTSPA was fitted as $Y = kt^{1/2}$ ($t < 2.6$), $Y = \sim 100$ ($t > 2.6$), $r^2 = 0.996$.

Although our FT-IR spectra (Figure 2.16) did not conclusively reveal any possible such interaction and while Suh et al. $^{55}$ have also not performed such an experiment, we believe that similar interactions as proposed by Suh et al. $^{55}$ may have also play roles in the differences in adsorption capacity and release properties among our materials. In another report by Vallet-Regí and co-workers $^{32,33}$, not only the functional groups but also the loading of drug or the drug: host ratio is reported to also play important role for drug release kinetics. Interestingly, the drug release profiles of our samples seem to show unusually slower drug release followed by faster release. This kind of release profile is peculiar and, to our best knowledge, has never been reported for mesoporous materials. However, we do not know what its underlying mechanism, which may warrant further investigations.
2.3 Supplementary of Characterization Results and Information

Table 2. 6 Percent weight loss between 100-550 °C and mmol/organic groups/g sample for the functionalized mesoporous samples synthesized by co-condensation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wt. % of Organic Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTS</td>
<td>6.5%</td>
</tr>
<tr>
<td>BTSPA</td>
<td>6.0%</td>
</tr>
<tr>
<td>MPTS</td>
<td>14.0%</td>
</tr>
<tr>
<td>VTS</td>
<td>4.5%</td>
</tr>
</tbody>
</table>

2.3.1 Solid-state NMR spectra (\(^{29}\)Si CP-MAS) of various functionalized mesoporous samples

(A)Grafting-APTS
(B) Grafting-BTSPA

(C) Grafting-MPTS
(D) Grafting-VTS

(E) Co-MPTS
(F) Co-VTS

Figure 2. 13 $^{29}$Si CP-MAS NMR spectra of various functionalized mesoporous samples.

2.3.2 Solid-state NMR spectra ($^{13}$C CP-MAS) of functionalized mesoporous sample

(A) Grafting-APTS
(B) Grafting-BTSPA

(C) Grafting-MPTS
(D) Grafting-VTS

(E) Co-MPTS
Figure 2.14 $^{13}\text{C}$ CP-MAS NMR spectra of functionalized mesoporous samples

2.3.3 Thermogravimetric (TGA) traces of various functionalized mesoporous materials
Figure 2.15 Thermogravimetric (TGA) traces of various functionalized mesoporous materials

2.3.4 FT-IR spectra of various functionalized mesoporous materials
2.4 Conclusion

The adsorption capacity and release properties of mesoporous materials for different drug molecules can be improved by functionalizing them with judiciously chosen organic groups. Rhodamine 6G and ibuprofen, which have different structures and surface properties, were used as model drug molecules to conduct comparative adsorption and release properties of organic functionalized mesoporous materials that were synthesized by post-grafting and co-condensation.
While mesoporous samples functionalized with mercaptopropyl and vinyl groups showed improved adsorption capacity for rhodamine 6G, samples functionalized with primary and secondary amine groups that were synthesized by grafting and co-condensation methods exhibited less adsorption capacity for rhodamine 6G. On the other hand, the trend in the degree of adsorption of ibuprofen was found to be the reverse of the results obtained for rhodamine 6G. The samples containing mercaptopropyl and vinyl groups showed less adsorption capacity for ibuprofen than the samples functionalized with primary and secondary amine groups. The release of the adsorbed molecules was also found to be dependent on the type of functional groups in the materials. The method of tuning adsorption capacity and release properties by organic functionalization of mesoporous materials could be extended to other organic functional groups and to a variety of other drug molecules. The resulting functionalized mesoporous materials may help to deliver drugs efficiently and, thus, minimize the drugs’ possible adverse effects. Introducing secondary bioactive groups onto the external surface of the materials may also allow targeted delivery of the drug cargo to specific cells.

2.5 **Experimental Section**

2.5.1 **Materials and reagents**

Tetraethoxysilane (TEOS), 3-aminopropyltriethoxysilane (APTS), rhodamine 6G (R6G), cetyltrimethylammonium bromide (CTAB), ibuprofen sodium salt, 3-mercaptopropyltrimethoxysilane (MPTS), vinyltrimethoxysilane (VTS), NaCl, NaHCO$_3$, KCl, K$_2$HPO$_4$·3H$_2$O, MgCl$_2$·6H$_2$O, CaCl$_2$, Na$_2$SO$_4$, NH$_2$C(CH$_2$OH)$_3$, and bis(triethoxysilylpropyl)amine (BTSPA) were obtained from Sigma-Aldrich. Hydrochloric acid (36.5%) and anhydrous toluene were purchased from Fisher Scientific.
2.5.2 Synthesis of Functionalized Mesoporous Materials via Co-condensation

A solution of 33.4 mL of distilled water and 15 mL of ammonium hydroxide was prepared and 2.30 mmol of CTAB was dissolved in it by stirring. Then, a mixture of 17 mmol TEOS and 3.0 mmol of one of the organosilanes (MPTS, VTS, APTS, or BTSPA) was added. The solution was stirred at room temperature for 2 h and then stored in oven at 80 °C for 2 days. The sample was cooled to room temperature and filtered using Whatman-1 filter paper. The solid was washed thoroughly with large amount of distilled water and dried under ambient condition resulting in organicfunctionalized mesostructured materials containing 3-mercaptopropyl, vinyl, 3-aminopropyl, or N, N-diproplyamine groups, respectively. The surfactant template was extracted by stirring 2 g of the functionalized mesostructured material with a solution of 50 mL methanol and 10 mL HCl for 5 h at 50 °C. The solution was filtered using Whatman-1 filter paper. The solid was washed three times with 20 mL of methanol and dried under vacuum for 30 min. This resulted in organic functionalized samples that were denoted as Co-MPTS, Co-VTS, Co-APTS, and Co-BPSPA, which contained 3-mercaptopropyl, vinyl, 3-aminopropyl, or N, N-diproplyamine groups, respectively.

![Chemical structure of orgasilica precursor and organosilanes](image)

**Figure 2. 17** Chemical structure of orgasilica precursor and organosilanes
2.5.3 Synthesis of Functionalized Mesoporous Materials via Post-Grafting

First, mesoporous silica was prepared and then functionalized with different organic groups. Typically, a solution of 960 mL distilled water, 10.9 mmol CTAB and 40 mL NaOH (2.0 M) was prepared at 80 °C. The solution was stirred at 80 °C for 30 min. After adding 22.6 mL of TEOS, the solution was stirred for 2 h at 80 °C. It was then filtered using Whatman-1 filter paper and the solid was washed thoroughly with copious amount of distilled water, and dried under ambient condition. The surfactant template was extracted by stirring 1 g of the functionalized mesostructured material with a solution containing 150 mL ethanol and 0.6 mL HCl for 5 h at 50 °C to produce mesoporous silica, MCM-41. It was dried at 80 °C for 4h before being used for grafting. The extracted MCM-41 was grafted with 3mercaptopropyl, vinyl, 3-aminopropyl, and secondary amine groups by stirring 600 mg MCM-41 with 4.4 mmol of MPTS, VTS, APTS, or BTSPA, respectively, in 100 mL toluene at 78 °C for 5 h. The samples were then washed with copious amount of ethanol and let to dry under ambient condition. The MCM-41 to organosilane ration of 600 mg/4.4 mmol was chosen in order to ensure that excess organosilane is present in the solution. Many of our and others’ previous studies have shown that this ratio is optimum for obtaining the maximum possible grafted groups in the materials.

2.5.4 Adsorption of Rhodamine 6G

Typically, a solution of 50 µM rhodamine 6G dye was prepared in distilled water at room temperature. Then, 100 mg of the the organic-functionalized mesoporous sample and 50 mL of a 50 µM rhodamine 6G solution were mixed and stirred. At 30 min intervals, the solution was centrifuged for 5 min and then the supernatant was analyzed with UV-Vis absorption
spectroscopy, by measuring adsorbance at 534 nm, which corresponds to the absorption maximum of rhodamine 6G. Additional absorption measurement was repeated, until the sample had stopped adsorbing more dye or until the absorption spectra of the supernatant had barely changed, indicating equilibrium was reached. Upon nearly complete adsorption of rhodamine 6G from the supernatant by the material, more rhodamine 6G solution was added into the sample and the above procedure was repeated.

In another experiment, 50 mg different functionalized samples were mixed with different concentrations of rhodamine 6G solutions and kept for 2 days, and after centrifugation, the absorbance of the supernatant was measured. This allowed us to plot adsorption versus concentration Figure 2.8 (A), which allowed us to determine the adsorption capacity of the samples.

2.5.5 Release of Rhodamine 6G

The experiments involving rhodamine 6G release by the samples were performed in a simulated body fluid (SBF) solution at 37 °C. The SBF solution was prepared by mixing NaCl (0.14 mol), NaHCO₃ (4.20 mmol), KCl (3.00 mmol), K₂HPO₄·3H₂O (1 mmol), MgCl₂·6H₂O (1.50 mmol), 1 N HCl (40 mL), CaCl₂ (2.50 mmol), Na₂SO₄ (0.50 mmol), and NH₂C(CH₂OH)₃ (49.90 mmol). A rhodamine 6G saturated sample was first prepared by mixing 100 mg of the functionalized mesoporous samples with 50 mL volume of 50 µM of rhodamine 6G solution until the mesoporous sample stopped adsorbing more rhodamine 6G, as monitored by UV-Vis absorption spectroscopy. The solution was filtered and the solid was washed with 20 mL of water. This washing procedure was used to remove excess rhodamine 6G molecules on the outer surface of the materials. The amount of rhodamine 6G on the external area was
calculated to be 2.0-4.5% by subtracting the amount of rhodamine 6G removed in the washing steps from the total amount adsorbed initially. The total loading of rhodamine 6G in the mesoporous channels is calculated to be 30 – 55 mg for 100 mg material (Table 2.2). Then, 100 mg of rhodamine 6G saturated samples was mixed 50 mL SBF solution of pH 7.4 at 37 °C and stirred for 2 h. The solution was centrifuged for 5 min and then the UV-Vis absorption of 5 mL of the supernatant was tested by UV-Vis spectroscopy, by monitoring the absorption maximum of rhodamine 6G at 534 nm. The SBF/rhodamine 6G supernatant solutions used for the measurement was discarded and the same volume of a fresh SBF solution was then added into the sample. After stirring and centrifugation of the solution, by following the same procedure above, the supernatant was analyzed again by UV-Vis spectroscopy. This experiment was repeated until the release of rhodamine 6G by the sample remained insignificant.

2.5.6 Adsorption of Ibuprofen

With the same functionalized mesoporous materials, adsorption and release experiments with ibuprofen were performed. First, ibuprofen was prepared by stirring 4.4 mmol of commercially available ibuprofen sodium salt in 40 mL, 0.2 M HCl solution overnight at room temperature. The ibuprofen was let to crystallize. The solution was then filtered over Whatman-1 filter paper and the solid ibuprofen was dried under vacuum for 30 min. A solution of ibuprofen with a concentration of 2.2 mM in ethanol was prepared. The UV-Vis adsorption experiments were carried out with this solution by following the procedure mentioned above for rhodamine 6G but by monitoring the absorption maximum of ibuprofen at 264 nm. To determine the % of adsorption of ibuprofen on the external surface of the materials, 50 mg of functionalized mesoporous sample was soaked in 50 mL of 24.2 mM ibuprofen solution for 24 h. It was then
filtered and quickly washed once with 20 mL ethanol to remove excess ibuprofen coating on the outer surface of the materials. The amount of ibuprofen on the external area was calculated by subtracting the amount of ibuprofen removed in the washing steps from the total amount adsorbed initially.

2.5.7 Ibuprofen Release Experiments

Typically, 50 mg of the functionalized mesoporous sample that was saturated with ibuprofen above was stirred in 10 mL of SBF solution at pH 7.4 at 37 °C. After centrifugation of the sample for 5 min, 5 mL of the supernatant was tested with UV-Vis every 30-60 min to ensure that the mesoporous sample was saturated with ibuprofen. The release experiments were generally carried out by following the same procedure as the one carried out for rhodamine 6G above, except monitoring the absorption maximum of ibuprofen at 264 nm.

2.5.8 Characterization

The UV-Vis absorption spectra were measured with a Lambda-950 spectrophotometer (PerkinElmer). The thermogravimetric (TGA) traces were collected by using a Q-500 Quantachrome Analyzer (TA-Instruments) with N₂ (99.999 %) as a carrier gas with a heating ramp of 5 °C/min. The low angle powder X-ray diffraction (XRD) patterns were obtained with a Scintag Diffractometer. The BET gas adsorption-desorption measurements were done with Micromeritics Tristar 3000 volumetric adsorption analyzer, after degassing the samples at 160 °C for 12 h. The solid-state $^{13}$C (75.5 MHz) and $^{29}$Si (59.6 MHz) NMR spectra were acquired on a Bruker AVANCE 300 NMR spectrometer. For $^{13}$C CP-MAS NMR experiments, 7.0 kHz spin rate, 5 s recycle delay, 1 ms contact time, $\pi/2$ pulse width of 5.6 $\mu$s, and 600 scans.
using TPPM $^1$H decoupling were employed. For the $^{29}$Si CP-MAS NMR experiments, 7.0 kHz spin rate, 10 s recycle delay, 10 ms contact time, $\pi/2$ pulse width of 5.6 μs, and 600 scans by using TPPM $^1$H decoupling were employed.

2.6 References


CHAPTER 3  RHODAMINE 6G AND IBUPROFEN ADSORPTION AND RELEASE STUDY OVER SBA-15

3.1 Introduction and Background

Among mesoporous materials, SBA-15 has large surface area, large pore size, and thick walls \(^1\)-\(^3\). It possesses a hexagonal array of mesopores 6.0-20 nm in diameter, which is much larger than the 3.0-nm pores characteristic of the MCM-41 and MCM-48. Their wall thickness is an important parameter for providing a high degree of hydrothermal stability and improved mechanical stability compared to mesoporous MCM-41 and related silicas \(^4\), and thus a sufficiently long lifetime in biological environments. \(^5\) Therefore, SBA-15 mesoporous materials could be modified with various surface functional groups and still preserve the large pore channels and surface areas for drug adsorption and desorption after modification. \(^6\)-\(^10\) We already investigated adsorption and release of R6G and ibuprofen over different MCM-41 of various functional groups, either hydrophobic or hydrophobic. Here we carried out the same study but over SBA-15 mesoporous materials, i.e. extracted SBA-15, SH-SBA-15 and COOH-SBA-15 and we studied the effect of functional groups. To learn how pore size affects the adsorption and release of drug and the difference of these properties between SBA-15 and MCM-41, carboxylic acid modified mesoporous materials were chosen as the carrier for this study. We consider that this acid group can have electrical interaction with the amine group of R6G and thus will have significant adsorption and release, making it better for the study.
In order to measure adsorption or uptake, nanomaterials were mixed into solutions of adsorbate of known concentration and kept mixed for 48 hours. As seen by desorption experiments results discussed below, 48 hours is long enough time to establish equilibrium between drug in solution and adsorbed drug. At various time intervals, samples were taken and centrifuged. The supernatant was separated and added into a cuvette, and analyzed by UV-Vis to obtain the adsorbate concentration (see Section 3.4.6 for details). The adsorption can be calculated based on Lambert–Beer law, which relates the absorption of light to the properties of the material through which the light is traveling. For liquid, it is described as $A = \varepsilon c L$, $A$ is absorbance, $\varepsilon$ is molar absorptivity and $L$ is the light path length. This implies that the absorbance becomes linear with the concentration of the solution under test. Thus, if the path length and the molar absorptivity are known and the absorbance is measured, the concentration of the solution can be deduced. Thermogravimetric analysis (TGA) was also used to confirm the adsorption. Samples before loading adsorbate and after loading adsorbate were analyzed by TGA and weight loss between 200-600 °C can be obtained. The weight loss difference indicates that drug molecules are really loaded onto the nanomaterials because this difference was the weight loss resulting from adsorbate loaded in the nanomaterials.
For release measurements, we mixed nanomaterials with solutions of known concentration for 48 h with stirring. The amount of drug adsorbed under these conditions was known from previous adsorption experiments. The loaded nanomaterials were separated from the solution by filtration and oven dried. Then they were mixed with SBF (Simulated Body Fluid). At various time intervals, samples were taken, centrifuged, and UV-Vis measurements were done on the supernatant to establish drug concentration in solution.

When nanoparticles possibly containing drug are in contact with a solution possibly containing drug, there is the possibility of drug transfer from particles to solution and also from solution to particles. In the simplest model, both these processes are first order with rate proportional to concentration. Let \( c(t) \) represent the solution drug concentration in moles per liter at time \( t \) and \( a(t) \) the drug concentration in the nanoparticles in moles of drug per gram of nanoparticles at time \( t \) so

\[
\frac{dc}{dt} = k_d a - k_a c
\]

where \( k_d \) is the desorption rate constant and \( k_a \) the adsorption rate constant. Units of \( k_d \) are \( \text{g nanomaterial}(\text{L solution})^{-1}t^{-1} \), units of \( k_a \) are \( t^{-1} \).

In an adsorption experiment, \( a(0) = 0 \) and \( am + cV = c_o V \) where \( m \) is the mass of nanomaterial in grams and \( V \) is the volume of solution in liters. Also, \( c_o = c(0) = \) initial drug concentration in solution. Equation (1) may be integrated to

\[
c = c_o \frac{k_a e^{-k_d(V/m)t} + k_d (V/m)}{k_a + k_d (V/m)} \tag{2}
\]

At \( t = 0 \), \( c = c_o \) and at \( t \to \infty \),

\[
c \to \frac{k_d c_o}{k_a \frac{m}{V} + k_d} \quad \text{and} \quad a \to \frac{k_a c_o}{k_a \frac{m}{V} + k_d}
\]
and \( a/c \rightarrow (k_a / k_d) \) as \( t \rightarrow \infty \). Thus at equilibrium the concentration of adsorbed drug \( a \) is proportional to the concentration of drug remaining in solution \( c \). The unitless equilibrium constant is defined as \( K_{eq} = (m/V)(a/c)_x \). To discuss release experiments, again start with equation (1) but with \( c(0) = 0 \) and \( a(0) = c_o m/V \). Then integration of equation (1) gives

\[
c(t) = c_o \frac{k_d(V/m)}{k_a + k_d(V/m)} \left(1 - e^{-(k_a + k_d(V/m))t} \right)
\]

so that \( c(\infty) = k_d c_o / [k_a(m/V) + k_d] \) again, and \( a/c \rightarrow (k_a / k_d) = K_{eq} (V/m) \) as \( t \rightarrow \infty \).

If in an adsorption experiment the concentration of drug left in solution after equilibrium is \( c_\infty \), the adsorbate has adsorbed \( (c_o - c_\infty)V \) moles so that \( a = a_\infty = (c_o - c_\infty)V/m \). Therefore

\[
\frac{a_\infty}{c_\infty} = \frac{c_o - c_\infty}{c_\infty} \frac{V}{m} = K_{eq} \frac{V}{m}
\]

so that equilibrium constant is easily calculated from concentrations before and after adsorption.

As shown below, \( K_{eq} \) can also be obtained from a release experiment, in which material previously loaded with drug is suspended in liquid and concentration of drug in liquid after long time is measured.

3.2 Results and Discussion

3.2.1 Characterization

3.2.1.1 Powder X-ray Diffraction

The X-ray diffraction (XRD) patterns of one of the functionalized mesoporous materials (ExSBA) are shown in Figure 3.2 I, II. The X-ray diffraction (XRD) patterns exhibited hexagonally ordered mesoporous structure that is characteristic of SBA-15. Furthermore, there is a slight decrease in their (100) Bragg reflection after R6G adsorption and no obvious decrease for ibuprofen adsorption. The samples remained mesostructured with the unit cell size changing a
little after adsorption and release of rhodamine 6G (R6G) and ibuprofen (Figure 3.2 I, II). The decrease in the intensity of Bragg reflection is probably a result of a slight decrease in electron contrast between the mesopores and the mesopore channel walls due to the confinement of the R6G and ibuprofen guest molecules inside the mesopores.\(^\text{11}\).

(I)

![Graph](image1)

(II)

![Graph](image2)

**Figure 3.2** (I) XRD of Ex-SBA (A) Before R6G adsorption, (B) After adsorbing R6G, (C) After R6G release study; (II) XRD of Ex-SBA (A) Before ibuprofen adsorption, (B) After adsorbing ibuprofen, (C) After ibuprofen release study.
3.2.1.2 Thermogravimetric analysis (TGA)

The mesoporous materials were further characterized by thermogravimetric analysis under nitrogen (Table 3.1, Table 3.2 and Figure 3.3). Thermogravimetric analysis of organic functionalized mesoporous materials under nitrogen or air were previously used by many groups for characterization of organic functionalized mesoporous materials. Our results further corroborated the results obtained from UV-Vis data. The TGA traces of the functionalized samples (Figure 3.3) showed a weight loss below 100°C due to physisorbed water while weight loss between ~200-600°C resulted from the loss of organic functional groups. In addition, there was a slight weight reduction after 600 °C because of the loss of water molecules from condensation of residual silanols. The order of adsorption and release capacity for the different functionalized silica particles are the same as calculated from UV-Vis data on the whole. The weight loss (%) of drug loaded samples before and after release is summarized in Table 3.1 and Table 3.2. By subtracting the weight loss of samples after release from that of the same functionalized samples before release, the amount of released drug can be calculated. It is summarized in Table 3.1 and Table 3.2. For R6G loaded samples, R6G load percent is 0.5% for Ex-SBA, 1.69% for SH-SBA and 4.12% for COOH-SBA and it decreases in the order of COOH-SBA > SH-SBA >Ex-SBA. This is the same as showed from UV-Vis data (see the following discussion). For ibuprofen loaded samples, load percent is 16.8% for Ex-SBA, 2.6% for SH-SBA and 9.0% for COOH-SBA. This shows that the ibuprofen adsorption amount decreases after -SH and -COOH group modification which is completely different from R6G adsorption case. It is reasonable because -SH is hydrophobic from our previous study and thus is not good for the hydrophilic ibuprofen attraction. For-COOH modified sample, it may be due to the repulsion
between the same charges and thus has low loading amount comparing with the parent Ex-SBA sample.

**Table 3.1** Weight losses (%) between 200-600 °C of samples loaded with R6G

<table>
<thead>
<tr>
<th></th>
<th>Ex-SBA</th>
<th>SH-SBA</th>
<th>COOH-SBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before release</td>
<td>6.12</td>
<td>31.11</td>
<td>16.87</td>
</tr>
<tr>
<td>After release</td>
<td>5.62</td>
<td>29.42</td>
<td>12.75</td>
</tr>
<tr>
<td>Amount loaded</td>
<td>0.50</td>
<td>1.69</td>
<td>4.12</td>
</tr>
</tbody>
</table>

**Table 3.2** Weight losses (%) between 200-600 °C of samples loaded with ibuprofen

<table>
<thead>
<tr>
<th></th>
<th>Ex-SBA</th>
<th>SH-SBA</th>
<th>COOH-SBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before release</td>
<td>22.8</td>
<td>31.1</td>
<td>19.5</td>
</tr>
<tr>
<td>After release</td>
<td>5.98</td>
<td>28.5</td>
<td>10.5</td>
</tr>
<tr>
<td>Amount loaded</td>
<td>16.8</td>
<td>2.6</td>
<td>9.0</td>
</tr>
</tbody>
</table>

(A)

(B)
Figure 3. Thermogravimetric (TGA) traces of various functionalized mesoporous materials before and after drug (R6G) release. (A) R6G-loaded Ex-SBA, (B) R6G-loaded SH-SBA, (C) R6G-loaded COOH-SBA, (D) Ibuprofen-loaded Ex-SBA, (E) Ibuprofen-loaded SH-SBA and (F) Ibuprofen-loaded COOH-SBA.

3.2.2 Adsorption study of Rhodamine 6G and ibuprofen over functionalized mesoporous materials

First, 1000µM solution of R6G and 10mg/ml solution (in hexane) of ibuprofen were prepared. Then 100 mg MCM-41 and SBA-15 materials were mixed with 10 ml of R6G or ibuprofen solutions prepared above, respectively. After 48 hours, the concentration of R6G remaining in the solution (original concentration 1000 µM) are 654 µM, 338 µM, 193 µM, and 674 µM for Ex-SBA, SH-SBA, COOH-SBA and COOH-MCM respectively. For functionalized SBA
samples, Ex-SBA adsorbs 34.6% of the R6G and SH-SBA adsorbs 66.2% of the R6G from solution at equilibrium (See the bar graphs below.) Thus, SH-SBA adsorbed more R6G than Ex-SBA which shows that thiol functionalized group help to improve the adsorption capacity of SBA for R6G. This result is consistent with Thiol functionalized MCM study. Carboxylic grafted SBA adsorbs 80.7% of R6G in solution, which is more than that of SH-SBA and indicates –COOH group is even better than –SH for the adsorption of R6G. The adsorption order for R6G is COOH-SBA > SH-SBA > Ex-SBA (Table 3.1 and Figure 3.4 A, B). This is consistent with the strength of interaction between R6G and grafted organic group. The functional mercaptopropyl group is hydrophobic and favorably interacts with the hydrophobic parts of rhodamine 6G, producing higher adsorption capacity for R6G compared to SBA without functional group. On the other hand, ibuprofen molecule contains a carboxylic acid group and carboxylic acid group could interact favorably with -NH- groups in the R6G molecule via hydrogen bonding and electronic attraction, producing adsorption capacity of R6G even higher than mercaptopropyl grafted SBA sample.

(A)
Figure 3.4 (A) Percentage of R6G in solution adsorbed by 100 mg Ex-SBA, SH-SBA and COOH-SBA materials, (B) µmol of R6G adsorbed by per gram Ex-SBA, SH-SBA and COOH-SBA of materials, (C) Percentage of R6G in solution adsorbed by 100 mg Ex-COOH-SBA and COOH-MCM materials and (D) µmol of R6G adsorbed by per gram COOH-SBA and COOH-MCM materials.
The table below shows the results and the calculated equilibrium constants $K_{eq}$ of the below equation for adsorption of R6G over the four materials.

$$R6G_{ad} \rightleftharpoons R6G_{f}$$

adsorbed free

Values of $K_{eq}$ are in the same order as per cent adsorption from solution. However, $K_{eq}$ varies much more in magnitude than per cent adsorption.

<table>
<thead>
<tr>
<th>Table 3.3</th>
<th>Results of R6G adsorption measurements (original R6G concentration = 1000 µM).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
<td>Ex-SBA</td>
</tr>
<tr>
<td>Concentration remaining, µM</td>
<td>654</td>
</tr>
<tr>
<td>$K_{eq}$</td>
<td>0.529</td>
</tr>
</tbody>
</table>

To investigate how pore size affects the adsorption of R6G into nanomaterials, SBA-15 and MCM-41 type materials were compared as carriers. Carboxylic functionalized materials were chosen since carboxylic acid grafted samples have highest adsorption capacity for R6G. Figure 3.4, C and D, shows COOH-SBA sample has more R6G adsorption than COOH-MCM sample, i.e. 80.7% of R6G in the solution was adsorbed by the former and 32.6% of R6G in the solution was absorbed by the latter. The values of $K_{eq}$ is 8.6 times greater for COOH-SBA than for COOH-MCM (Table 3.3). This is due to physical parameters of the parent SBA-15 and MCM-41.. SBA-15 has pore size up to 300Å while MCM-41 pore size is up to 100 Å, while MCM-41 has larger surface area, around 1000m²/g, and SBA-15 has only 500-600 m²/g. Considering they have the same -COOH functional group, the main reason for the larger adsorption by SBA-15 must be the bigger pore size, making it easier for R6G to enter SBA-15.

Besides R6G adsorption, ibuprofen adsorption was investigated over these functionalized mesoporous materials. The concentration of Ibuprofen remaining in the solution is 6.49 mg/ml,
8.01 mg/ml, 8.20 mg/ml for Ex-SBA, SH-SBA, COOH-SBA and 8.75 mg/ml for COOH-MCM (original concentration, 10 mg/ml) (Table 3.4 and Figure 3.5 A, B). For functionalized SBA samples, Ex-SBA adsorbs 35.1% of ibuprofen in solution while SH-SBA adsorbs 19.9% of ibuprofen from solution. So, SH-SBA loaded less ibuprofen, showing thiol functionalized group reduced adsorption capacity of ibuprofen. This result is consistent with our previous thiol functionalized MCM study. Carboxylic acid grafted SBA adsorbs 18% of ibuprofen in the solution, which is less than that of SH-SBA and Ex-SBA and indicates -COOH group also reduces the adsorption of ibuprofen. The adsorption amount of ibuprofen decreases in the order Ex-SBA > SH-SBA > COOH-SBA  The reason may be that ibuprofen can form hydrogen bonding with -OH in the channel of Ex-SBA, while SH-SBA has less -OH group for forming hydrogen bonding and -SH group is hydrophobic and has weak interaction with the ibuprofen which has a hydrophilic -COOH group. For COOH-SBA, the attraction is even weaker because carboxylic groups in SBA channel and carboxylic groups of ibuprofen molecules have the same charge and thus will repel each other.

(A)
Figure 3.5 (A) Percentage of ibuprofen in solution adsorbed by 100 mg Ex-SBA, SH-SBA and COOH-SBA materials, (B) mmol of ibuprofen adsorbed per gram of Ex-SBA, SH-SBA and COOH-SBA, (C) Percentage of ibuprofen in solution adsorbed by 100 mg Ex-COOH-SBA and COOH-MCM materials and (D) mmol of ibuprofen adsorbed per gram COOH-SBA and COOH-MCM materials.
To investigate how pore size affects ibuprofen adsorption, the same carboxylic acid grafted SBA-15 and MCM-41 were used as for the R6G adsorption study. Figure 3.5, C shows COOH-SBA sample has more ibuprofen adsorption than COOH-MCM sample. About 18.0% of ibuprofen in the solution was adsorbed by the former and around 12.5% was absorbed by the latter (see Figure 3.5, D). The size difference between these two mesoporous silica materials plays a crucial role for the adsorption capacity as for the R6G adsorption study above.

Table 3.4 Results of ibuprofen adsorption measurements (original ibuprofen concentration = 10 mg/ml)

<table>
<thead>
<tr>
<th>Material</th>
<th>Ex-SBA</th>
<th>SH-SBA</th>
<th>COOH-SBA</th>
<th>COOH-MCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration left, mg/ml</td>
<td>6.49</td>
<td>8.01</td>
<td>8.20</td>
<td>8.75</td>
</tr>
<tr>
<td>Concentration absorbed, mg/ml</td>
<td>3.51</td>
<td>1.99</td>
<td>1.80</td>
<td>1.25</td>
</tr>
</tbody>
</table>

Equilibrium constants $K_{eq}$ were calculated for ibuprofen adsorption by all the samples (Tables 3.4) and compared with $K_{eq}$ for R6G adsorption (Table 3.5), $K_{eq}$ for R6G adsorption is bigger than $K_{eq}$ for ibuprofen adsorption in every case, but the ratio is far from constant. It is 1.5 for EX-SBA, and more than 23 for COOH-SBA.

Table 3.5 Drug adsorption equilibrium constant ($K_{eq}$) of the nanomaterials

<table>
<thead>
<tr>
<th>$K_{eq}$</th>
<th>EX-SBA</th>
<th>SH-SBA</th>
<th>COOH-SBA</th>
<th>COOH-MCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>R6G</td>
<td>0.529</td>
<td>1.959</td>
<td>4.18</td>
<td>0.484</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>0.541</td>
<td>0.248</td>
<td>0.220</td>
<td>0.143</td>
</tr>
</tbody>
</table>

3.2.3 Release study of Rhodamine 6G and ibuprofen over functionalized mesoporous materials

For the release studies, 1000µM solution of R6G and 10mg/ml solution (in hexane) of ibuprofen were prepared first. Then, 100 mg MCM-41 or SBA-15 materials were mixed with
10ml of R6G or ibuprofen solutions prepared above, respectively, for 48h. The drug-saturated materials were removed, and mixed with 10 ml solvent at \( t = 0 \). Solution drug concentration was measured as a function of time \( t \). Shown in the desorption graphs below (Fig.3.6, Fig 3.7) is solution concentration as function of sampling time. Concentration has leveled off well before 48 hr in every case.

Plots of release amount of R6G, in \( \mu \text{mol per gram nanomaterial, vs. time} \) are given in Figure 3.6. Figure 3.6 also shows the best fits of release amount \( R \) to the function

\[
 R = A(1 - e^{-Qt}) \tag{5}
\]

(see Eq. 3) which guarantees \( R = 0 \) at \( t = 0 \) (initial time) and gives \( A \) as the maximum amount released. It is clear that, after 5 h, the release amounts of R6G from all three SBA samples have approached the maximum.

The parameters \( A \) and \( Q \) of the above equation for R6G release were obtained after fitting the release curves and are summarized in Table 3.6. According to equation (3),

\[
 A = c_o \frac{k_a V / m}{k_a + k_d V / m} = c_o \frac{1}{K_{eq} + 1}
\]

Since \( A \) is \( c_o \) this shows that \( K_{eq} = (c_o / c_x) - 1 \). Here \( c_o \) is the total amount of drug (in solution and adsorbed), expressed as a concentration. Therefore the equilibrium constant \( K_{eq} \) can be calculated from the following equation:

\[
 K_{eq} = (1-f)/f
\]

where \( f \) is the fraction of the drug released of the total drug loaded in the nanomaterials (\( f = c_o/c_x \)). Calculated equilibrium constants (\( K_{eq} \)) are summarized in Table 3.6, last line.
Table 3. 6 Overall results for R6G release test

<table>
<thead>
<tr>
<th>Material</th>
<th>Ex-SBA</th>
<th>SH-SBA</th>
<th>COOH-SBA</th>
<th>COOH-MCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (μmol /g material)</td>
<td>4.57</td>
<td>0.250</td>
<td>14.4</td>
<td>1.76</td>
</tr>
<tr>
<td>Q (see equation 5)</td>
<td>0.465</td>
<td>1.28</td>
<td>3.53</td>
<td>6.26</td>
</tr>
<tr>
<td>Total adsorbed (μmol/g)</td>
<td>34.6</td>
<td>66.2</td>
<td>80.7</td>
<td>32.6</td>
</tr>
<tr>
<td>f = fraction released</td>
<td>0.132</td>
<td>0.00377</td>
<td>0.179</td>
<td>0.0540</td>
</tr>
<tr>
<td>$K_{eq}$ = equil. constant</td>
<td>6.57</td>
<td>263.8</td>
<td>4.60</td>
<td>17.5</td>
</tr>
</tbody>
</table>

The maximum release amount of R6G from the SBA samples is 4.57 μM/g material for Ex-SBA, 0.250 μM/g material for SH-SBA, and 14.4 μM/g material for COOH-SBA (see Table 3.6). The maximum release amount decreases in the order COOH-SBA > Ex-SBA > SH-SBA. The per cents released, 13.2%, 0.377%, and 17.9%, are in the same order. The order is not the same for the equilibrium constants, $K_{eq}$.

COOH-SBA has highest release amount and release percentage. SH-SBA has less release amount and less release percentage than Ex-SBA. This can be explained from the interaction between the SBA and drug molecules. The reason is that for COOH-SBA, the interaction involves hydrogen bonding and electronic attraction. When drug is released, hydrogen bonding is broken and so R6G can easily escape without the attraction by COOH group.

Values of $K_{eq}$ (Table 3.6) are significantly larger than corresponding values of $K_{eq}$ from adsorption measurement, except for COOH-SBA (Table 3.5). This may be because some amount of adsorbed drug in the materials can not escape, so it is not in equilibrium with solution. Note that, before release study, drug-loaded nanoparticles were kept in oven for at least 48 hours.
to dry. During this process the bonding of drug to nanoparticles may be changed, or the structure of the nanomaterials may be changed, preventing escape of some of the drug from the materials.

After we fitted the release curve, the release rate of R6G from each sample was calculated. According to \[5\],

$$\frac{dR}{dt} = AQe^{-\sigma t}$$

the initial rate is \(AQ\). Using data in Table 3.6, release rates from Ex-SBA, SH-SBA, COOH-SBA, and COOH-MCM are 2.13 µmol/g/h, 0.320 µmol/g/h, 50.8 µmol/g/h, and 11.0 µmol/g/h respectively. Dividing through by the total amount adsorbed initially (see Table 3.6) we get apparent first-order rate constants 0.616 h\(^{-1}\), 0.00483 h\(^{-1}\), 0.629 h\(^{-1}\) and 0.337 h\(^{-1}\). The result for SH-SBA is very small because \(A\) is much smaller than the total amount adsorbed. Release rates are in the same order as the release amounts, i.e., COOH-SBA > Ex-SBA > SH-SBA. For SH-SBA and Ex-SBA samples, the bonding between host materials and guest drug molecules has no significant change, and thus R6G release rate from Ex-SBA is faster than that from SH-SBA because the binding in the former is weaker than that in the latter.

The role of pore size on the R6G release of drug loaded samples was also investigated. After 5 hours, the release amounts of R6G from carboxylic grafted samples approach the maximum, i.e., 14.4 mg/g for COOH-SBA sample and 1.76 mg/g COOH-MCM. Release amount from COOH-SBA is larger than that from COOH-MCM sample. The release percentage of R6G out of total amount loaded in the sample is 17.9% for COOH-SBA sample and 5.4% for COOH-MCM sample. It has the same order as the release amount, i.e., COOH-SBA > COOH-MCM. Release rate for COOH-MCM is 11.0 µmol/g/h, which is also less than that of COOH-SBA (50.8 µmol/g/h). It is consistent with the release amounts order. Considering two samples have the same functional group, R6G molecule should have nearly same strength of attraction to the host
materials. So, the main reason for this difference should be due to the pore size difference. SBA has larger pore size than MCM, so it is easier for R6G molecules to release from channels.

(A)

(B)
Figure 3. 6 (A) Release percentage of R6G loaded Ex-SBA, SH-SBA and COOH-SBA materials over different time, (B) Release amount of R6G loaded Ex-SBA, SH-SBA and COOH-SBA materials over different time, (C) Release percentage of R6G loaded Ex-COOH-SBA and COOH-MCM materials and (D) Release amount of R6G loaded Ex-COOH-SBA and COOH-MCM materials over different time.
Table 3. 7 Release (mg/g material) of ibuprofen as function of time.

<table>
<thead>
<tr>
<th>Material</th>
<th>t/h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Ex-SBA</td>
<td>0.143</td>
</tr>
<tr>
<td>SH-SBA</td>
<td>0.124</td>
</tr>
<tr>
<td>COOH-SBA</td>
<td>0.133</td>
</tr>
<tr>
<td>MCM-COOH</td>
<td>0.0664</td>
</tr>
</tbody>
</table>

Table 3. 8 Release per cent of ibuprofen as function of time.

<table>
<thead>
<tr>
<th>Material</th>
<th>t/h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Ex-SBA</td>
<td>0.0407</td>
</tr>
<tr>
<td>SH-SBA</td>
<td>0.0623</td>
</tr>
<tr>
<td>COOH-SBA</td>
<td>0.0739</td>
</tr>
<tr>
<td>MCM-COOH</td>
<td>0.0531</td>
</tr>
</tbody>
</table>

(A)
The release data of ibuprofen from SBA functionalized samples are summarized in Table 3.7 and Table 3.8 and plotted in Figure 3.7. After 2.5 hours, the release amount of ibuprofen from three SBA samples approaches the maximum. Figure 3.7 also shows the best fits to Equation 5, which give $A$, maximum released, as 0.146 mg/g for Ex-SBA, 0.133 mg/g for SH-SBA, and 0.140 mg/g for COOH-SBA sample. Table 3.9 gives other quantities calculated from the fits. The release amount is almost the same, i.e., Ex-SBA≈SH-SBA≈COOH-SBA. The
release percentage of ibuprofen out of total amount loaded in the SBA samples is 0.041% for Ex-SBA, 0.067% for SH-SBA, and 0.077% for COOH-SBA sample. It decreases in the order COOH-SBA > SH-SBA > Ex-SBA. From $A$ and the amount of ibuprofen adsorbed originally, we calculate $K_{eq}$, the equilibrium constant for adsorption.

**Table 3.9** A and Q values for ibuprofen release test

<table>
<thead>
<tr>
<th>Material</th>
<th>Ex-SBA</th>
<th>SH-SBA</th>
<th>COOH-SBA</th>
<th>COOH-MCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (mg/g material)</td>
<td>0.146</td>
<td>0.133</td>
<td>0.140</td>
<td>0.0820</td>
</tr>
<tr>
<td>Q (see equation 5)</td>
<td>4.00</td>
<td>2.67</td>
<td>3.00</td>
<td>1.53</td>
</tr>
<tr>
<td>Total adsorbed (mg/g)</td>
<td>351</td>
<td>199</td>
<td>180</td>
<td>125</td>
</tr>
<tr>
<td>$f =$ fraction released</td>
<td>0.000416</td>
<td>0.000668</td>
<td>0.000778</td>
<td>0.000656</td>
</tr>
<tr>
<td>$K_{eq}$ for adsorption</td>
<td>2403</td>
<td>1495</td>
<td>1285</td>
<td>1523</td>
</tr>
</tbody>
</table>

Table 3.9 shows that very large values are obtained, because the fraction released is always very small. These values are so large that the explanation, that most of the ibuprofen is unavailable for desorption because of changes occurring during the 48-hour drying and storage process between the adsorption and desorption is not correct. We consider the other explanation, saturation of binding sites. The theory is essentially the Langmuir theory and has been used for the drug adsorption study$^{19}$.

Suppose the adsorbent can be saturated, i.e. there are only a limited number of adsorption sites. The rate of adsorption is proportional to the number of empty sites. Let $c =$ concentration of adsorbate in solution. Let $n =$ concentration of adsorbate adsorbed by the material. We keep everything in concentration units; $n$ may be calculated as the moles adsorbed divided by the volume of solution. Let $m$ be the maximum concentration of adsorbate that can be adsorbed. The
rate of adsorption, in concentration per unit time, is $k_a c(m-n)$ and the rate of desorption is $k_d n$.

At equilibrium,

$$\frac{k_d}{k_a} = \frac{c(m-n)}{n}$$  \hspace{1cm} [7]

so, defining the equilibrium constant $K$ as $k_a/k_d$, we show

$$n = \frac{Kmc}{1 + Kc}$$  \hspace{1cm} [8]

For small $c$, $n$ is about $Kmc$, but, for large $c$, $n$ approaches $m$ (saturation).

There are two unknown constants, $K$ and $m$, requiring two measurements. Suppose that in an adsorption measurement on a drug we get equilibrium with solution concentration $c_a$ and adsorbed concentration $n_a$, and then in a desorption measurement we get equilibrium with solution concentration $c_d$ and adsorbed concentration $n_d$. Then equation 7 shows

$$m = n_a n_d - \frac{c_d - c_a}{c_d n_a - c_a n_d}$$  \hspace{1cm} [8]

Also,

$$K = \frac{(n_d / c_d) - (n_a / c_a)}{n_a - n_d}$$  \hspace{1cm} [9]

Table 3.10 and Table 3.11 shows the results of calculations for R6G and ibuprofen using equation [8].

<table>
<thead>
<tr>
<th>Material</th>
<th>$c_a$ (µM)</th>
<th>$n_a$ (µM)</th>
<th>$c_d$ (µM)</th>
<th>$n_d$ (µM)</th>
<th>$m$ (µM)</th>
<th>$K$ (µM$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex-SBA</td>
<td>654</td>
<td>356</td>
<td>45.7</td>
<td>300.3</td>
<td>350</td>
<td>0.132</td>
</tr>
<tr>
<td>SH-SBA</td>
<td>338</td>
<td>662</td>
<td>2.5</td>
<td>659.5</td>
<td>662</td>
<td>104.7</td>
</tr>
<tr>
<td>COOH-SBA</td>
<td>193</td>
<td>817</td>
<td>144</td>
<td>673</td>
<td>2201</td>
<td>0.003</td>
</tr>
<tr>
<td>COOH-MCM</td>
<td>674</td>
<td>326</td>
<td>17.6</td>
<td>308.4</td>
<td>326</td>
<td>0.968</td>
</tr>
</tbody>
</table>
Table 3.11 results of calculations for ibuprofen

<table>
<thead>
<tr>
<th>Material</th>
<th>$c_a$ (mg/mL)</th>
<th>$n_a$ (mg/mL)</th>
<th>$c_d$ (mg/mL)</th>
<th>$n_d$ (mg/mL)</th>
<th>$m$ (mg/mL)</th>
<th>$K \times 10^5$ (mL/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex-SBA</td>
<td>6.49</td>
<td>3.51</td>
<td>0.00146</td>
<td>3.51</td>
<td>3.51</td>
<td>16.5</td>
</tr>
<tr>
<td>SH-SBA</td>
<td>8.01</td>
<td>1.99</td>
<td>0.00133</td>
<td>1.99</td>
<td>1.99</td>
<td>11.2</td>
</tr>
<tr>
<td>COOH-SBA</td>
<td>8.2</td>
<td>1.8</td>
<td>0.0014</td>
<td>1.80</td>
<td>1.80</td>
<td>9.2</td>
</tr>
<tr>
<td>COOH-MCM</td>
<td>8.75</td>
<td>1.25</td>
<td>0.00082</td>
<td>1.25</td>
<td>1.25</td>
<td>18.6</td>
</tr>
</tbody>
</table>

From definition of the equilibrium constant $K$ as $k_a/k_d$, we can know the smaller $K$ stands for larger release capacity. For R6G release data in Table 3.10, the equilibrium constant $K$ for COOH-SBA is 0.003, 0.132 for Ex-SBA and 104.7 for SH-SBA. The release capacity is in the order of COOH-SBA > Ex-SBA > SH-SBA. For ibuprofen release data in Table 3.11, the equilibrium constant $K$ for COOH-SBA is $9.2 \times 10^5$, $16.5 \times 10^5$ for Ex-SBA and $11.2 \times 10^5$ for SH-SBA. The release capacity is in the order of COOH-SBA > SH-SBA > Ex-SBA.

The pore size effect on ibuprofen release was also investigated. After 2.5 h release, the release amount of ibuprofen from carboxylic grafted samples approach the maximum, i.e., 0.140 mg/g for COOH-SBA sample and 0.0820 mg/g COOH-MCM. Release amount decreases in the order COOH-SBA > COOH-MCM. The release percentage of ibuprofen of total amount loaded in the samples is 0.077% for COOH-SBA sample and 0.0656% for COOH-MCM sample which has same order as the release amount, i.e., COOH-SBA > COOH-MCM. Release rate for COOH-MCM is 0.0010 mg/g$^{-1}$/h$^{-1}$ and it is also less than that of COOH-SBA. Considering both have the same functional group, the main reason should be the pore size as we discussed above. SBA has big pore size and it is easy for ibuprofen to come out of the mesoporous pores.
After we fitted the release curve, the release rate of ibuprofen from each sample was calculated. It is 0.00231mg/g/h for COOH-SBA, 0.00165 mg/g/h for EX-SBA and 0.00178 mg/g/h for SH-SBA. Release rate is consistent with the release percentage order. This can be easily understood from strength of interaction between grafted functional group and ibuprofen molecule. The sample which has weaker binding with the ibuprofen molecule will have more release amount.

3.3 Conclusion

TGA and UV-Vis were conducted to characterize the loading and release of the drug. For R6G loaded samples, it was found that R6G load percent decreases in the order of COOH-SBA > SH-SBA > Ex-SBA. For ibuprofen loaded samples, it showed that the ibuprofen adsorption amount decrease after –SH and –COOH group modification which is completely different from R6G adsorption case. The adsorption amount of ibuprofen decreases in the order Ex-SBA > SH-SBA > COOH-SBA. After the first 5 h, the release amount of R6G from three SBA samples all approaches the maximum. Release amount decreases in the order COOH-SBA > Ex-SBA > SH-SBA. The R6G release percentage out of total amount loaded in the SBA samples and release rate has the same order as the release amount order, i.e., COOH-SBA > Ex-SBA > SH-SBA, which is different from order of adsorption amount indicated above. It indicates that sample with larger adsorption amount doesn’t necessarily have larger release amount or larger release percentage than sample with less adsorption amount.

Release amount from COOH-SBA is larger than that from COOH-MCM sample. The release percentage of R6G out of total amount loaded in the sample and release rate has the same order as the release amount, i.e., COOH-SBA > COOH-MCM. Considering two samples have the
same functional group, R6G molecule should have nearly same strength of attraction to the host materials. So, the main reason for this difference should be due to the pore size difference.

For ibuprofen loaded samples, the release amount of ibuprofen from three SBA samples reach the maximum after release in 2.5 hours. Release amount is almost same, i.e., Ex-SBA≈SH-SBA=COOH-SBA. The release percentage of ibuprofen out of total amount loaded in the SBA samples and release rate decreases in the order COOH-SBA > SH-SBA > Ex-SBA. However, the adsorption amount decrease in the order Ex-SBA > SH-SBA > COOH-SBA which is exactly opposite to the release order. This can be easily understood from strength of interaction between grafted functional group and ibuprofen molecule. The sample which has weaker binding with the ibuprofen molecule will have more release amount. The pore size effect on ibuprofen release was also conducted. After 2.5 h release, the release amount of ibuprofen from carboxylic grafted samples approaches the maximum and it decreases in the order of COOH-SBA >COOH-MCM. The release percentage of ibuprofen out of total amount loaded in the samples and release rate has same order as the release amount, i.e., COOH-SBA >COOH-MCM. Considering with the same functional group, the main reason should be the pore size as we discussed above. SBA has big pore size and it is easy for ibuprofen to come out of the mesoporous pores. This is consistent with the ibuprofen adsorption result for the two samples.

3.4 Experiment Section

3.4.1 Materials and reagents

Tetraethylorthosilicate (TEOS), cetyltrimethylammonium bromide (CTAB), and poly (ethylene oxide)-block-poly (butylene oxide)-block-poly (ethylene oxide) (P123, EO_{20}PO_{70}EO_{20}) were purchased from Sigma-Aldrich, 3-(trimethoxysilyl)-propionitrile (95%, Aldrich), 3-mercaptopropyltrimethoxysilane (MPTS), sulfuric acid (H_{2}SO_{4}, 98%, SCR)
3.4.2 Synthesis of SBA materials

SBA-15 was synthesized as reported by using P123 in acidic solution as a template.\textsuperscript{21,22} Usually, a solution of EO\textsubscript{20}PO\textsubscript{70}EO\textsubscript{20} : 2 M HCl : tetraethoxysilane (TEOS) : H\textsubscript{2}O = 2 : 60 : 4.25 : 15 (mass ratio) was stirred at 40 °C for 20 h, and then aged at 80 °C for another 24 h in the oven. The solution was then filtered, and the solid was washed with a large amount of water and dried to get “as-synthesized” SBA-15. The surfactant was extracted by stirring 1g of above sample in 300 mL ethanol and stirred at 50°C for 24 h. The resulting material was filtered and washed again with copious amount of water until it became neutral (checked with pH paper), and then washed with 500 mL ethanol and then 1000 mL distilled water and last 300 mL ethanol again. Dry the sample in the air and get extracted SBA-15.

3.4.3 Synthesis of carboxylic acid functionalized-MCM-41

The extracted MCM-41 was grafted with 3-(trimethoxysilyl)- propionitrile by stirring 400 mg MCM-41 with 2.92 mmol of 3-(trimethoxysilyl)- propionitrile in 500 mL toluene for 6 h at 80°C. The samples were then washed with copious amount of ethanol and let to dry under ambient condition resulting propionitrile functionalized MCM-41 sample, denoted as CN-MCM41. Then, the above sample was hydrolyzed to get carboxylic modified MCM-41. Briefly, 400 mg CN-MCM41 was mixed with 50 mL 48% H\textsubscript{2}SO\textsubscript{4} solution and stirred at 95°C for 24 h to get carboxylic functionalized-MCM-41, referred as COOH-MCM-41. Then, the resulting material was filtered and washed again with copious amount of water until it became neutral (checked with pH paper), and then washed with 500ml ethanol and dried in the air.

3.4.4 Synthesis of carboxylic acid functionalized SBA-15
The extracted SBA-15 was grafted with 3-(trimethoxysilyl)-propionitrile by stirring 400 mg SBA-15 with 2.92 mmol of 3-(trimethoxysilyl)-propionitrile in 500 mL toluene for 6 h at 80°C. The samples were then washed with copious amount of ethanol and let to dry under ambient condition resulting propionitrile functionalized SBA-15 sample, denoted as CN-SBA-15. Then, the above sample was hydrolyzed to get carboxylic modified SBA-15. Briefly, 400 mg CN-SBA-15 was mixed with 50 mL 48% H₂SO₄ solution and stirred at 95°C for 24 h to get carboxylic functionalized- SBA-15, referred as COOH-SBA-15. Then, the resulting material was filtered and washed again with copious amount of water until it became neutral (checked with pH paper), and then washed with 500 ml ethanol and dried in the air.

3.4.5 Synthesis of 3-mercaptopropyl-functionalized SBA-15

The extracted SBA-15 was grafted with 3-mercaptopropyltrimethoxysilane (MPTS) by stirring 400 mg SBA-15 with 2.92 mmol of 3-mercaptopropyltrimethoxysilane (MPTS) in 500 mL toluene for 6 h at 80°C. The samples were then filtered and washed with copious amount of ethanol, and let to dry under ambient condition resulting 3-mercaptopropyl functionalized SBA-15 sample, denoted as SH-SBA-15.

3.4.6 Adsorption of R6G and ibuprofen

1000µM solution of R6G was prepared by dissolving R6G powder in distilled water and 10mg/ml solution of ibuprofen was prepared by dissolving ibuprofen powder in hexane. The R6G solution was covered with aluminum foil to avoiding light and ibuprofen solution was sealed in container in case of evaporation. For R6G adsorption, we took 100 mg nanomaterials and mixed them with 10ml 1000µM solution of R6G at room temperature. The solution was
stirred for 5 minutes and then kept at static condition for 48h to establish equilibrium. For ibuprofen adsorption, we took 100 mg nanomaterials and mixed them with 10ml 10mg/ml solution of ibuprofen at room temperature. The solution was sealed in the container and stirred for 5 minutes and then keep at static condition for 48h. Then the solutions were centrifuged in small tube for 30min and supernatant were taken to be monitored by UV-Vis. When tested under UV-Vis, the R6G supernatant was diluted 100 times and tested at 534 nm, and the supernatant of ibuprofen was diluted 20 times in a quartz cuvette and test at 264nm. From Lambert–Beer law, we calculated the adsorption amounts of the drugs.

### 3.4.7 Release of R6G and ibuprofen

For R6G release study, we took 100 mg functionalized samples and mixed with 10 ml R6G water solution of 1mM at static condition for 48h. For ibuprofen release study, we took 100 mg functionalized samples and mixed with 10ml ibuprofen hexane solution of 10mg/ml at static condition for 48h. After all functionalized samples were loaded with ibuprofen or R6G, samples were filtered and dried in oven overnight for release study. For the release study, we mixed 100 mg functionalized samples loaded with ibuprofen or R6G with 40 ml SBF solution and stir constantly at 37 °C. At various time intervals, we took 1.5ml solution into 1.9ml centrifuge tube to centrifuge for 30min before test UV-Vis. At each test, refill 1.5 ml fresh SBF to the release system and also put the samples left in each test solution to the release system, which make sure the samples for release stay same.

### 3.5 References


CHAPTER 4 ACCELERATED OXIDATION OF EPINEPHRINE BY SILICA NANOPARTICLES

4.1 Introduction

Catecholamines are biogenic amines responsible for the acute stress response perceived by mammals upon unexpected stimuli coming from either external or internal surroundings.\textsuperscript{1, 2} In the view of chemistry, they are hormones and can be bio-synthesized from tyrosine and phenylalanine via hydroxylation to produce, among others, dopamine, norepinephrine and epinephrine.\textsuperscript{3} Among them, epinephrine (Figure 4.1) is the most potent agonist to adrenergic receptors in a various cells.\textsuperscript{4-7} Epinephrine will transmit neuronal signals to $G$-protein coupled receptors on the cell membrane once bound and a cascade of intracellular enzymatic reactions occur. As a result, much cyclic adenosine monophosphates are produced to activate glycogen phosphorylases which speed up the glycogenolysis to liberate glucoses into the bloodstream.\textsuperscript{5-7} The resulting sudden energy burst will stimulates the body to make to fight or flight.

High level of epinephrine due to the frequent alternation of stress and depression can put our lives under the danger of tumorigenesis and cardiac dysfunction.\textsuperscript{8, 9} In addition, it is reported that this hormone could sabotage cancer therapy by inactivating the pro-apoptotic proteins.\textsuperscript{10} Thus, the control of epinephrine production becomes necessary to either keep a normal physical condition or reinforce cancer treatment.

![Epinephrine molecule](image)

**Figure 4.1** Structure of epinephrine molecule
As a neurotransmitter, the function of epinephrine can be aborted if it is oxidized. But the autoxidation of epinephrine is very slow under the physiological conditions (pH=7.4). The oxidation process produces \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) as intermediates and adrenochrome.\(^{11,12}\) Epinephrine oxidation involves a cyclization of the quinone moiety in the presence of superoxides.\(^{13}\) This step help to produce more reactive oxygen species and appears even more dominant at a higher pH condition.\(^{13}\) \( \cdot\text{O}_2^- \) reacts with \( \text{H}_2\text{O}_2 \) at neutral or basic condition to give \( \cdot\text{OH} (+ \text{O}_2 + \text{OH}^-) \), which may be the active species in epinephrine oxidation. In acidic solutions, \( \cdot\text{O}_2^- \) becomes \( \text{HO}_2^- \) which reacts with \( \text{H}_2\text{O}_2 \) to give \( \cdot\text{OH} + \text{O}_2 + \text{H}_2\text{O} \).\(^{14}\) Many enzymes, such as catechol oxidase and amine oxidase can catalyze the oxidation reaction of catecholamines and many other organic and inorganic materials.\(^{15,16}\)

In this dissertation, we investigated the accelerated oxidation of epinephrine in the presence of two MSN, SBA-15 and MCM-41. In order to emphasize the effect of mesoporous structure on the oxidative reaction, silica microspheres (SMS) were also tested in this study as a control.

It was reported that oxidative stress and free radical-mediated lipid peroxidation may have a crucial role in various diseased states.\(^{21-26}\) To prevent and treat such diseases which relates to oxygen species, lots of study on antioxidants have been carried out. Some biologically important reactive oxygen species include the superoxide radical, the hydroxyl radical, peroxyl radical, singlet oxygen, and hydrogen peroxide. Caffeine (1,3,7-trimethylxanthine) is a ingredient that can be found in a great amount of beverages such as tea, coffee, cola and food containing chocolate.\(^{27,28}\) And it was also reported as a scavenger of the hydroxyl radical at millimolar concentrations in the study of electron spin resonance (ESR) spin trapping.\(^{29}\) So, we also studied the epinephrine oxidation in the presence of caffeine. Antioxidant effects of caffeine and its
metabolites were also evaluated by the epinephrine oxidation rate. Same experiment was also investigated with its isomer, isocaffeine.

4.2 Results and Discussion

4.2.1 Characterizations of nanoparticles

4.2.1.1 Transmission Electron Microscopy (TEM)

The as-synthesized MCM-41 or SBA-15 was calcined to remove the template and result in ordered MCM-41 and SBA-15 nanoparticles. The transmission electron microscopy (TEM) images (Figure 4.2 A-B) indicate that SBA-15 particles are of irregular shape of different sizes and MCM-41 particles and silica spheres are of rather regular spheres.
Figure 4. 2 Transmission Electron Micrographs of (A) calcined SBA-15, (B) calcined MCM-41 and (C) silica nanospheres.

4.2.1.2 Nitrogen Gas Adsorption (BET)

The BET gas adsorption-desorption measurements were done with Micromeritics Tristar 3000 volumetric adsorption analyzer for these two kinds of MSN after degassing the samples at 160 °C for 12h, and both of the gas adsorption showed Type IV isotherms with steep capillary condensation steps (Figure 4.3). This is indicative of the presence of mesoporous structure in these materials with large surface areas. BET surface area of MCM-41 was 1143 m²/g. Porosity by the Barrett-Joyner-Halenda (BJH) method shows a 28.4 Å average pore diameter and cumulative pore volume is 0.91cm³/g. For SBA-15 particles, the surface area is 883m²/g, the average pore width is 63.8 Å and the cumulative pore volume is 1.10cm³/g. These results are summarized in Table 4.1. Thus, compared to SBA-15, MCM-41 has a bigger surface area but smaller pore volume and width. Spherical silica nanoparticles were synthesized by following the
procedure stated in the Experimental Section. By adjustment of ammonia concentrations, quite symmetrical silica spheres of around 300 nm in diameter was synthesized (Figure 4.2 C). The BET gas adsorption-desorption measurements showed a surface area of 11 m$^2$/g. Such a small surface indicates SMS nanoparticles are non-porous which correspond to their solid-cored structure.

![Graph A](image1)

![Graph B](image2)

**Figure 4. 3** (A) Nitrogen gas adsorption (blue) and desorption (pink) isotherm of calcined MCM-41 (diamonds), SBA-15 (triangles) and silica spheres (circles). (B) BJH pore size distribution of calcined MCM-41 (diamonds) and SBA-15 (squares) mesoporous silica nanomaterials.
Table 4. 1 Characteristics of silica nanoparticles. Surface area, pore volume and average pore width are measured for three different nanomaterials as indicated.

<table>
<thead>
<tr>
<th>Type of particles</th>
<th>Surface area, m²/g</th>
<th>Pore volume, cm³/g</th>
<th>BJH pore width, Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCM</td>
<td>1143</td>
<td>0.91</td>
<td>28.4</td>
</tr>
<tr>
<td>SBA</td>
<td>883</td>
<td>1.10</td>
<td>63.8</td>
</tr>
<tr>
<td>Silica sphere</td>
<td>11</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

4.2.1.3 UV-Vis Spectra of Epinephrine

Firstly we investigate the UV-Vis spectrophotometry of epinephrine solutions. Epinephrine of various concentrations from 20 to 240 µM in dH₂O were prepared and immediately scanned at wavelengths from 800 to 200 nm. Results are shown in Figure 4.4 A. There are two absorbance peaks in the spectrum with one peak at ~200 nm and the other at ~280 nm. Moreover, one absorbance shoulder was evident lying in the wavelengths between 210-230 nm. All the spectra depend on epinephrine concentration. For higher concentration of epinephrine, the peak intensity is strong while the intensity of peak for lower concentration of epinephrine is weaker. To compare and evaluate the change of absorbance over epinephrine, we calculate the sum of intensities for a number of wavelengths rather than absorbance at a single wavelength because different concentration of epinephrine result a little shift of the absorbance peak. In Figure 4.4 B, the sum of intensities for the band of 195-205 nm, 210-230 nm, and 270-290 nm are plotted as a function of epinephrine concentration.

For the band of 195-205 nm, the sum of intensities (diamonds) first increases with the addition of epinephrine, but then reaches a plateau. The later independency of the integrated intensities on the concentrations of epinephrine is associated with an instrumental limitation. So we fit the sum of intensities of the band 195-205 nm as a two-line function of [epinephrine] (i.e., [EP]): $S_{195-205}$ (the sum of intensities) = 0.250 [EP] (when [EP] < 129.6 µM) and $S_{195-205} = 32.4$
(when [EP] > 129.6 μM). But the sum intensities for band 210-230 nm and 270-290 nm (squares and triangles) increased with the [EP] over the whole concentration range. The fitting of the sum of intensities for each individual band vs. [EP] into a linear function passing through zero gives: for the 210-230 nm band, $S_{210-230} = 0.121 \ [EP] \ (r^2 = 0.979)$; and for the 270-290 nm band, $S_{270-290} = 0.044 \ [EP] \ (r^2 = 0.983)$, respectively. Therefore, except the instrumental limitation, the integrated intensities for each band demonstrated proportionality to [EP]. Thus, the ratio between any two of three variables ($S_{195-205}$, $S_{210-230}$, and $S_{270-290}$) in a spectrum remains unchanged, so that these three absorbances very likely result from the same epinephrine species, which prevails in the freshly made epinephrine solutions.

Extensive studies on transient free radical forms of catecholamines have enabled a good understanding of in situ oxidation of epinephrine to adrenochrome through a sequential one-electron loss, although it is possible that other intermediate steps also exist. Instead of reacting directly with O$_2$ dissolved in solutions, epinephrine is much more easy to react with the hydroxyl radical “·OH” which is resulted from the reaction between O$_2^-$ and H$_2$O$_2$ and then epinephrine converts to o-semiquinone radical. Under neutral or acidic conditions, the concentration of O$_2^-$ and ·OH produced from reaction between O$_2^-$ and H$_2$O$_2$ is very low, so oxidation rate of epinephrine is low correspondingly. On the contrary, O$_2^-$ can be formed quickly in basic condition, and then reduced to H$_2$O$_2$ rapidly which can further reacts with remaining O$_2^-$ to produce ·OH. Furthermore, in basic medium the oxidative product adrenochromes are not stable because they are liable to interact with OH-. These processes result a rapid oxidation of epinephrine at elevated pH. It has been reported that the rate of epinephrine oxidation at pH = 8 is almost four times higher than that at pH = 4.33.
Figure 4.4 (A) UV absorption spectrum for epinephrine for concentrations of 20-200 µM. (B) Band intensities for epinephrine as functions of epinephrine concentration with fits. ♦ = 195-205 nm, ■ = 210-290 nm, ▲ = 270-290 nm. Linear fits are given for the first two, whereas the third is fit to two intersecting lines.
(A)

(B)
Figure 4.5 Absorbance of adrenochrome (product of epinephrine oxidation) at 480 nm as a function of time. The different plots correspond to different pH of the original epinephrine solutions. The slopes of the plots give the rate of epinephrine oxidation. Figure 4.5 A: Empty circles = pH 1, Filled triangles = pH 2, Empty triangles = pH 3, Filled diamonds = pH 4, Filled circles = pH 5, Empty squares = pH 6. Figure 4.5 B: Empty squares = pH 6, Filled triangles = pH 7, Empty triangles = pH 8, Empty circles = pH 9. Figure 4.5 C: Empty circles = pH 9, Filled squares = pH 10, Empty triangles = pH 11, Filled diamonds = pH 12.

So we investigate the effect of pH on the epinephrine oxidation rates by monitoring the absorbance of the adrenochrome produced in the solution at 480 nm over time. When investigating the effect of pH on epinephrine oxidation reaction, all water solutions of different pH values were prepared with two times higher [H+] by adding NaOH or HCl, and then mixed with the same volume of 250 µM epinephrine immediately right before test. Thus various epinephrine solutions of 125µM in dH₂O with different pH (pH = 1-12) was prepared. The same mixture without addition of epinephrine was tested to confirm the desired starting pH values. The pH can be adjusted by adding HCl for acidic conditions and NaOH for basic conditions.
Absorbance readings were taken for every 5 s for 30 min and the results are shown in Figure 4.5 and the absorbance is expected to increase with the time because there are gonna more adrenochrome being produced with the oxidation of epinephrine going on. The negative value of in the graphs in Figure 4.5 is due to instrument limitation when the adsorbance is very low. At acidic conditions, the production of adrenochrome is minimal (Figures 4.5 A and 4.5 B). The gradual decrease in intensity, which appears similar in most of these conditions, may be due to the drift in the instrument. However, the decrease over the first few minutes likely represents an induction time, required for the formation of ·OH to oxidize the epinephrine. For a quantitative measure of the epinephrine oxidation, we obtain linear fits to only the data for $t \geq 200s$. This gives the slopes ($\times 10^{-7}$ s$^{-1}$): $-3.12 + 0.07$ ($r^2 = 0.85$), $-4.85 + 0.11$ ($r^2 = 0.87$), $0.37 + 0.08$ ($r^2 = 0.07$), $-0.88 + 0.07$ ($r^2 = 0.36$), $-0.13 + 0.09$ ($r^2 = 0.01$), and $3.65 + 0.13$ ($r^2 = 0.72$) for solutions at pH = 1, 2, 3, 4, 5 and 6, respectively.

At pH = 7-10 (Figures 4.5 B and 4.5 C), the absorbance at 480 nm first declines a little bit, but then increases linearly with time. (Note the difference in scale between Figures 4.5 A, 4.5 B, and 4.5 C). As before, the initial drop could be due to an induction time required to generate active oxygen radicals. These plots were fit to V-shaped functions (the overall $r^2$ values are all greater than 0.98); in all cases, the bottom of the V was at $\sim 220$ s, suggesting a certain fixed time is required for the fast oxidization of epinephrine to begin under these neutral or weakly alkaline conditions. The slopes after the minimum, when [adrenochrome] increases linearly with time, were ($\times 10^{-6}$ s$^{-1}$) are 1.20, 3.68, 3.51, and 2.53 for the solutions of pH = 7, 8, 9 and 10, representing an order-of-magnitude increase in rate over the slopes at acidic conditions.

In solution with pH = 11 (Figure 4.5 C), the production of adrenochrome drops off initially, and then quickly increases, showing the rapid oxidization of epinephrine. Later it
declines again, probably due to the instability of high concentrations of adrenochrome in this solution. Further evidence for the destruction of adrenochrome is the shape of the plot for pH 11. Excluding the first decreasing part before oxidation occurs, we fit the increasing part of the data to a cubic, i.e., \[\text{Intensity} = -9.6 \times 10^{-9} t^2 + 2.2 \times 10^{-5} t - 3.4 \times 10^{-3} \quad (75 < t < 1030 \text{ s}, r^2 = 0.99)\]. The rate of adrenochrome production is then equal to \(2.2 \times 10^{-5} - 1.9 \times 10^{-8} t\) s\(^{-1}\), much higher than those at smaller pH. At pH = 12, the oxidation of epinephrine starts immediately. After a sharp increase, the production of adrenochrome slows down abruptly, so that the concentration almost levels off.

By fitting these data to a two-line function, we get the slope for the rapid oxidation \(3.3 \times 10^{-5}\) and the slope for the slow one \(9.9 \times 10^{-7}\) (overall \(r^2\) value = 0.95). The apparent “slow oxidation” is really a net rate of production of adrenochrome, i.e., its rate of production minus that of destruction. These results show that the oxidation of epinephrine is highly pH-dependent, and the rate of adrenochrome production is clearly higher at higher pH.

### 4.2.1.4 Effect of SBA-15, MCM-41 and silica sphere on epinephrine Oxidation

In the following experiments we measure the rates of epinephrine oxidation in dH\(_2\)O with or without silica nanoparticles. 10 mg/mL SBA-15, MCM-41 or silica spheres were individually dispersed in dH\(_2\)O and sonicated until well suspended. Each suspension was then centrifuged, and the resulting supernatant was mixed with 125 \(\mu\)M freshly prepared epinephrine solution and instantly collected for the spectroscopic scan. Our previous studies on drug adsorption by silica nanoparticles showed that centrifugations were not sufficient to remove all nanoparticles from the supernatant.\(^{35}\) Therefore, these experiments measure epinephrine oxidation in the presence of these silica nanoparticle residues.

The results are shown in Figure 4.6. The absorbance at 480 nm was fit to a linear function of \(t\) in each case, the slope giving the rate of epinephrine oxidation. The different intercepts in
the beginning of oxidation reveal the “pseudo-adsorption” due to the scattering of 480-nm radiation from the nanoparticle residues; only the slopes are significant. In the absence of nanoparticles, epinephrine (solid curve) oxidizes to adrenochrome at a rate of $(10.00 + 0.07) \times 10^{-7} \text{ s}^{-1} (r^2 = 0.98)$. With particles present, the oxidation rate becomes $(9.76 + 0.06) \times 10^{-7} \text{ s}^{-1} (r^2 = 0.99)$ for MCM-41 addition (long-dashed curve), $(15.74 + 0.08) \times 10^{-7} \text{ s}^{-1} (r^2 = 0.99)$ for SBA-15 addition (hatched curve), and $(9.89 + 0.10) \times 10^{-7} \text{ s}^{-1} (r^2 = 0.96)$ for silica sphere addition (short-dashed curve). Obviously, the presence of MCM-41 or silica spheres does not accelerate the oxidation, whereas SBA-15 exhibits an exceptional ability to enhance the oxidation rate of epinephrine to adrenochrome (by 57.4%), although all the oxidations proceed very slowly in dH$_2$O. The same experiments done with no epinephrine show that the slopes of absorbance at 480 nm vs. $t$ (due to nanoparticles) are essentially zero for all nanoparticles (data not shown).

Figure 4.6 Absorbance at 480 nm due to adrenochrome as a function of time for epinephrine solutions without (solid line) and with silica nanoparticles. Long-dashed line = addition of MCM-41, hatched line = addition of SBA-15, short-dashed line = addition of silica spheres.
We next examine the oxidation of epinephrine in PBS, with or without silica nanoparticles. The experiments were performed the same as the above, except dH$_2$O was replaced with PBS solutions. Results are shown in Figure 4.7. With no nanoparticles added (hatched line), epinephrine oxidizes much more quickly in PBS than in dH$_2$O. The absorbance due to the produced adrenochrome increases at a rate of $5.04 \times 10^{-5}$ s$^{-1}$, over 50 times higher than in dH$_2$O. This enhancement of oxidation rate could be due to the weak acidity of dH$_2$O (pH = 6), at which we have illustrated previously that oxidation occurs slower. Moreover, the higher ionic strength in PBS solutions (~ 0.15 M, as that in physiological condition) may contribute to this increased oxidation velocity as well. Later, the addition of silica spheres (short-dashed line) leads to a rate of oxidation of epinephrine to adrenochrome of $5.19 \times 10^{-5}$ s$^{-1}$, so there is no sign of silica spheres accelerating the oxidation in PBS. However, with either MCM-41 (solid line) or SBA-15 (long-dashed line) nanoparticles present, the oxidation is clearly enhanced. These curves are better fit by polynomials than straight lines, since the apparent rates decrease with time. The later reductions in the rate of production of adrenochrome reflect the instability of this product in these solutions. By fitting each absorbance vs. $t$ in to a quadratic form, we have $\text{[Intensity]}_{\text{MCM}} = -1.42 \times 10^{-8} t^2 + 9.61 \times 10^{-5} t + 8.16 \times 10^{-3}$ ($r^2 > 0.99$) and $\text{[Intensity]}_{\text{SBA}} = -1.39 \times 10^{-8} t^2 + 8.60 \times 10^{-5} t + 1.68 \times 10^{-2}$ ($r^2 > 0.99$). Thus, in the presence of MCM-41 and SBA-15, the oxidative reaction of epinephrine to adrenochrome occurs at a rate of $(9.61 \times 10^{-5} - 2.84 \times 10^{-8} t)$ s$^{-1}$ and $(8.60 \times 10^{-5} - 2.78 \times 10^{-8} t)$ s$^{-1}$, respectively. The initial rates are $9.61 \times 10^{-5}$ s$^{-1}$ and $8.60 \times 10^{-5}$ s$^{-1}$, both higher than that for epinephrine oxidation in PBS with no nanoparticles. The same experiments were also done without epinephrine and the result shows that the slopes of absorbance at 480 nm vs. $t$ in PBS (due to nanoparticles) are essentially zero.
To further investigate the accelerated oxidation of epinephrine by MSN in PBS and/or dH₂O, we perform experiments in which dithionite is added into epinephrine solutions in different time sequences. Sodium dithionite (Na₂S₂O₄) is well known to consume O₂ molecules rapidly in aqueous solutions, producing bisulfate and bisulfite, where the reaction involves free radical intermediates, including ∙O₂⁻.³⁶,³⁷ In the present experiments, dithionite was first dissolved in dH₂O at a high concentration (1 M) and then diluted into dH₂O or PBS solutions (final concentration [S₂O₄²⁻] = 500 μM), either 5 min prior to, or simultaneously with, the addition of 125 μM epinephrine. Results are shown in Figure 4.8. Time zero corresponds to the addition of epinephrine. In dH₂O, dithionite added 5 min before (dotted curve) or at the same time as (long-dashed curve) the epinephrine addition prevents production of adrenochrome (which was low in any case). However, in PBS solutions, rapid oxidation of epinephrine occurs when epinephrine is added concurrently with dithionite (solid curve), whereas epinephrine is not oxidized if it is added 5 min after the dithionite addition. The addition of dithionite alone to either dH₂O or PBS gives no rise in the absorbance at 480 nm (data not shown).

Since 500 μM dithionite can scavenge dissolved O₂ in seconds and simultaneously generate radical intermediates like ∙O₂⁻,³⁶,³⁷ this result confirms that, rather than O₂ molecules, oxygen radicals are responsible for the rapid oxidation of epinephrine in a phosphate buffer environment. Concomitantly, it suggests that the accelerated epinephrine oxidations with MSN present in PBS are due to the accumulation of reactive oxygen species by the nanoparticles. Apparently, MCM-41 or SBA-15 silica nanoparticles, but not silica spheres, provide a hotbed for oxygen radicals. In dH₂O the epinephrine oxidation is not accelerated by dithionite, even when it is added simultaneously with epinephrine. A possible explanation for this is that dithionite generates, in addition to oxygen radicals that should be able to execute the epinephrine oxidation,
$\text{HSO}_3^-$ and $\text{HSO}_4^-$ ions. In an unbuffered solution, these ions create an acidic environment which undermines the reaction. In fact, the measured pH of a 500 µM dithionite solution in dH$_2$O is 3.7 + 0.1.

**Figure 4. 7** Absorbance of adrenochrome at 480 nm as a function of time, from epinephrine solutions in PBS, with and without silica nanoparticles.

**Figure 4. 8** Absorbance of adrenochrome at 480 nm as a function of time, from solutions of dithionite + epinephrine. Dotted curve = dH$_2$O with dithionite added 5 min before epinephrine, long-dashed curve = dH$_2$O with dithionite and epinephrine added simultaneously, short-dashed curve = PBS with dithionite added 5 minutes before epinephrine, solid curve = PBS with dithionite and epinephrine added simultaneously.
4.2.1.5 UV-Vis Spectra of Caffeine and Isocaffeine

The spectra of caffeine and isocaffeine solutions were first investigated using UV-Vis spectrophotometry. Different concentrations of both trimethylxanthines (0-120 μM) in PBS were prepared and immediately scanned at wavelengths from 350 to 190 nm (no noticeable peaks appeared after 350 nm in the visible range). Results are shown in Figure 4.9. In each spectrum of caffeine, two absorbance peaks can be observed, one at ~205 nm and the other at ~273 nm. In addition, one absorbance shoulder was evident, in the wavelength region of 225-240 nm. All the spectra are concentration-dependent, showing the stronger intensity resulting from the higher concentration of caffeine. However, changing concentration also produced a small shift in the wavelength of the absorbance peak, which could be resulted from the instrumental limitations. Therefore, we here adopted an integrated absorbance over a band, i.e., the sum of intensities under a number of wavelengths, in replacement of the absorbance at a single wavelength. Shown by the inserted graph in Figure 4.9 A, the summed intensities for 200-205 nm (solid squares), 225-235 nm (solid triangles), and 270-275 nm (empty squares) are plotted as functions of caffeine concentrations, respectively. For the 200-205 nm band, clearly, the sum of intensities increases with addition of caffeine, and we thereby fit the two variables into a linear function (solid line). That is, $S_{200-205}$ (the sum of intensities in 200-205 nm band) = $(0.131+0.004) \cdot [\text{caf.}] + (0.572+0.224) \cdot (r^2 = 0.996)$, where [caf.] stands for [caffeine]. Intercept turns out to be positive, although numerically this small intercept can be neglected when high [caf.] (e.g., 100 μM) is used. Therefore, under circumstances where high concentration of caffeine is employed, proportionality still applies between $S_{200-205}$ and [caf.]. For the 225-235 nm and 270-275 nm bands (empty squares and triangles, respectively), the integrated intensities both increased with
The fitting of the sum of intensities for each individual band vs. [caf.] into a linear function passing through zero gives: for the 225-235 nm band (dashed line), $S_{225-235} = (0.055+0.000) \text{[caf.]} + (-0.046+0.022) (r^2 > 0.999)$; and for the 270-275 nm band (dotted line), $S_{270-275} = (0.057+0.000) \text{[caf.]} + (-0.011+0.012) (r^2 > 0.999)$. Intercepts are both statistically zero. Obviously, both integrated intensities for each band demonstrated proportionality to [caf.]. In brief, at a relatively high concentration of caffeine, the ratio of any two of the three variables ($S_{200-205}$, $S_{225-235}$, and $S_{270-275}$) remains nearly unchanged; thereby, these three absorbance ranges could result from the same caffeine derivatives, prevailing in the freshly made caffeine solutions.

Compared to the spectra of caffeine, those of isocaffeine displayed many a difference. First, the absorbance shoulder in caffeine spectra vanished; instead, an absorbance peak formed around 239 nm. By adding together the intensities from 235 nm to 240 nm, we fit this sum ($S_{235-240}$) into a linear function versus [isocaffeine] (i.e., [isocaf.]) and obtained: $S_{235-240} = (0.055+0.002) [\text{isocaf.}] + (-0.034+0.097) (r^2 = 0.991)$ (dotted line as shown in the insert of Fig.4.9 B). Statistically, intercept was zero. Second, the long-wave band in previous caffeine spectra (~273 nm) made a hypsochromic shift in isocaffeine solutions, about 5 nm downwards to the blue. As a result, peaks under ~ 268 nm were observed in isocaffeine spectra, and the sum of intensities covering 265 nm to 270 nm was therefore fit into a function of [isocaf.] (dashed line). The linear regression returns as $S_{265-270} = (0.069+0.003) [\text{isocaf.}] + (-0.071+0.124) (r^2 = 0.991)$, where the intercept was basically zero. Third, the short-wave band including 200-205 nm stayed as it showed in caffeine aqueous solutions, but the corresponding intensities were clearly augmented. By fitting the sum of intensities versus [isocaf.] into a linear function (solid line), we got $S_{200-205} = (0.155+0.005) [\text{isocaf.}] + (0.753+0.231) (r^2 = 0.993)$. Here, intercept was positive, although a large value of [isocaf.] (> 100 µM) would leave this number negligible. We noticed that the
slope was higher in $S_{200-205}$ of isocaffeine than that of caffeine. That is, in PBS solutions, the isocaffeine owns a higher value of extinction coefficient than its isomer. All different characteristics existing between caffeine and isocaffeine underlined a structural variation, reflecting the effect of –CH$_3$ position change on physicochemical properties of xanthine compounds. Be noted - the repeated occurrence of positive intercepts in the 200-205 nm range pointed out one possibility that the PBS solution containing no drug might may have a non-zero absorbance in this region, which was later proven true by scanning the spectra of PBS solution (results are not shown). However, with [caf.] or [isocaf.] $\geq$ 100$\mu$M, The summed intensities from 200-205 nm band, coming from the PBS solution, is insignificant.
4.2.1.6 Accelerated Oxidation of Caffeine and Isocaffeine by MSN

In order to investigate the oxidation of caffeine and its isomer in aqueous solutions, using UV-Vis spectrometry, we first monitored the spectral density over time when dissolving 100μM isocaffeine or caffeine in PBS. As a result, there came no noticeable change in their absorbance spectra during 24 hours (results are not shown), suggesting either a very sluggish oxidation or an indistinguishable difference between isocaffeine, caffeine and their oxidized forms in absorbance spectra. Thereby, to visualize the oxidation of isocaffeine and caffeine in a much shortened timeframe or in a convenient manner, we examined the oxidation of epinephrine at 480 nm in the presence of silica nanoparticles, when isocaffeine or caffeine were added or kept clear. Because of the crucial role of hydroxyl radicals (OH·) produced inside the mesoporous channels in a phosphate-buffered environment in the accelerated oxidation of epinephrine by MSN, these hydroxyl radicals have been thought capable to induce the oxidation of caffeine. Therefore, once hydroxyl radicals are formed due to the presence of MSN in PBS, oxidation of caffeine may take...
place which will compete with the oxidation of epinephrine. Thus, the expecting accelerated oxidation of epinephrine by MSN would be retarded. 10 mg/mL SBA-15 was dissolved in PBS solution and the precipitates of nanoparticles were removed by centrifugation, keeping supernatants for further use. Centrifugation was believed not sufficient to remove all nanoparticles in the solution, leaving residues of nanomaterials in the supernatant. Epinephrine of 125 μM was then added into such PBS solutions, further added with or without various concentrations of (iso) caffeine. Solutions were immediately collected for spectroscopic measurements and were monitored at 480 nm for 30 min with absorbance reading taken every 5 s.

In the following experiments we measure the rates of epinephrine oxidation in PBS with or without silica nanoparticles. To study the caffeine and isocaffeine effect on oxidation rate, we also dissolve caffeine or isocaffeine of different concentration in epinephrine before mix with silica particles. 10 mg/mL SBA-15 or silica spheres were individually dispersed in 2×PBS and sonicated until well suspended. Each suspension was then centrifuged, and take 1ml resulting supernatant and mix with 1ml 250 μM freshly prepared epinephrine solution containing caffeine or isocaffeine of different concentration (0.5mM, 1.0mM, 2.0mM, 4mM) and instantly collected for the spectroscopic scan. So, in the test solution, the epinephrine concentration is 125μM with caffeine or isocaffeine of 0.25mM, 0.5mM, 1.0mM, 2.0mM and 1×PBS. Our previous studies on drug adsorption by silica nanoparticles showed that centrifugations were not sufficient to remove all nanoparticles from the supernatant. Therefore, these experiments measure epinephrine oxidation in the presence of these silica nanoparticle residues. The results are shown in the Figures below. The absorbance at 480 nm was fit to a linear function of t in each case, the slope giving the rate of epinephrine oxidation. In the absence of nanoparticles, epinephrine oxidizes to
adrenochrome at a rate of \((7.49 + 0.008) \times 10^{-5} \text{ s}^{-1} (r^2 = 0.99)\). With SBA-15 present, the oxidation rate increases to \((8.01 + 0.005) \times 10^{-5} \text{ s}^{-1} (r^2 = 0.99)\).

**Figure 4.10** From top to down: Epinephrine SBA caffeine(2mM), Epinephrine SBA caffeine(0.25mM), Epinephrine, Epinephrine SBA caffeine(0.5mM), Epinephrine SBA caffeine(0mM), Epinephrine SBA caffeine(1mM)

Figure 4.10 shows the result of a series of test with caffeine of various concentrations. The oxidation rate decreases with the increase of caffeine concentration. With the caffeine concentration increasing from 0.25mM to 2mM, the oxidation rate reduced as the following: \((8.15 + 0.008) \times 10^{-5} \text{ s}^{-1} (r^2 = 0.99)\), \((7.53 + 0.008) \times 10^{-5} \text{ s}^{-1} (r^2 = 0.99)\), \((7.08 + 0.008) \times 10^{-5} \text{ s}^{-1} (r^2 = 0.99)\), \((6.18 + 0.008) \times 10^{-5} \text{ s}^{-1} (r^2 = 0.99)\). In general, the oxidation rate decreases after addition of caffeine. Also, oxidation rate decreases more when the caffeine concentration is higher.

The result of same test with isocaffeine was plotted in Figure 4.11. In the absence of silica nanoparticle, oxidation rate is \((8.57+0.01) \times 10^{-5} \text{ s}^{-1} (r^2 = 0.99)\). After adding SBA particle, it increase to \((9.02+0.02) \times 10^{-5} \text{ s}^{-1} (r^2 = 0.99)\); After addition of isocaffeine, with the concentration changing from 0.25mM to 2mM, the oxidation rate decreased from to \((9.45+0.01) \times 10^{-5} \text{ s}^{-1} (r^2 = 0.99)\).
\((r^2=0.99), (9.15+0.009)\times10^{-5}\text{ s}^{-1} (r^2=0.99), (8.49+0.009)\times10^{-5} \text{ s}^{-1} (r^2=0.99), (6.73+0.008)\times10^{-5} \text{ s}^{-1} (r^2=0.99).\) It has same effect as caffeine does.

**Figure 4.11** From top to down: Epinephrine SBA isocaffeine(0.25mM), Epinephrine SBA isocaffeine(0.5mM), Epinephrine SBA isocaffeine(0mM), Epinephrine SBA isocaffeine(1mM), Epinephrine, Epinephrine SBA isocaffeine(2mM)

Replacing SBA-15 with silica sphere, a series of same experiment was also conducted on the oxidation of epinephrine. As showed in Figure 4.12, in the absence of nanoparticles, epinephrine oxidizes to adrenochrome at a rate of \((8.23 + 0.02)\times10^{-5}\text{ s}^{-1} (r^2=0.99).\) With particles present, the oxidation rate becomes \((8.68 + 0.01)\times10^{-5}\text{ s}^{-1} (r^2=0.99),\) there is a little increase though not a big change. With the addition of caffeine of different concentration, the oxidation rate decreases with the concentration increase. Corresponding concentration from 0.25mM to 2mM, the oxidation rate are \((9.45 + 0.02)\times10^{-5}\text{ s}^{-1} (r^2=0.99), (8.95 + 0.009)\times10^{-5} \text{ s}^{-1} (r^2=0.99), (8.69 + 0.008)\times10^{-5} \text{ s}^{-1} (r^2=0.99), (8.59 + 0.006)\times10^{-5} \text{ s}^{-1} (r^2=0.99),\) respectively.
After addition of isocaffeine, with the concentration of isocaffeine changing from 0.25mM to 2mM, the oxidation rate changes to $(9.02 \pm 0.02) \times 10^{-5} \text{ s}^{-1} \ (r^2 =0.99)$, $(8.88 \pm 0.02) \times 10^{-5} \text{ s}^{-1} \ (r^2 =0.99)$, $(8.21 \pm 0.02) \times 10^{-5} \text{ s}^{-1} \ (r^2 =0.99)$, $(7.38 \pm 0.02) \times 10^{-5} \text{ s}^{-1} \ (r^2 =0.99)$. The result is illustrated in Figure 4.13.
Figure 4. From top to down: Epinephrine MCM caffeine(0mM), Epinephrine MCM caffeine(2mM), Epinephrine MCM caffeine(0.25mM), Epinephrine MCM caffeine(0.5mM), Epinephrine MCM caffeine(1mM), Epinephrine

Lastly, MCM-41 effect was also carried out in the presence of caffeine. Figure 4.14 shows before adding MCM and caffeine, oxidation rate is $(7.57 + 0.02) \times 10^{-5} \text{ s}^{-1} (r^2=0.999)$, After addition of caffeine, with the concentration changing from 0.25mM to 2mM, the oxidation rate changes to $(9.36 + 0.014) \times 10^{-5} \text{ s}^{-1} (r^2 > 0.99)$, $(7.76 + 0.014) \times 10^{-5} \text{ s}^{-1} (r^2 > 0.99)$, $(7.47 + 0.003) \times 10^{-5} \text{ s}^{-1} (r^2 > 0.99)$, $(7.36 + 0.002) \times 10^{-5} \text{ s}^{-1} (r^2 > 0.99)$, $(7.44 + 0.006) \times 10^{-5} \text{ s}^{-1} (r^2 > 0.99)$.

4.3 Conclusions and Discussions

Influence of mesoporous silica (MCM-41 and SBA-15) nanoparticles and dense silica nanoparticles on epinephrine oxidation is pH-dependent. Oxidation rate is small in acidic or neutral solutions and will be increased significantly at higher pH. MCM-41 and silica spheres does not accelerate the oxidation rate in dH$_2$O while SBA-15 does, indicating that the difference in the structures of nanomaterials leads to different effects on the epinephrine oxidative process. Comparing to MCM-41 and silica sphere, SBA-15 has a unique microporosity and interconnectivity in the mesopore walls, which contributes to a substantial part of total surface area.
This feature could lead to more traps of oxygen radicals inside the mesoporous channel and thus significantly accelerate the oxidation of epinephrine even in a weak acidic condition. In PBS solutions, the presence of SBA-15 and MCM-41 makes the oxidation even more rapid. Silica spheres have no noticeable influence on the oxidation both in PBS and dH₂O. The possibility that the catalytic effect of MSN results from the residue of templating chemicals could be excluded due to the post-synthesis calcinations. Experiments with dithionite, added either earlier than or at the same time as the epinephrine addition, show that fast oxidation takes place only when dithionite and epinephrine are simultaneously added into PBS solution. This confirms a vital role of oxygen radicals (probably ·O₂⁻) in the oxidation of epinephrine. These oxygen radicals are likely to accumulate within the phosphate buffer or in the presence of mesoporous silica nanoparticles. Comparing the three kinds of silica nanoparticles applied, we note that mesoporous SBA-15 and MCM-41 materials own much larger surface area than solid silica particles do, whereas MCM-41 possesses a much narrower pore size (0.4-fold) than SBA-15. It seems, therefore, that large surface area plus characteristic mesoporosity and surface structures aid in the deposit of oxygen radicals inside MSN particles, which catalyze the epinephrine oxidation in a favorable phosphate environment.

In the presence of either caffeine or isocaffeine, the oxidation rate of epinephrine by MSN will be slowed down. Also, with the increase of caffeine or isocaffeine concentration, the oxidation rate decrease accordingly.

4.4 Experimental section

4.4.1 Materials and Methods
Tetraethylorthosilicate (TEOS), cetyltrimethylammonium bromide (CTAB), and poly (ethylene oxide)-block-poly (butylene oxide)-block-poly (ethylene oxide) (P123, EO$_{20}$PO$_{70}$EO$_{20}$) were purchased from Sigma-Aldrich. Sodium dithionite (m.w. 174.11) was purchased from Fluka, UK. Caffeine (m.w. 194.2), isocaffeine (1, 3, 9-Trimethylxanthine, m.w. 194.2) were ordered from Sigma-Aldrich, and dissolved in dH$_2$O or PBS immediately before use. Epinephrine was purchased from Sigma-Aldrich (St.Louis, MO). Working solutions (1.0 M) were freshly made in dH$_2$O under argon shield. Phosphate-buffered salt solution (1x PBS, without Mg$^{2+}$ and Ca$^{2+}$, pH=7.4) was purchased from Mediatech (Herndon, VA).

4.4.2 Synthesis of Silica Nanoparticles

SBA-15 was synthesized as reported by using P123 in acidic solution as a template.$^{38,39}$ Usually, a solution of EO$_{20}$PO$_{70}$EO$_{20}$ : 2 M HCl : tetraethoxysilane (TEOS) : H$_2$O = 2 : 60 : 4.25 : 15 (mass ratio) was stirred at 40 °C for 20 h, and then aged at 80 °C for another 24 h in the oven. The solution was then filtered, and the solid was washed with a large amount of water and dried to get “as-synthesized” SBA-15. The synthesis of MCM-41 was obtained by following a previously reported method with minor modification.$^{40,41}$ 4.0 g (1.1 mmol) CTAB was dissolved in 960 mL Millipore water and then added 14 mL 2.0 M NaOH solution. The mixed solution was moderately stirred at 80 °C for half hour and then 22.6 mL (101.2 mmol) TEOS was added. After stirring for another 2 h at 80 °C, the solution was filtered and the precipitate was washed four times with Millipore water, each time 80 mL, followed by rinsing with ethanol four times, each time 80 mL and dried in the oven at 80 °C.

Silica microspheres were synthesized by following the well-known Stöber method. 5.84 g of TEOS was added to 10 mL of 5 M ammonia solution (30 wt %) in a mixture of 50 ml ethanol and 3.6 g Millipore water under stirring to allow the hydrolysis of TEOS. After stirring for 12 h,
a colloidal solution of silica spheres was obtained. The solution was centrifuged at 6500 rpm for
five minutes and the supernatant was dumped. The precipitate was then re-dispersed in a mixture
of 20 mL Millipore water and 20 mL ethanol. The centrifugation and re-dispersing process was
repeated several times to remove any unreacted chemicals. The resulting silica microspheres
were dried before further modification.

4.4.2 Preparation of PBS solutions containing silica nanoparticles, caffeine, isocaffeine, or
epinephrine

Oxidation rates of epinephrine in various PBS solutions were measured in the experiments
and the absorbance of adrenochrome (one of oxidized products of epinephrine) at 480 nm versus
time was plotted, followed by fitting the data into linear functions and obtaining the slope. More
specifically, 10 mg/mL SBA-15 or silica spheres were individually dispersed in 2×PBS (freshly
diluted from 10× PBS stock) and sonicated until well suspended. Each suspension was then
centrifuged, and 1 ml supernatant was taken to mix with 1 ml 250 μM freshly prepared
epinephrine solution that contained caffeine or isocaffeine in different concentrations (e.g., 0.5,
1.0, 2.0 and 4 mM). The mixtures were instantly collected for the spectroscopic scans. That is,
finally the mixture contained 125 μM epinephrine, final concentrations of caffeine or isocaffeine
(e.g., 0.25, 0.5, 1.0, and 2.0mM) and 1×PBS, plus certain amount of nanomaterial residues. Our
previous studies on drug adsorption by silica nanoparticles showed that centrifugations were not
sufficient to remove all nanoparticles from the supernatant.

4.5 References


42. Stöber, W; Fink, A.; Bohn, E., Controlled Growth of Monodisperse Silica Spheres in the Micron Size Range, J. Colloid Interface Sci., 1968, 26, 62-69.


CHAPTER 5 pH CONTROLLABLE DRUG RELEASE STUDY USING PAA ENCAPSULATED MESOPOROUS SILICA MATRIX

5.1 introduction

In order to better control drug release process and drug administration, controlled delivery systems has become the research field of interest of many scientists recently. Nowadays, many organic delivery systems like polymeric nanoparticles, micelles and liposomes have been investigated for delivery system. However, one of the disadvantages of these organic delivery carriers is their poor stability due to biochemical attack. More and more interest of scientists has focused on mesoporous silica materials to use as carriers in controlled drug release for their stability, nontoxic nature, adjustable pore size, high internal and external surface area\(^1\) and more importantly, plenty of Si-OH bonds on the surface which can be further functionalized with various groups.

To achieve controlled release purpose, usually the mesoporous materials are functionalized with stimuli-responsive materials, typically polymers. Most common stimuli are pH \(^2\text{-}^9\), temperature \(^10\text{-}^12\), redox potential\(^13\), electric field \(^14, 15\), light \(^16\text{-}18\), magnetic field \(^19\), enzyme\(^21\), salt concentration\(^22\) and etc. Upon a little change in surroundings, stimuli-responsive materials can show dramatic and different responses: conformational or shape change, solubility, degradation, swelling or collapsing, change of hydrophilicity or hydrophobicity and etc. Through these stimuli response, the drug release loaded in the materials can be controlled as expected. However, to achieve controlled release in medicine, stimuli-responsive delivery systems have to show their response properties within the setting of biological conditions. Now that different organs, tissues and cellular compartments may have large differences in pH, for example, tumor and inflammatory tissues (pH 6.8), endosomes (pH~5.5-6), and lysosomes (pH ~ 4.5-5.0), stomach
(pH ~1.0-3.0) and intestine (pH ~5-8), so a release strategy that employs pH as a stimulus for drug release would be particularly useful. In this study, pH responsive release study was conducted. Ibuprofen and R6G were used as model drug molecules. For the ibuprofen loading, amine functionalized MCM (NH$_2$-MCM) was chosen as carrier because -NH$_2$ group is helpful for the adsorption of ibuprofen, while amine and thiol bi-functionalized MCM (NH$_2$-MCM-SH) was chosen as carrier for the adsorption R6G because –SH is suitable for R6G loading according our previous study. Poly(acrylic acid) PAA was encapsulated onto the silica matrix as pH stimuli because it is a well-known bioadhesive hydrogel and often used in drug formulation.

5.2 Result and discussion

5.2.1 Release study via UV-Vis test

The UV-Vis absorption spectra were measured with a Lambda-950 spectrophotometer (PerkinElmer). Calculated from the UV-Vis absorption spectra data, amount of R6G loaded into NH$_2$-MCM-SH sample is 0.0327 mmol/g, while the amount loaded to NH$_2$-MCM-SH after PAA encapsulation is 0.0321 mmol/g. There is no obvious change in the loading amount of R6G, which indicates that the R6G release during encapsulation step of the PAA can be neglected.

![Figure 5.1](image)

**Figure 5.1** Release amount (mmol/g) of R6G from various functionalized mesoporous samples. (a) NH$_2$-MCM-SH at pH 1.0 (b) NH$_2$-MCM-PAA at pH 1.0 (c) NH$_2$-MCM-SH at pH 7.4 (d) NH$_2$-MCM-PAA at pH 7.4
For all the samples, the release rate of R6G was fast in the initial first hour and then slowed down. Table 5.1 shows after 30.5 h hour, release amount of R6G reached the maximum of 0.00525 mmol/mg at pH 1.0 which is more than the release amount (0.00227 mmol/mg) at pH 7.4. The increase in rate and amount released can be attributed to decreased electrostatic interactions between the positively charged rhodamine 6G molecules and the surface of silica at the different pH conditions. It is known that the surface of mesoporous silica is negatively charged above isoelectric point (pH 2-3). At pH 1.0, the surface of silica is positively charged and it is negatively charged at pH 7.4. Also, amine groups on the silica surface were protonated to a greater extent at pH 1.0 than at pH 7.4, which also contribute to the positive charge on the silica surface at pH 1.0. So, at pH 1.0, surface of silica has more positive charge which will repulse cationic rhodamine 6G molecules and thus contribute the larger release amount.

For NH2-MCM-PAA sample, besides the above discussed electric interaction between drug molecules and silica surface or protonated amine groups, the dissociation of PAA become another factor needed to be considered for the R6G release. The reason is that PAA is a weak polyelectrolyte with repeated pendant carboxylic groups with a pK_a of 4.5-5 and its shape mainly depends on the pH of the surroundings. At pH 1.0 (pH <pK_a 4.5), the side carboxylic groups cannot be dissociated and remains contracted and block the openings of the materials and thus retard the escaping of R6G molecules while at pH 7.4 (pH >pK_a 5), the side carboxylic groups can be dissociated and negative charge on the PAA will repulse each other and dissolve in the release media, so, opening of the samples will make the R6G molecules release easier. The release result will depend on which factor dominates the process. If electric interaction dominate the release process, there will be more release amount at pH 1.0 than at pH 7.4 as for sample without PAA encapsulation while if PAA dominate the process, there will be less release at pH
1.0 because PAA remains contracted and block the openings of mesoporous matrix. Table 5.1 shows that after 30.5 h, the release amount is 0.00356 mmol/mg at pH 1.0, which is more than the release amount (0.00117 mmol/mg) at pH 7.4. It indicates that electric interaction still dominates the release process instead of PAA encapsulation.

**Table 5.1** R6G release (mmol/g) at pH 1.0 and pH 7.4 over the time

<table>
<thead>
<tr>
<th>time/h</th>
<th>Different conditions</th>
</tr>
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<tr>
<td></td>
<td>pH 1.0</td>
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<td>0.00525</td>
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<tr>
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</table>

**Figure 5.2** Release of ibuprofen from various functionalized mesoporous samples (A) NH$_2$-MCM at pH 7.4 (B) NH$_2$-MCM-PAA at pH 7.4 (C) NH$_2$-MCM at pH 1.0 and (D) NH$_2$-MCM-PAA at pH 1.0

For all the samples, the release rate of ibuprofen was fast in the initial first hour and then slowed down. For NH$_2$-MCM sample, Table 5.2 shows after 9 hour, release amount of ibuprofen reached the maximum of 94.4 mg/g at pH 1.0 which is less than the release amount (302.6 mg/g) at pH 7.4. For NH$_2$-MCM -PAA sample, ibuprofen release amount of ibuprofen at pH 1.0 is 85.3
mg/g which is also less than the 304.1 mg/g release amount at pH 7.4. Release amount at pH 1.0 is less than that at pH 7.4 for both samples (with or without PAA encapsulation), which is different from the above discussed R6G release result.

This result can also be explained from the electric interaction between ibuprofen molecules and silica surface, protonated amine group and dissociation of PAA. For NH$_2$-MCM sample, on one hand there is positive charge on silica surface at pH 1.0 and negative charge at pH 7.4. On the other hand, ibuprofen molecules have negative charge after dissociation at pH 7.4 (pH $>$ pk$_a$) while not at pH 1.0 (pH $<$ pk$_a$=4.5) because ibuprofen cannot be dissociated. So there is repulsion between ibuprofen and silica surface and thus more release at pH 7.4. For NH$_2$-MCM-PAA sample, on one hand, electric interaction factor favors the releasing of ibuprofen. On the other hand, PAA was dissociated at pH 7.4 which also favors the ibuprofen releasing.

Lastly, drug (R6G or ibuprofen) release amount from PAA covered samples is less than the corresponding samples without PAA cover at the same pH condition (pH 1.0 or pH 7.4). It is easy to understand that PAA acts as a barrier when the drug molecules release from the mesoporous matrix and thus reduce the release amount.

Table 5.2 Ibuprofen release (mg/g) at pH 1.0 and pH 7.4 over the time (h)

<table>
<thead>
<tr>
<th>time/h</th>
<th>Different conditions</th>
</tr>
</thead>
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<tr>
<td></td>
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</tr>
<tr>
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<td>3</td>
</tr>
<tr>
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<td>9</td>
</tr>
<tr>
<td></td>
<td>23.5</td>
</tr>
</tbody>
</table>
5.2.2 TGA data of different samples

The thermogravimetric (TGA) traces were collected by using a Q-500 Quantachrome Analyzer (TA-Instruments) with N₂ (99.999 %) as a carrier gas with a heating ramp of 5 °C/min. The TGA traces of samples (Figure 5.3-Figure 5.6) show a weight loss below 100 °C due to physisorbed water and a weight loss at ~200-600 °C due to the loss of organic functional groups.

Figure 5.3 Weight loss of R6G loaded NH₂-MCM-SH (A) after release at pH 7.4, (B) before release and (C) after release at pH 1.0.

Figure 5.4 Weight loss of R6G loaded NH₂-MCM-SH-PAA (A) before release (B) after release at pH 1.0 and (C) after release at pH 1.0.
Table 5.3 shows the weight loss data of ibuprofen loaded samples before and after release at different pH condition from TGA test. The total release amount of ibuprofen can be calculated from the difference of the TGA data before and after release.

For NH$_2$-MCM sample, Table 5.3 shows that release amount of ibuprofen at pH 7.4 is 12.1% which is larger than the release amount of 3.0% at pH 1.0. For NH$_2$-MCM -PAA sample, ibuprofen release amount of ibuprofen at pH 7.4 is 7.0% which is also larger than the 0.1% release amount at pH 1.0. For NH$_2$-MCM-PAA sample, it is noted that there are 0.1% ibuprofen release at pH 1.0 comparing 7.00 % release at pH 7.4. The TGA result is consistent with the Uv-vis data we discussed above.

Figure 5.5 Weight loss of ibuprofen loaded NH$_2$-MCM (A) after release at pH 7.4, (B) after release at pH 1.0 and (C) before release.

Figure 5.6 Weight loss of ibuprofen loaded NH$_2$-MCM-PAA (A) after release at pH 7.4, (B) after release at pH 1.0 and (C) before release
### Table 5.3 Weight loss of Ibuprofen loaded samples at pH 1.0 and pH 7.4

<table>
<thead>
<tr>
<th>Sample</th>
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<th>Before release (%)</th>
<th>After release (%)</th>
<th>Release amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₂-MCM</td>
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<td>23.29</td>
<td>20.29</td>
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<td>23.29</td>
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<td>23.24</td>
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<td>pH 7.4</td>
<td>23.14</td>
<td>16.15</td>
<td>7.00</td>
</tr>
</tbody>
</table>

### 5.3 Experiment section

#### 5.3.1 Synthesis of Organoamine functionalized MCM-41

Mesoporous materials, MCM-41 was synthesized as reported previously.\(^\text{23}\)

#### 5.3.2 Loading and release

To load R6G into mesoporous matrix, 30 mg NH₂-MCM-SH was mixed with 10 mL 1mM solution of R6G. The suspension was vortexed and then kept undisturbed for 48 h. Then, the solution was centrifuged and the R6G supernatant was taken out by pipette. The remaining silica particles were dried in the oven overnight for the release study. For ibuprofen loading, 30 mg NH₂-MCM was mixed with 3 mL 20 mg/mL solution of ibuprofen/hexane solution for 48h and then, centrifuge the suspension and take ibuprofen supernatant out of the stock solution.

To get PAA encapsulation mesoporous matrix, 10ml 3mg/mL solution of PAA (Fw=1800) was added to the above R6G or ibuprofen loaded samples and keep for 10 min. Then centrifuge the solution and take out the PAA supernatant. Lastly, dry all the samples for 48h before release test. The resulted samples were denoted as NH₂-MCM-PAA and NH₂-MCM-SH-PAA, respectively. For the release study, 30 mL solution (HCl/KCl buffer) of pH 1.0 or pH 7.4 ×1 PBS
solution was mixed with the above R6G or ibuprofen loaded samples. Then the solution was stirred consistently at 37°C. Take 1mL solution at different time interval and centrifuge for 30min before UV-Vis test. Take the upper supernatant for test. Last, add 1mL fresh same release solution back to the release system.

5.4 Conclusion

For R6G release study, NH$_2$-MCM-SH was used and it is concluded that there are more release amount at pH 1.0 than at pH 7.4 for sample with or without PAA encapsulation. For ibuprofen release study, NH$_2$-MCM was used and there is less ibuprofen release at pH 1.0 than at pH 7.4 for sample for both with and without PAA encapsulation. On one hand, electric interaction factor favors the releasing of ibuprofen. On the other hand, PAA was dissociated at pH 7.4 which also favors the ibuprofen releasing. Lastly, drug (R6G or ibuprofen) release amount from PAA covered samples is less than the corresponding samples without PAA cover at the same pH condition (pH 1.0 or pH 7.4).

5.5 References


CHAPTER 6 CONCLUSIONS

The lower efficiency of traditional ways of delivering drugs, like oral administration and injection, to humans for some therapies makes new delivery systems necessary. A class of mesoporous silicates materials such as MCM-41 and SBA-15 has attracted the attention of many scientists as drug delivery vehicles for their high surface area, high porosity, well-ordered, tunable nanometer pores and “non-cytotoxic” properties. Because MCM-41 and SBA-15 are promising candidates of drug delivery vehicles, in this dissertation, comparative investigation of the adsorption capacity and drug release properties of MCM-41 and SBA-15 was conducted. The surfaces of the materials were functionalized with judiciously chosen organic groups via post-grafting or co-condensation of various organosilanes and two different hydrophobic and hydrophilic molecules, i.e., ibuprofen and R6G, were used as model drugs in the study. Finally, oxidation of epinephrine in the presence of the two MSN, SBA-15 and MCM-41, as well as silica microspheres was also investigated. Antioxidant effects of caffeine and its metabolites were also evaluated by the epinephrine oxidation rate. The same experiments were also performed with its isomer, isocaffeine. Based on the findings of this study, the following conclusions could be drawn:

The adsorption capacity and release properties of MCM-41 for different drug molecules can be controlled by functionalizing them with judiciously chosen organic groups. Some functional groups can increase adsorption capacity while some groups can decrease adsorption capacity.

Functional groups have different effects on adsorption and release capacity for different drug molecules. Groups that can increase adsorption of R6G may decrease adsorption of ibuprofen.
When SBA-15 was functionalized with mercaptopropyl and carboxylic acid, it was found that R6G adsorption capacity increased and ibuprofen adsorption decreased after functionalization. Mercaptopropyl and carboxylic acid affected adsorption to different extents. Samples with larger adsorption amounts did not necessarily have larger release amounts or larger release percentages than samples with smaller adsorption amounts.

For both R6G and ibuprofen, release amount, release percentage of total amounts loaded in the sample and release rate from COOH-SBA are larger than that from COOH-MCM sample.

MCM-41 and silica spheres do not accelerate the epinephrine oxidation rate in dH₂O while SBA-15 does. In PBS solutions, the presence of SBA-15 and MCM-41 makes the oxidation even more rapid than in dH₂O. The effect of MCM-41 and SBA-15 nanoparticles and dense silica nanoparticles on epinephrine oxidation is pH-dependent.

Silica spheres have no noticeable influence on epinephrine oxidation in PBS and dH₂O. It seems, therefore, that large surface area plus characteristic mesoporosity and surface structures aid in the deposit of oxygen radicals inside MSN particles, and thus deposited oxygen radicals help to catalyze the epinephrine oxidation in a favorable phosphate environment.

In the presence of either caffeine or isocaffeine, the oxidation rate of epinephrine by MSN is slowed down. Also, with the increase of caffeine or isocaffeine concentration, the oxidation rate decreases.

As a candidate for pH-responsive release, PAA works well for ibuprofen release study, but works only slightly for R6G release study.

### 6.1 Limitation and recommendation for future study

For the drug adsorption and release studies of MCM-41 and SBA-15 described in this dissertation, only two drug molecules were used and all the conclusions were based on these
results. For these drugs, adsorption capacity and release rates can be tuned by organic functionalization. Probably, the method of tuning adsorption and release properties by organic functionalization of mesoporous materials could be extended to other organic functional groups and to a variety of other drug molecules. Such additional studies should be performed to further confirm our findings.

In addition, introducing secondary bioactive groups or ligands onto the surfaces of the materials may allow targeted delivery of the drug cargo to specific cells, such as cancer cells. The mesoporous silica can be loaded with drug cargo and then targeting ligand can be conjugated to the surface of the mesoporous silica. Selective targeting strategies employ ligands that specifically interact with receptors expressed on the cell surface of interest to promote nanocarrier binding and internalization.

Another extension of the procedures we have investigated could be the functionalization of the mesoporous materials’ internal and external pores with two groups of different hydrophobicity, followed by loading two different drug molecules. Drug combination is most widely used in treating the most dreadful diseases, such as cancer and AIDS. The main aims are to achieve synergistic therapeutic effect, dose and toxicity reduction, and to minimize or delay the induction of drug resistance.

Drug delivery study focus on the adsorption and release property by changing physical interaction, such hydrogen bonding, hydrophilic and hydrophobic interaction, or electronic interaction between drug molecules and mesoporous materials. There are no chemical interaction between drug molecules and mesoporous materials in the studies in this thesis. Actually, Epinephrine oxidation studies is a separate project, but from this study, we also can get some
information about the possible chemical reaction between drug molecules and mesoporous materials in future study.
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