Trinity University
Digital Commons @ Trinity

Chemistry Student Honors Theses | Chemistry Department

4-23-2008

Micellar Templates and Spectroscopic Rulers for Synthesis and Characterization of Site-Isolated Inorganic Catalysts

Brittni Scruggs
Trinity University

Follow this and additional works at: http://digitalcommons.trinity.edu/chem_honors

 Part of the Chemistry Commons

Recommended Citation
Scruggs, Brittni, "Micellar Templates and Spectroscopic Rulers for Synthesis and Characterization of Site-Isolated Inorganic Catalysts" (2008). *Chemistry Student Honors Theses*. 1.
http://digitalcommons.trinity.edu/chem_honors/1

This Thesis open access is brought to you for free and open access by the Chemistry Department at Digital Commons @ Trinity. It has been accepted for inclusion in Chemistry Student Honors Theses by an authorized administrator of Digital Commons @ Trinity. For more information, please contact jcostanz@trinity.edu.

TRINITY UNIVERSITY DEPARTMENT OF CHEMISTRY

Micellar Templates and Spectroscopic Rulers for Synthesis and Characterization of Site-Isolated Inorganic Catalysts

---

Honors Thesis

Micellar Templates and Spectroscopic Rulers for Synthesis and Characterization of Site-Isolated Inorganic Catalysts

Brittni Scruggs

A departmental senior thesis submitted to the Department of Chemistry at Trinity University in partial fulfillment of the requirements for graduation with departmental honors.

April 23, 2008

_____ _____

Thesis Advisor Department Chair

_____

Associate Vice President
for
Academic Affairs

This thesis is licensed under the Creative Commons Attribution-NonCommercial-NoDerivs License, which allows some noncommercial copying and distribution of the thesis, given proper attribution. To view a copy of this license, visit http://creativecommons.org/licenses/ or send a letter to Creative Commons, 559 Nathan Abbott Way, Stanford, California 94305, USA.

Table of Contents

Contents	Page(s)
Acknowledgments	6
List of Figures	7
Chapter 1: Introduction	9
Chapter 2: Preparation and Amine Quantification of APS Modified Silica	26
Background	27
Experimental Procedures	30
Preparation of Grafted APS Slica	
APS-SBA-15 Synthesis	
Cobalt Titrations	
Ninhydrin Assays	
Results and Discussion	33
Figures	40
Chapter 3: Micelle Deposition of APS for Isolated Amine Sites on Silica	48
Background	49
Experimental Procedures	52
Critical Micelle Concentration (CMC) Determination	
Reflectance UV-visible Spectroscopy	
Micelle Monolayer Capacity of Davisil Silica	
Encapsulation of APS by CTAB Micelles	
Removal of Surfactants and Surface Alkylation	
Results and Discussion	55
Figures	64
Chapter 4: Fluorescence Resonance Energy Transfer (FRET) Development for APS Silica	73
Background	74
Experimental Procedures	75
Anchoring of FRET Fluorophores to APS Silica	
FRET Studies	
Results and Discussion	77
Figures	87
Applications in Heterogeneous Catalysis	95
Conclusion	96
References	97

Appendix A.....100

Appendix B.....101

Appendix C.....102

Appendix D.....103

Abstract

The controlled preparation of active sites on heterogeneous catalysts could improve several catalyst systems by optimizing catalyst efficiency and increasing reaction selectivity. Cetyltrimethylammonium bromide (CTAB) micelles were used as templating structures to encapsulate and deposit aminopropyltriethoxysilane (APS) onto silica. Methods of determining the number of available surface amines were explored; in particular, cobalt(II) titrations and ninhydrin tests were developed. Further characterization of the surface amines after micelle removal and surface alkylation with tetramethyldisilazane (TMDS) was performed by using fluorescence resonance energy transfer (FRET). Indole-3-propionic acid, a fluorescence donor, and a variety of fluorescence acceptor molecules have been reacted with the surface amines on APS micelle-templated and APS grafted silica to provide a distribution of distances between the amines using the Förster radii of the specific donor and acceptor pairs. These FRET studies can be used to determine the extent of isolation of the active sites on oxide supports using the proposed templating scheme.

Acknowledgments

To my family: I would like to thank my family, especially my father, Dr. Jeffrey H. Scruggs, and my mother, Stephanie J. Weeks, for supporting me throughout my undergraduate career. It is because of them that I have had the opportunity to attend Trinity University.

To Dr. Bert Chandler: I will be forever grateful to Dr. Chandler for providing me with the tools to think like a researcher and for his guidance throughout the process of applying to M.D./Ph.D. programs. His passions for research and teaching have inspired me to pursue academic medicine, and I am thankful for his support, encouragement, and countless conversations throughout the last four years.

Dr. Brent Shanks and Sarah Hruby: for their collaborative efforts on this project

Dr. Steven Bachrach: for his advising and revisions

Suzanna Kilgore: for her research contributions and discussions

Trinity University Chemistry Department: for the opportunity to learn, research, and teach chemistry

The Chandler Research Laboratory: for their suggestions and hard work

Funding: I would like to acknowledge the National Science Foundation (CTS-0455965) for financial support of this work and Trinity University and the Mach Research Scholarship Program for a Mach Research Scholarship

List of Figures

		Page
1.1	Various methods for silica functionalization	19
1.2	Amine Group Interactions	20
1.3	Aminoalkyl-Modified Silica	21
1.4	Micellar Templating Proposed Scheme	22
1.5	Ninhydrin Reaction with APS functionalized Silica	23
1.6	Anchored Fluorophore and Fluorescence Quenching Molecules	25
2.1	Cobalt Titrations of Silica Materials	40
2.2	Calibration Curve for Ninhydrin Assays	42
2.3	Ninhydrin Tests on Grafted APS Silica Materials	43
2.4	Ninhydrin Tests on APS-SBA-15	45
2.5	Parity Plot for Ninhydrin Titrations	47
3.1	Spectroscopy Determination of the Critical Micelle Concentration	66
3.2	Conductivity Determination of the Critical Micelle Concentration	67
3.3	Reflectance UV-visible Spectrum of Silica	68
3.4	UV-visible spectrum of a TRIS buffer solution with Phenol Red	69

3.5 Micelle Monolayer Determination 70

4.1 Fluorophore Anchoring Synthetic Scheme 87

4.2 Fluorescence Spectra of APS Grafted Davisil Silica 88

4.4 Quenching of Various Donor Loaded APS Silicas..... 89

4.3 Fluorescence Spectra of APS Grafted Silica 90

4.5 Fluorescence Resonance Energy Transfer Study.....91

4.6 FRET Estimated Amine-Amine Average Distances 93

Introduction

Chapter 1

Amine-Functionalized Silica

Heterogeneous catalysts are often implemented in industrial settings because they dramatically increase reaction rates and are easily separated from reaction mixtures. Amine-functionalized silica has promising applications as solid adsorbents for CO_2 and can be used in separations, chemical sensing, and base catalysis, such as in the Knoevenagel condensation reaction (1,2). In addition, amine modified solids can be used to prepare liquid chromatography packing materials and various chelating agents (3). Anchored amines on the surface of oxide supports can also serve as versatile synthetic platforms for organic moieties and catalyst molecules (1,4). Unfortunately, these materials can be less effective catalysts compared to their homogeneous analogues due to a reduction in reactivity and selectivity. Current research in this area has focused on increasing the catalytic efficiency of such materials by modifying the material's synthesis, isolating amine sites, and improving cooperation between individual sites (4,5).

Two primary methods are utilized for preparing amine-functionalized silica. One method involves a direct synthesis of SBA-15 material by co-condensing an alkoxyaminosilane and tetraethyl orthosilicate (TEOS) around a pore templating agent such as Pluronic 123 (P123) (1, 6). The resulting SBA-15 mesoporous materials, which have high surface areas and tunable pore size, include ordered arrays of uniform nanochannels with diameters as large as 300 Å (6). X-ray and electron microscopy show these materials to have a periodically ordered structure, which is a desirable property for solid phase catalysts as it improves mass transport (7). The second method of preparing amine-functionalized silica utilizes a grafting procedure in which silica is immersed in an alkoxyaminosilane solution (1). As shown in Figure 1.1, the two methods result in materials of

varying functionality; the SBA-15 co-condensation material contains organic groups primarily within the porous channels, whereas the grafted material may be functionalized mainly on the external surface of individual silica particles. Synthesis of amine-functionalized SBA-15 materials provides for the possibility that the amine moieties are spatially organized within the backbone of the silica. The grafting procedure, in contrast, has been used for quick modifications of silica, and such post-synthesis techniques usually involve the preparation of silicas with high amine surface densities where site isolation is of lesser importance (8,9).

In order to improve reaction rates and increase catalyst selectivity, the molecular properties of the system must be understood and well characterized. Surface characterization of amine-functionalized silica has been performed by using a variety of tools, including thermogravimetric analysis (TGA), nitrogen physisorption, FT-Raman spectroscopy, and NMR spectroscopy (1,4). Specifically, the number of reactive surface amines is of interest for evaluating the catalytic activities of supported amine catalysts (10). Elemental analyses give total nitrogen content, but an alternative that would only measure available surface amine groups would be more relevant for characterizing catalytic materials. Traditional aqueous and non-aqueous acid-base titrations are complicated by the buildup of surface charges, which shift pKa values and require extremely long equilibration times (24+ hours) (11). For organic reactions that deal with potentially large substrates or reaction products (e.g. from coupling reactions), it is also important to distinguish amines that can readily react with larger substrates from those that may be occluded in micropores or are otherwise inaccessible.

Amine Site Isolation

In many catalytic systems, a greater number of reactive amine sites results in faster rates. For example, it is advantageous to have high amine loadings (>6 mmol/g) on material used as a solid adsorbent for CO_2 capture from flue gas streams (2). However, amine loadings have been restricted (0.09-0.2 mmol/g) in many studies of grafted silica to prevent clustering, which is the interaction between two adjacent amine groups. Clustering arises from hydrogen bonding and from the ability of aminosilanes to react in close proximity via siloxane bridges (1,9). Interactions between neighboring groups affect reaction rates (sometimes positively and sometimes negatively); thus, significant research has been devoted to maximizing surface amine loadings while preventing amine-amine interactions (9). Figure 1.2 shows the some of these interactions.

Prevention of amine-amine interactions is important for creating well-defined functional materials. The immediate environment around an individual amine affects its ability to participate in catalysis and its ability to serve as a scaffold for further surface functionalization (1,4). Immobilizing metal complex catalysts as single sites has been hypothesized to be a major advance for these materials, such as for the immobilization of Ti ethylene polymerization catalysts (4). To avoid the basic amines from interacting with the acidic silica silanols or hydrogen bonding to adjacent amine sites, the amine can be functionalized before incorporation onto the silica surface (1). An SBA-15 material has been synthesized with a patterning molecule, an alkoxyaminosilane functionalized with a trityl or benzyl group, to ensure adequate spacing between adjacent amine sites; the catalytic activity of this amine dense material is comparable to that of the homogeneous analogues and much greater than that of traditionally prepared materials (7). Amine-functionalized silica can also be modified after amine deposition with an alkylating reagent, such as

hexamethyldisilazane (HMDS), tetramethyldisilazane (TMDS), or isobutyltrimethoxysilane (IBTMS) to cap the surface silanols and thus prevent interactions between the amine with the surface (Figure 1.2). A schematic of modified silica with alkylated, protected hydroxyl groups and isolated amine sites is shown in Figure 1.3.

Patterned sites, which provide an efficient assembly of surface species, may be more accessible for catalysis (1,12). Because mass transport is slower in the pores of the solid, accessibility to active centers is crucial in heterogeneous catalysis. Greater access to binding sites may produce faster remediation processes, greater sensitivities in electrochemical processes, and higher catalytic efficiencies and turnovers (12). Mesostructured SBA-15 silica materials, with spatial arrangements of pores, have increased catalytic activities and selectivities as compared to comparable grafted materials (7). Therefore, facile methods of isolating amine with post-synthetic grafting would be advantageous for using other silicas in heterogeneous catalysis. This thesis proposes such a method incorporating micellar structures for encapsulating alkoxyaminosilanes to prepare site-isolated amine-silicas.

Micelle Properties and Uses

Surfactants, which are amphiphilic molecules containing a hydrophobic tail and a hydrophilic head group, can aggregate in solution to form spherical structures called micelles. Adsorbed surfactants on silica prepare the materials for use in paints, detergents, plastics, pharmaceuticals, cosmetics, pesticides, and separations (13); the ability of micelles to adsorb to silicas is a key component in the proposed templating scheme. Increasing the surfactant concentration in solution beyond a certain value, the critical micelle concentration (CMC), provides that only micelles form. Further addition of surfactant molecules results in a transition from

spherical micelles to rod-like micellar structures (14). The exact value of the CMC is important for many micelle studies to ensure both the presence of micelles in solution and absence of rod-like structures.

The CMC for a surfactant is usually associated with a variation of some property as a function of concentration (15). Accurate measurements of this value can be performed by using light scattering, conductivity, ultrasonic velocity, surface tension, spectrophotometry, and ionization equilibrium shift experiments (16, 14, 17, 18). In addition, alterating the acid-base equilibrium of a reporter molecule can indicate the presence of micelle formation and thus serve to determine the CMC (16). This value, which depends on the pH, temperature, and ionic strength of the solution, can be used to assemble micelles in solution for the purpose of adsorbing these structures onto silica (19). Deposited surface micelles have been imaged by using soft contact atomic force microscopy (AFM). These adsorbed micelles have been shown to arrange themselves in a hexagonal lattice with equal distances of separation. Cetyltrimethylammonium bromide (CTAB), a common surfactant, has been shown to self-aggregate to form micelles with a diameter of approximately 4 nm composed of 70-90 monomers of surfactant (13, 17); the aggregation number and diameter of micelles can be determined by optical probe diffusion (20).

Aqueous micelles act as globular proteins capable of stabilizing intermediates and increasing rates of certain catalytic reactions. In addition, these structures solubilize organic compounds by incorporating the organic group into the hydrophobic micelle interior (21, 18). Related studies have provided evidence that the CMC decreases with increasing concentration of organic probes due to the increased separation of the ionic head groups of the surfactant monomers (18). CTAB micelles could serve as templating agents for amine deposition onto silica for the purpose of active

site isolation due to the tendency for the hydrophobic alkyl region of APS to become encapsulated by the micelle. The proposed scheme, shown in Figure 1.4, includes the formation of micelles (Step 1), addition of an alkoxyaminosilane (Step 2), subsequent contact with silica (Step 3), removal of surfactant, and protection of surface hydroxyls using an alkylating reagent (Step 4).

Surface Amine Quantification

Recent studies have been aimed at developing new and advanced systems for selective amine sensing; such research has made use of colorimetric probes for detecting primary aliphatic amines (22). A surface titration for amines, developed by Taylor and Howard, involves the reaction between surface amines and ninhydrin, a reagent used to detect primary and secondary amines via solution colorimetry (3). Shown in Figure 1.5, the ninhydrin reaction is advantageous because it cleaves the C-N bond of primary amines, resulting in a soluble highly colored analyte. The production of an extended aromatic product, along with four equivalents of water, provides the strong driving force necessary to cleave the C-N bond. However, the material studied by Taylor and Howard was epoxy-modified silica functionalized with ethylenediamine, and no studies have been performed that apply such a titration technique to the surfaces of either amine-modified SBA-15 or APS grafted silica. Due to the synthetic techniques used in preparating these materials, such an assay could distinguish between amines that readily react with organic molecules and those that may be protonated, occluded in the material, trapped in micropores, or poisoned by remnants of synthetic templates.

Fluorescence Resonance Energy Transfer

Characterization of the resulting silica materials is critical to verify site isolation; however, quantitative measurements of the distance between adjacent amines on amine-functionalized silica are unavailable. Site-isolated materials have been characterized by using steady-state fluorescence emission experiments; specifically, pyrene species anchored to the amine sites were tested for monomer and excimer emissions. It was found that patterned surfaces showed strong monomer emission whereas traditionally grafted material provided strong excimer emissions (9). This technique, although important for qualitatively differentiating between materials, does not quantitatively determine the amine separation. A technique capable of providing such an estimate would be invaluable in evaluating heterogeneous catalysts capable of tunable cooperativity between sites, and this thesis is directed towards characterizing various amine-functionalized silica samples, particularly developing fluorescence resonance energy transfer (FRET) techniques, as a quantitative measure of amine distances.

Fluorescence, a type of luminescence, occurs when a substance, typically an aromatic molecule, emits light due to electronically excited states. Fluorescence resonance energy transfer (FRET) results when the emission spectrum of a fluorophore overlaps with the absorption spectrum of an acceptor molecule (23). FRET involves nonradiative energy transfer, i.e. without the presence of an intermediate photon, when the fluorescent donor molecules and acceptor molecules are brought into close proximity. This process is extremely sensitive to the separation distance between the donor and the acceptor molecules and thus has been used to study receptor-ligand interactions and changes in protein conformations (24, 25). In fact, the distance between the donor and acceptor can be calculated based on the transfer efficiency. As provided in Equation 1, the Förster radius (R_o), which is the distance at which 50% energy-transfer occurs, and the

experimental transfer efficiency (%E) are used to determine the center-center distance between the donor and acceptor molecules (23, 25).

If applied to amine-functionalized silica, FRET could be used to estimate the separation between adjacent amine moieties, and various preparative methods could be compared based on their ability to create isolated sites. This thesis develops FRET methods for various silica materials, such as amine-functionalized SBA-15 silica, grafted silica, and micelle-templated silica, in order to evaluate amine site isolation techniques. Specifically, this research involves anchoring indole moieties, which are excited at 280 nm, and various acceptor molecules, such as levulinic acid. Figure 1.6 shows the proposed anchoring of an indole donor and a levulinic acid acceptor on silica. This allows for varying the donor-acceptor pairs' Förster radii, thus providing a general technique for evaluating a range of inter amine differences.

Catalytic Reactions of Acid-Base Anchored Catalysts

Isolation of the amine sites on silica increases the basicity of the amine site and could also allow the amines to serve as isolated anchor sites for further surface modification. In recent studies, bifunctional, solid-supported materials have been of interest in catalysis, sensor design, drug delivery, and nanotechnology (26). Hybrid materials with anchored NH_2 and COOH groups exhibit amphoteric properties and could serve to mimic the active site of enzymes (26). In addition, it has been proposed that bifunctionalized heterogeneous catalysts are more capable of obtaining reactivity not achievable in homogeneous catalysis (27). A promising extension of the research provided in this thesis is the anchoring of acid-base moieties on the surface of silica. Specifically, the use of a spacer, diaminobenzophenone, functionalized with various acid and base groups could

be anchored to the isolated amine sites and could provide a system where cooperative catalysis is enhanced between the spacer functional groups but is nonexistent between adjacent sites.

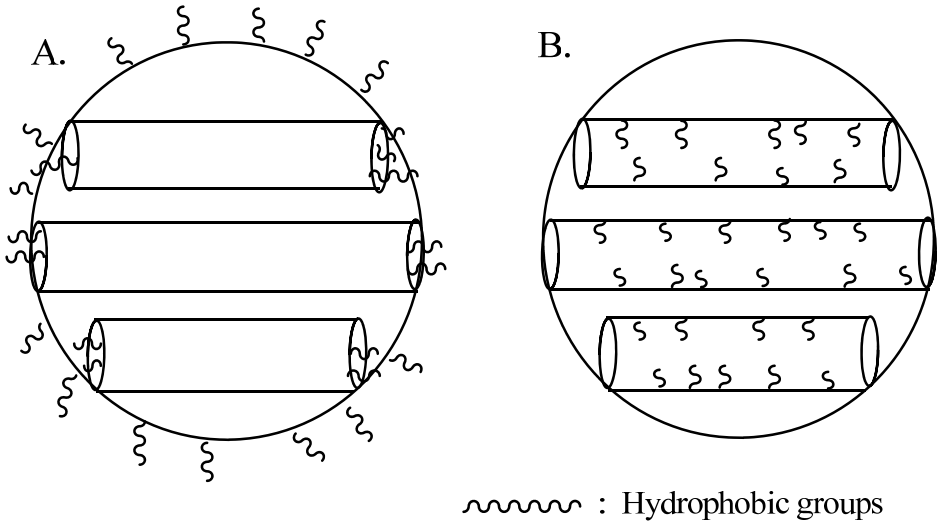


Figure 1.1. Various methods for silica functionalization include (a) post-synthetic modifications and (b) one-step co-condensation techniques (28).

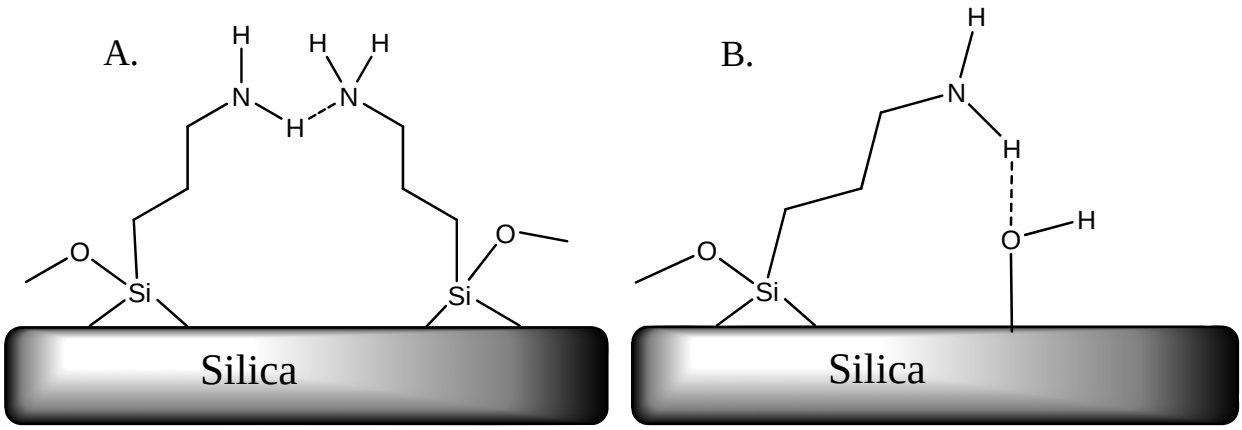


Figure 1.2. Amine Group Interactions. Amine groups are capable of interacting with (a) adjacent amine groups and (b) free hydroxyls on the surface of silica.

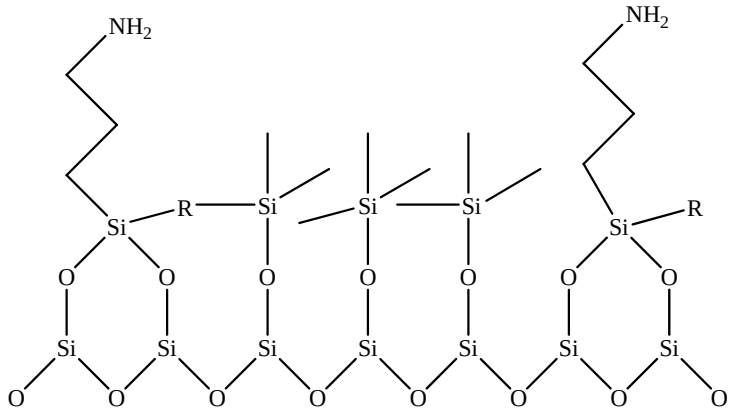


Figure 1.3. Aminoalkyl-Modified Silica. Isolated aminopropyltriethoxysilane grafted onto silica with subsequent protection of surface hydroxyls with tetramethyldisilazane.

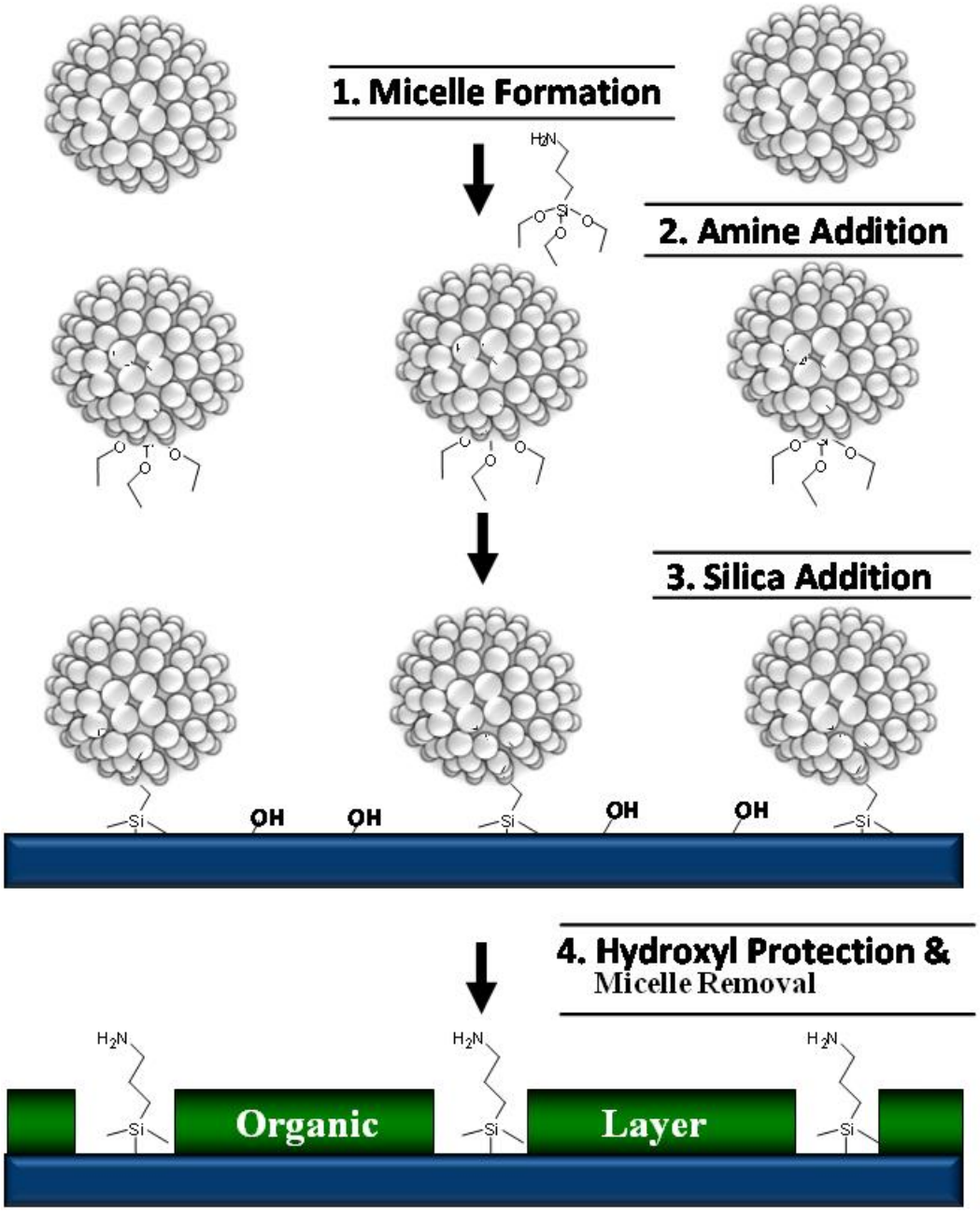


Figure 1.4. Micellar Templating Proposed Scheme. Micellar deposition of alkoxyaminosilane groups on silica for the creation of isolated amine site.

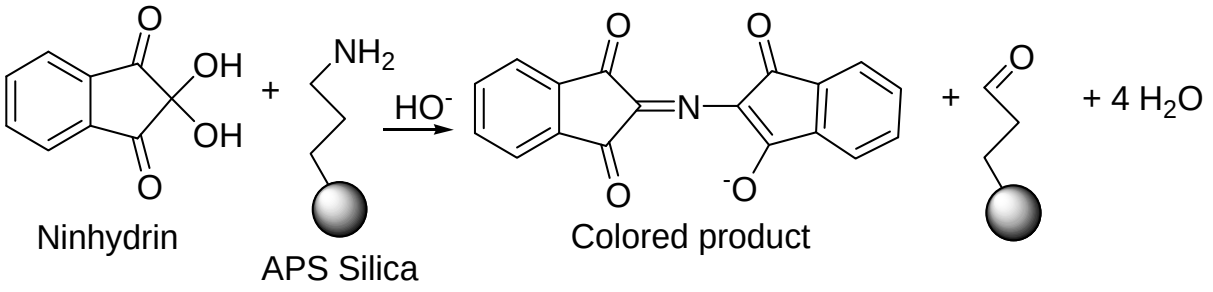


Figure 1.5. Ninhydrin Reaction with Aminopropyltriethoxysilane (APS) Functionalized Silica

$$\%E = \frac{R_0^6}{r^6 + R_0^6}$$

Equation 1.1. Efficiency transfer between a fluorophore donor molecule and an acceptor and its relationship to the pair's Förster radius (R_0) and interatomic distance (r) (23).

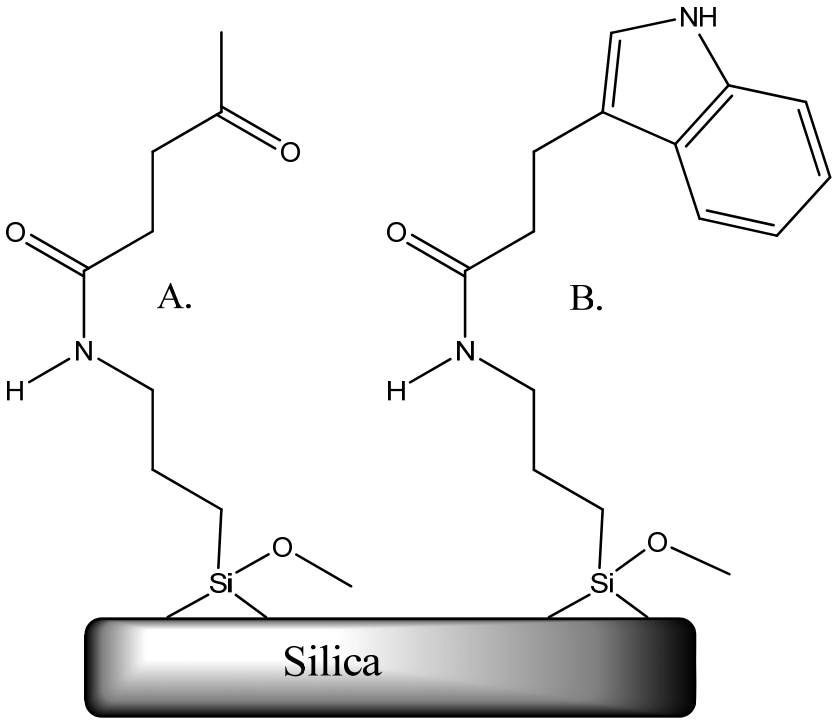


Figure 1.6. Silica Anchored Fluorophore and Fluorescence Quenching Molecules. Silica can be modified with various organic moieties such as (a) the fluorescence acceptor, levulinic acid, and (b) a fluorophore, indole propionic acid. Such modifications involve the synthetic coupling to the anchored amine sites on the silica surface.

Preparation and Amine Quantification of APS Modified Silica

Chapter 2

Background

Amine-functionalized silica has been used in adsorption (4), separations, and catalysis (1). Specifically, supported amine materials are being investigated as catalysts for a number of important organic reactions including Michael additions(29, 30), Knoevenagel condensation (31), aldol condensation (32,33), and cyano-O-ethoxycarbonylation (32). Two different synthetic techniques for preparing amine-functionalized silica include the incorporation of aminosiloxanes into SBA-15 material synthesis and post-synthetic grafting of aminosiloxanes onto pre-formed silicas (28).

SBA-15 is a mesoporous organic-inorganic hybrid material synthesized by co-condensing an alkoxyaminosilane with tetraethyl orthosilicate (TEOS) around a pore templating agent. Electron microscopy and x-ray diffraction have verified that these SBA-15 materials have periodically ordered arrays of cylindrical pores (34). Conversely, grafting of silica involves anchoring siloxane molecules such as aminopropyltriethoxysilane (APS) onto the silica surface by reacting surface hydroxyls with the silane moieties. For catalytic reactions, it is desirable to maximize the surface amine loading to maximize catalytic activity; however, densely loaded amines may induce interactions that can be detrimental to the catalytic efficiency. These interactions include hydrogen bonding between adjacent amine molecules and additional interactions hydrogen bonding between the basic amines and to the acidic oxide support.

The controlled preparation of active sites on heterogeneous catalysts could improve several catalyst systems by optimizing catalyst efficiency and by increasing reaction selectivity. Specifically, using anchored amines as scaffolds for catalyst molecules and as base catalysts,

such as for the Knoevenagel reaction, requires that significant interactions between adjacent molecules and the surface are minimized. Characterizing the resulting surface is a key component for providing a controlled preparation of active sites and for developing a complete understanding of the system's molecular properties. Necessary characterization for APS silica would include determining the number of amine sites and the average distance between adjacent molecules. Developing such characterization techniques is essential before a scheme for molecule isolation can be implemented.

Thermogravimetric analysis (TGA), nitrogen physisorption, FT-Raman spectroscopy, and NMR spectroscopy are currently used to characterize APS silicas, and elemental analysis is normally performed to determine the total nitrogen content (1). Elemental analysis is the most common tool for determining the number of surface amines; however, it often requires waiting days to weeks for sample analysis and can be expensive if multiple materials need to be tested. A bench-scale characterization technique that quickly determines the number of reactive surface amines and can distinguish between the total number of amines and reactive centers would be more useful for evaluating supported amine catalysts. Developing such a technique would serve as a time and cost-efficient method for characterizing multiple APS silica samples.

One literature technique, reported by Taylor and Howard, describes the determination of primary amine content for epoxy-modified silica functionalized with ethylenediamine (3). This technique involves reacting the solid with ninhydrin, a reagent that is capable of cleaving the C-N bond of primary amines, and monitoring the colored product in solution using UV-visible spectroscopy. This assay has been shown to differentiate between primary and

secondary amines (3); however, the ability to distinguish reactive primary amines from total amine content has not been explored with silica materials. A technique that could differentiate between inaccessible and accessible amines would allow for quantifying active amines; applications of such a tool include determining degree of protonation after a specific treatment, evaluating the fraction of nitrogen occluded in the material, and determining the fraction of amines poisoned by remnants of synthetic templates.

Experimental

Materials. 1,1,3,3-tetramethyldisilazane (Gelest) and ninhydrin (Aldrich Chemical Co.) were all used without further purification. Davicat SI-1403 silica powder ($245 m^2/g$) was supplied by Grace-Davison. Solution UV-visible absorption spectra were collected by using a Jasco V-530 spectrophotometer. Infrared spectroscopy was performed on all APS samples by using a Nicolet Nexus 470 FT-IR spectrometer. Elemental analyses were performed by Desert Analytics. Water was purified to a resistivity of 17-18 MΩ-cm with a Barnstead Nanopure system.

Preparation of Grafted Amine-Functionalized Silica. Silica was pressed, crushed, and sieved to 40-60 mesh particles and calcined at 550°C overnight. To anhydrous toluene (40 mL), silica was added under N_2 and stirred for an hour. Aminopropyltriethoxysilane (APS, 500µL) was pipetted into a 25.00 mL volumetric flask and diluted to volume with ethanol. This APS solution was pipetted into the silica mixture and stirred for 24 hours at room temperature under nitrogen. The volume of APS solution added to the silica was adjusted to obtain various loadings of APS functionalized silica. The functionalized silica was then filtered, washed with toluene three times, and dried under vacuum at 50°C.

Silica Alkylation. To tetramethyldisilizane (TMDS, 1.0 g) in anhydrous toluene (30 mL), amine-functionalized silica was added and stirred overnight at room temperature under N_2. The silica was then filtered, washed with toluene three times, and dried under vacuum at 50°C. Ethanol (190 mL) and nanopure water (10 mL) were mixed, and the pH was adjusted to 4.7 with acetic acid. Isobutyltrimethoxysilane (4.0 mL) was added to the solution, stirred for

five minutes, and the modified silica was added. After ten minutes, the solution was decanted, the solid was washed with ethanol, cured at 110°C for ten minutes, and dried under vacuum.

APS-SBA-15 Synthesis. SBA-15 materials were prepared by Sarah Hruby at Iowa State University as described in the literature (35, 36). The structure-directing agent, Pluronic P123 (BASF Co.), was dissolved in 125 ml deionized water and 25 ml hydrochloric acid (12.1 N) with stirring. Tetraethyl orthosilicate (TEOS) was added as the silica precursor (98%, Acros Organics) at 40°C. 3-aminopropyltriethoxysilane (APS) (99%, Aldrich) was added after a TEOS prehydrolysis period of one hour. The resulting mixture (1 TEOS: 0.1 APS/IPTES: 7.76 HCl: 171 H_2O molar ratio) was stirred at 40°C for 20 hours and aged at 90°C for 24 hours before being filtered. The surfactant template was removed by refluxing in ethanol with 10% hydrochloric acid for 24 hours. The catalyst was then filtered and washed with ethanol. Excess protons from the acidic synthesis conditions were removed with 5 ml tetramethylammonium hydroxide (TMAH) solution (25 wt. % in methanol, Acros Organics) in 45 ml methanol with stirring for 30 min. The solid was filtered, washed 3 times with methanol, and dried under vacuum.

Cobalt Titrations. TMDS protected silica (15.3 mg), which had either no organic moieties or various loadings of APS, was added to minimal amounts of anhydrous toluene in dialysis tubing, which was clamped at both ends. This dialysis tubing with the silica suspension was stirred in a flask containing anhydrous toluene (100 mL). A 12.4 mM $CoCl_2 \cdot 6H_2O$ ethanol solution was prepared, and aliquots of 300 µL (3.72 µmol) were added to the flask. After each aliquot addition of the cobalt solution, the toluene and tubing were allowed to stir for five minutes. The absorbance of the toluene solution at 656 nm was monitored by using UV-visible spectrophotometry, and the sample was immediately returned to the flask. Aliquots of the

cobalt solution were added until the toluene solution turned a dark blue color. Amount of APS present on silica surface was determined by using graphical representations of the data obtained.

Ninhydrin Assays. Ninhydrin tests were performed by using a modified procedure of Taylor, et al. (3) APS Silica (10-75 mg) of various loadings (0.857, 0.571, and 0.343 mmol NH_2/g Silica) was added to phosphate buffer (5 mL, 100mM, pH 6.5), and 1 mL of a 5% w/v solution of ninhydrin in ethanol was added to the slurry. After stirring for an hour in a boiling water bath, the mixture was allowed to cool slowly to room temperature. The silica was then filtered and washed three times with warm (70°C) distilled water. The filtrate was collected, added to a volumetric flask, diluted to 100 mL, and the absorbance of this solution at 565 nm was measured by using a UV-visible spectrophotometer. The reference solution was prepared as above with unmodified amine-free silica. Calibration standards were prepared with aliquots of a 1 mg/mL solution of APS in ethanol.

Results

Cobalt Titrations. In an attempt to quantify the surface amine density on APS modified silica, titrations using $CoCl_2 \cdot 6H_2O$ in ethanol were performed. Alkylated APS functionalized silica was added to dialysis tubing and stirred with anhydrous toluene while a 12.4 mM $CoCl_2 \cdot 6H_2O$ in ethanol solution was added. For each addition, the solution's absorbance at 656 nm was monitored by using UV-visible spectroscopy. As a control experiment, comparable aliquots of the $CoCl_2 \cdot 6H_2O$ in ethanol solution were added to a similarly prepared alkylated silica (no surface amines).

Figure 2.1 shows changes in the solution absorbance with increasing $CoCl_2 \cdot 6H_2O$ concentration for titrations using alkylated silica (red, A) and an APS functionalized and alkylated silica (blue, B). The addition of cobalt solution to alkylated silica resulted in a linear increase in solution absorbance at 656 nm due to increasing cobalt(II) concentration in solution. Conversely, the same addition of cobalt solution to APS silica resulted in a negligible increase in absorbance at 656 nm until 21 µmol cobalt(II) had been added. Increasing the cobalt(II) concentration from 21 µmol to 37 µmol resulted in a linear increase in solution absorbance at 656 nm. Both experiments had similar slopes for their linear regions, although the APS modified silica was somewhat lower.

The control experiment shows that cobalt(II) stays in solution and does not appreciably bind to the alkylated silica. The cobalt titration of the APS material resulted in removal of solution $CoCl_2$, presumably by the primary amines on the silica surface. The increase in solution color occurring after addition of 21 µmol $CoCl_2$ is expected to occur after saturating the surface

amines with cobalt (II), presumably with a 1:1 Co(II):N ratio. The determined number of surface amine sites for a given sample is indicated by the intersection of the two linear regions, shown by the arrow in Figure 2.1.

Four APS SBA-15 materials were tested by using the cobalt titration, as shown in Table 1. (Figure 2.1 shows data for sample D and the control material). Elemental analysis was performed to determine the expected number of surface amines. Table 2.1 shows that the cobalt titrations consistently overestimated amine surface density. Cobalt(II) is highly sensitive to the presence of water, and binding of cobalt(II) to the water present in the solvent or within the silica pores may have affected the titration. Even though care was taken to dry the samples immediately prior to use, the cobalt titration was not sufficiently precise for our purposes.

Ninhydrin Assays of Grafted Silicas. The reaction between ninhydrin and a primary amine, based on the work by Taylor and Howard, is shown in Scheme 1 (3). For a surface titration, this reaction is advantageous because it cleaves the C-N bond of primary amines, resulting in a soluble highly colored analyte. The production of an extended aromatic product, along with four equivalents of water, provides the strong driving force necessary to cleave the C-N bond. Calibration curves prepared with APS were linear and could be prepared daily for direct comparison with anchored amine materials (Figure 2.2).

Figure 2.3 shows the results of ninhydrin assays of grafted, alkylated silica samples with various amine loadings. The first sample was repeated on different days (4 times each day) with freshly prepared solutions to evaluate day-to-day reproducibility. These values were 0.86±0.00 and 0.90±0.04 mmol N/g, indicating good day-to-day reproducibility.

Ninhydrin assay data for a series of grafted silicas is compiled in Table 2.2. The ninhydrin assays were typically within one standard deviation of the elemental analysis value, and the % errors were generally less than 10%. The lowest amine loading had slightly larger errors, which may be due to the lower total nitrogen content, or possibly due to partial protonation (*vida infra*). In general, however, the ninhydrin assays gave reasonable results and were much faster and less expensive than commercial elemental analysis.

Ninhydrin Assays of Amine Functionalized SBA-15 Materials. The ninhydrin assay can also be used to enhance traditional elemental analysis, as it can potentially distinguish between total N content and available/free amines. The latter are, of course, of primary concern for catalysis, and the assay offers an opportunity to distinguish between amines that readily react with organic molecules and those that may be protonated, occluded in the material, trapped in micropores, or poisoned by remnants of synthetic templates. SBA-15 materials, which are prepared by using an oxide polymer template, make for a good test of the ninhydrin assay because they require polymer removal. For APS-SBA-15 materials, where the amine is incorporated during the SBA-15 synthesis, the polymer cannot be calcined without destroying the amine functionality. Less forcing conditions are therefore necessary, and it is important to assess the availability of the remaining surface amines.

Table 2.3 provides the ninhydrin assay values, the elemental analysis data, and the SBA-15 materials after various treatments. The ninhydrin assay performed after the acid reflux clearly shows that only a fraction of the total amines react with ninhydrin. A substantial fraction of the amines may be either protonated (and therefore unreactive) or poisoned by polymer remnants (Figure 2.4). Treating the materials with $N(CH_3)_4OH$ removes any excess

protons and may help to dislodge any remaining polymer from the solid. After this treatment, the SBA-15 materials behave similarly to the amorphous silicas, as shown in Figure 2.5. Additionally, this parity plot shows the consistency of the titration over several materials and several amine loadings.

Discussion

Amine-functionalized silica can be used in liquid chromatography (37), base catalysis (29), and as a solid adsorbent for CO_2 capture (38). Some of these applications, which only represent a small number of possible examples, would benefit from increasing amine loadings, site isolation, and increased catalytic rates. Assessing the amine accessibility is crucial for these applications. However, there are few simple quantitative tests that can differentiate between accessible and inaccessible amines. Most silicas are analyzed by thermogravimetric analysis, nitrogen physisorption, and elemental analysis (29). Elemental analysis, which can be used to determine total nitrogen content, may require a waiting time of weeks, can become expensive with multiple materials, and does not distinguish between available and poisoned surface amines.

Multiple methods were developed by our research group to assess the number of amine groups present on commercial and SBA-15 material. The cobalt titration method using $CoCl_2 \cdot 6H_2O$ in ethanol was not reliable in quantifying the surface amine density on aminoalkyl silicas. Our experiments indicated that the cobalt does not bind to the silica surface without amines present (Figure 2.1, A), and it was shown that cobalt does bind to surface amines (Figure 2.1, B). However, the determined amine density was significantly larger than the elemental analysis data. Elemental analysis provides total nitrogen content, and any method overestimation of nitrogen content (relative to elemental analysis) is likely to be inaccurate.

The overestimations may be due to the presence of water in the reaction. Although precautions were taken to remove water from the silica materials, a method that requires scrupulous drying techniques (e.g., glovebox) is unlikely to be practical. Cobalt(II) has a high

affinity for water, and water may have been residing within the pores of the silica or within the solvent. The molar absorptivity of cobalt(II) in water is much lower than in ethanol, and the water converts the cobalt to its aqua complex, which appears as a loss of cobalt from solution. In addition, $CoCl_2 \cdot 6H_2O$ in ethanol results in a blue solution that can be monitored at 656 nm, and the addition of water causes the solution to shift from a blue to a light pink, which does not absorb light strongly at 656 nm.

A relatively simple and fast assay for accessible surface amines that can be readily applied without scrupulously excluding water (e. g., with glovebox or schlenk techniques) was developed by using ninhydrin to oxidize an anchored primary amine and cleave the C-N bond. Figure 2.2 shows the calibration curve used to determine the number of amines present in solution. This curve was reproduced daily to account for slight differences in UV-visible spectra baselines and reaction conditions. The ninhydrin assay was generally in good agreement with known values for amines grafted onto commercial silicas and SBA-15 materials that had amines incorporated into the mesopores during synthesis. Ninhydrin assays of the SBA-15 materials also distinguished between functional and unreactive amines and highlighted the importance of a base treatment after polymer removal if active amines are desired.

A parity plot that relates the expected values based on elemental analysis to ninhydrin titrations, such as in Figure 2.5, can be used to evaluate the utility of this method. The parity plot for these materials shows that the expected results and titration results are linearly related with a slope close to 1. This provides strong evidence that the ninhydrin assay provides reasonable results for various types of materials. In addition, the ninhydrin titration values for the acid refluxed SBA-15 were plotted against the elemental analysis values to construct a line

with a smaller slope and smaller y-intercept than that of the grafted or treated SBA-15 materials.

This assay will aid in future characterization schemes (discussed in Chapter 4) and provides a rapid means of evaluating catalytic activity on a per amine basis. This characterization technique can be applied to other silane molecules if the active center is displaced with an amine via ammonia displacement and subsequent ninhydrin characterization is performed. In particular, using ninhydrin characterization for iodosilanes (which can be readily converted to amines) will be an important step in our future work, for we have proposed anchoring iodopropyltriethoxy silanes onto silica for use as ideal scaffolds for catalyst molecules.

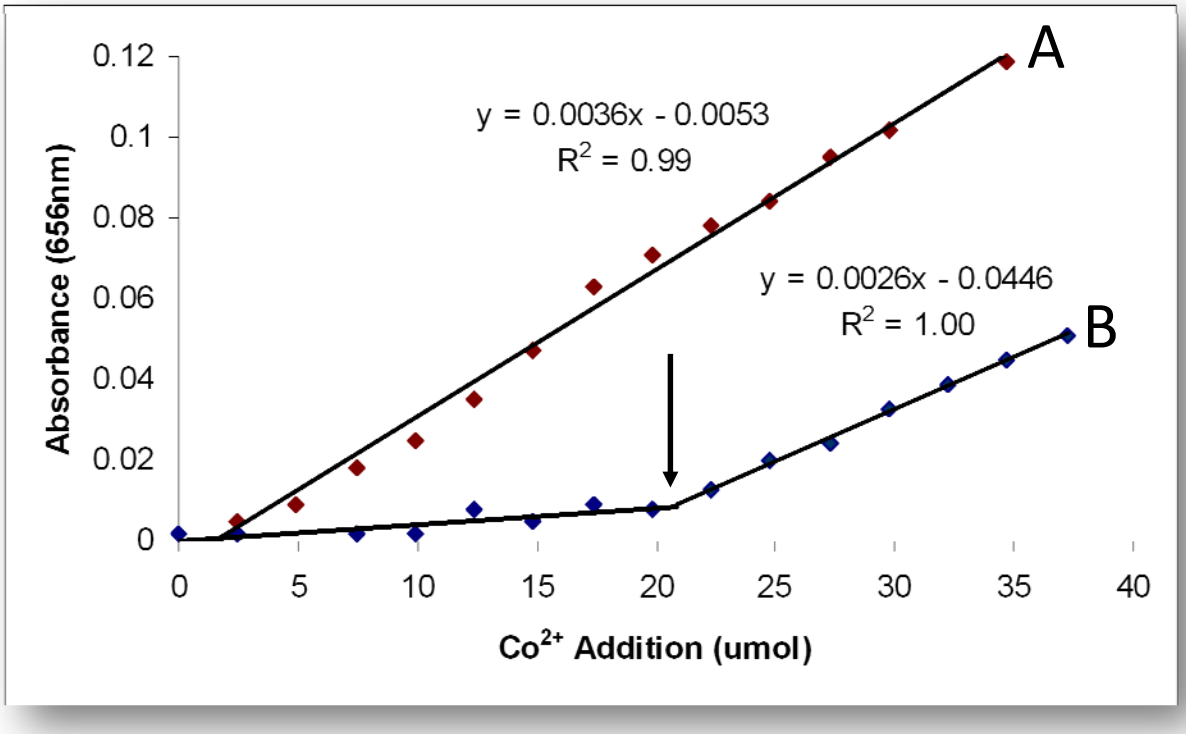


Figure 2.1. Cobalt Titrations of Silica Materials. 12.4 mM $CoCl_2 \cdot 6H_2O$ additions to unmodified silica (A) and APS SBA-15 modified silica (B) in anhydrous toluene as a function of solution absorbance monitored by UV-visible spectroscopy. All silica materials were alkylated using tetramethyldisilazane (TMDS) and added to dialysis tubing in anhydrous toluene. The APS modified silica, provided in Table 2.1 as Sample D, was synthesized by Sarah Hruby of Iowa State University and analyzed by elemental analysis. Experimental amine density of the material was determined as 21 µmol (arrow). Four APS SBA-15 materials were tested (not shown) and all elemental analysis data and experimental amine densities are provided in Table 2.1.

Silica Sample	Expected Density[a] (μmol)	Cobalt Titration Results[b] (μmol)	Error (μmol)	Percent Error
A	22.4	32.7	10.3	46%
B	22.4	33.4	11.0	49%
C	22.4	26.2	3.8	17%
D	17.0	21.0	4.0	24%

Table 2.1. Titration measurements using 12.4 mM $CoCl_2 \cdot 6H_2O$ for the determination of experimental amine densities on four APS SBA-15 materials. A representative plot of the $CoCl_2 \cdot 6H_2O$ titrations is shown in Figure 2.1, and sample D was used as the modified SBA-15 material in Figure 2.1. All experimental results are overestimates based on the expected densities. [a]Determined using elemental analysis. [b]Graphically determined from the cobalt titrations as the cobalt(II) concentration necessary to significantly increase the solution's absorbance at 656 nm (Figure 2.1, arrow).

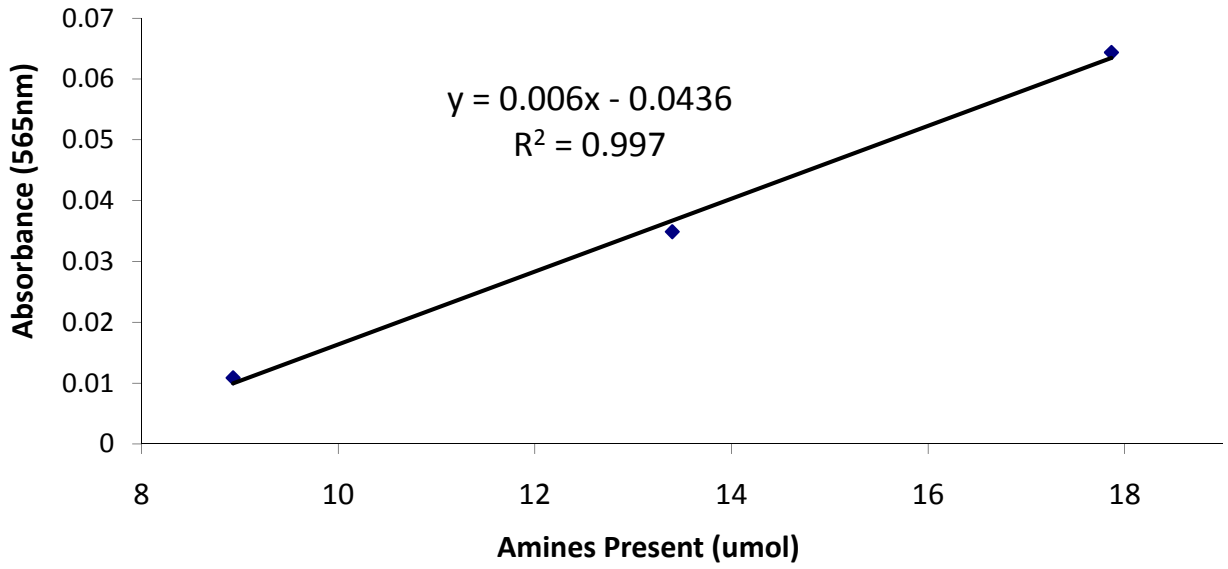


Figure 2.2. Typical Calibration Curve for Ninhydrin Assays. Absorbance values as a function of amine concentration for three calibration standards prepared with aliquots of a 1 mg/mL APS solution in ethanol. Calibration standards were prepared daily for all ninhydrin runs provided in Figure 2.3 and Figure 2.4, and the associated calibration curve and equation were used to quantify the amine density for the ninhydrin reactions performed with APS silica.

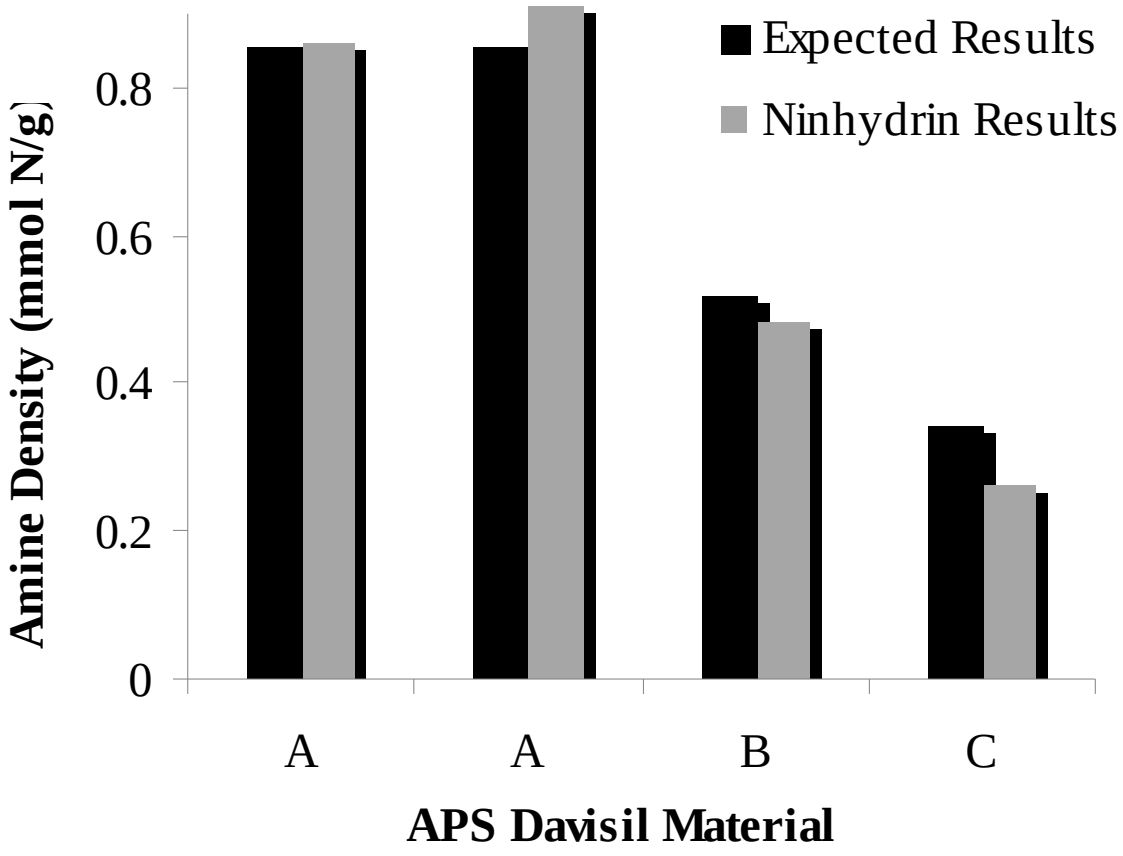


Figure 2.3. Ninhydrin Tests on Grafted APS Silica Materials. The two samples with 0.857 mmol/g loadings (Sample A) were tested by using different solutions on different days to test reproducibility from day-to-day. All expected loadings and experimental results can be found in Table 2.2.

Grafted Davisil	Expected Loading[a] (mmol N/g)	Experimental Results[b] (mmol N/g)	Error (mmol N/g)	Percent Error
Davisil A	0.86	0.86	0	0.4%
Davisil A	0.86	0.90	0.04	5%
Davisil B	0.52	0.48	0.04	7%
Davisil C	0.34	0.26	0.08	23%

Table 2.2. Ninhydrin assays for surface amines using grafted silicas. [a]Determined from grafting synthesis and elemental analysis; [b]Typical standard deviations were 0.08 mmol N/g.

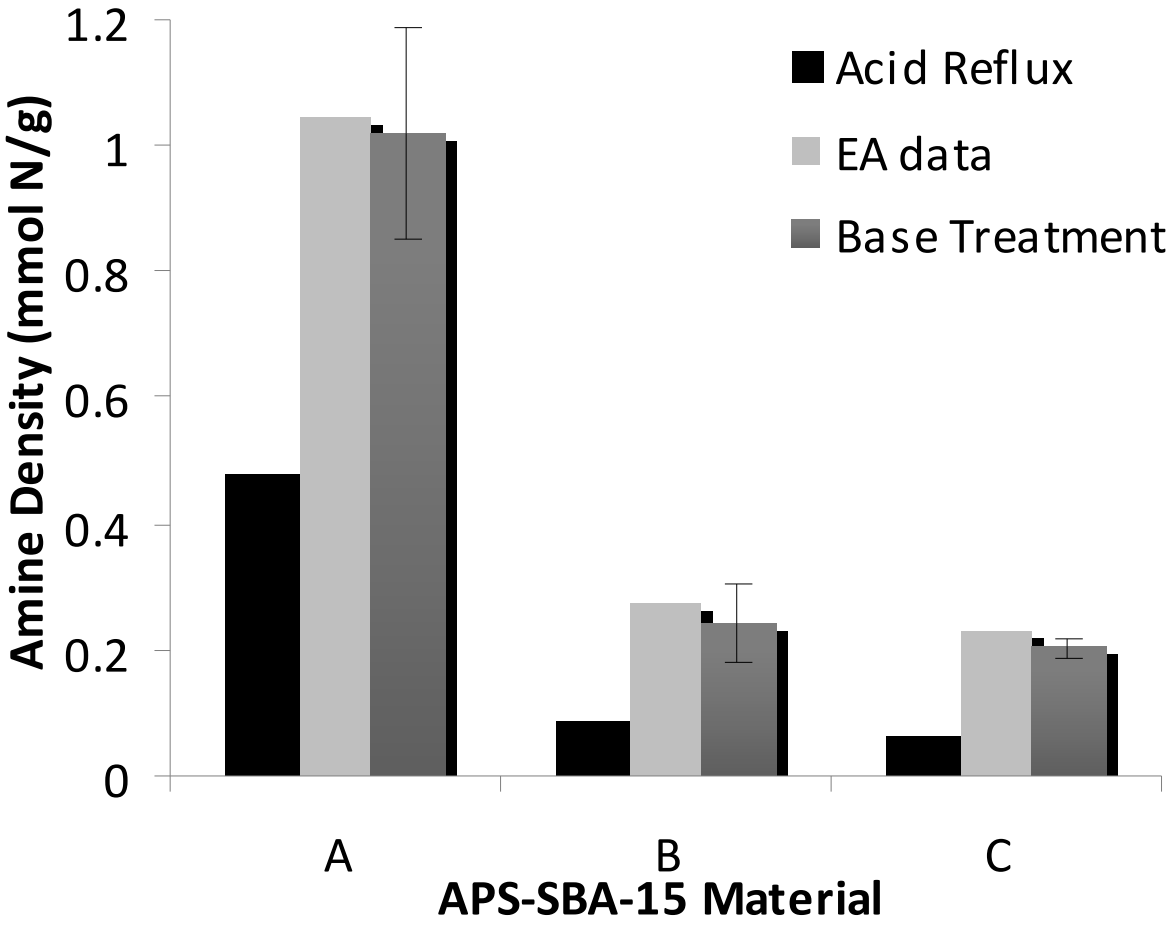


Figure 2.4. Ninhydrin Tests on APS-SBA-15. Tests were performed after treatment in refluxing ethanol and after an additional treatment with $N(CH_3)_4OH$ in refluxing methanol. Ninhydrin tests are compared to the expected results determined from elemental analysis.

SBA Material[a]	N Loading[b] (mmol N/g)	Acid Reflux[c] (mmol N/g)	Base Treatment[d] (mmol N/g)
APS-SBA-15 A	1.0	0.48	$1.0 \pm .17$
APS-SBA-15 B	0.27	0.09	$0.24 \pm .06$
APS-SBA-15 C	0.23	0.06	$0.21 \pm .02$

Table 2.3. Ninhydrin assays for surface amines on APS-SBA-15 materials. [a]All materials were alkylated after polymer removal; [b]determined from elemental analysis; [c]refluxed in 10% HCl in ethanol for 24 hours; and [d]stirred with 25 wt% $N(CH_3)_4OH$ in methanol for 30 minutes.

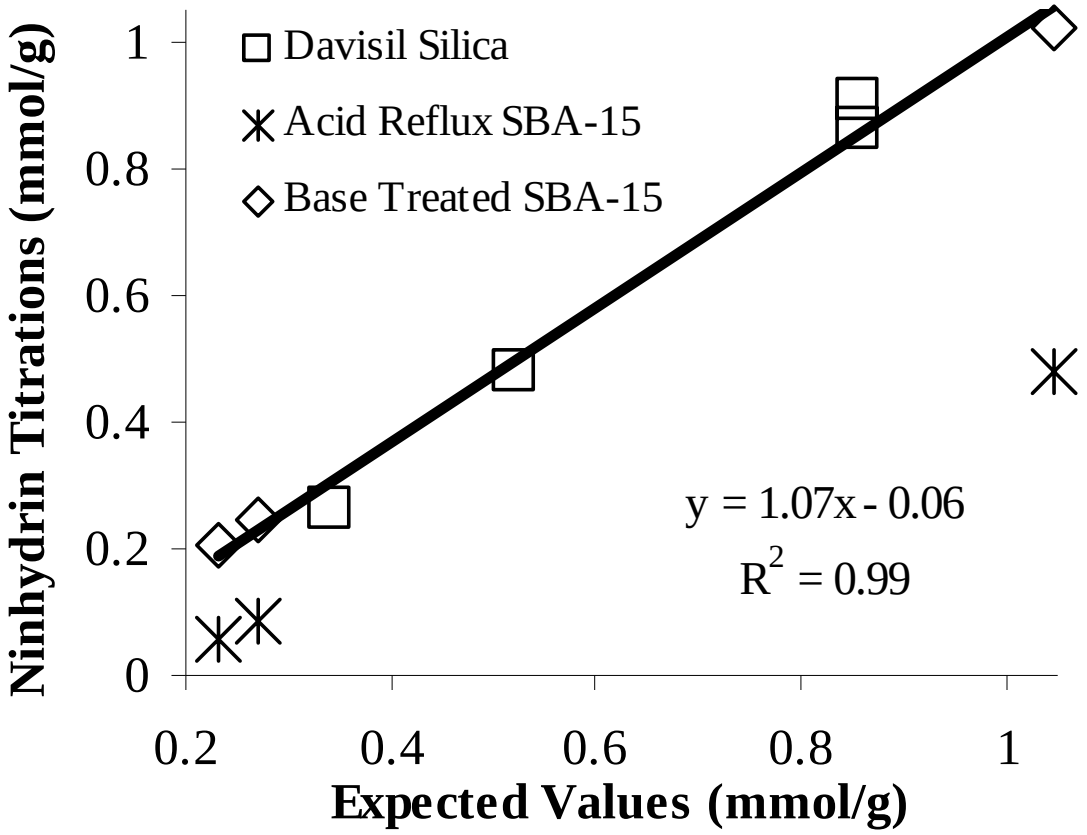


Figure 2.5. Parity Plot for Ninhydrin Titrations. Measured amine densities are plotted against the expected values.

Micelle Deposition of APS for Isolated Amine Sites on Silica

Chapter 3

Background

Multiple types of amines may reside on aminoalkyl-functionalized silica when the material is prepared using grafting techniques. Preparing well-defined functionalized materials involves separating the surface amine groups and capping the surface silanols. Hydrolyzable templates (28), molecular imprinting (30), and patterning molecules, such as immobilized tritylimine groups (31), provide amine separation, but the loadings can be significantly lower than traditional grafted materials. Preventing amine clustering at comparable surface loadings has been achieved with benzyliminosilane deposition (32); however, only limited amine-spacing is available and the techniques utilized to prepare, deposit, and hydrolyze the patterning molecule are time consuming and labor intensive. Developing methods for preparing site-isolated amines without lengthy syntheses and with greater amine spacing would be advantageous for increasing surface amine catalytic activity.

This thesis proposes using micellar templates to encapsulate and deposit aminosilanes for the preparation of site-isolated amine-functionalized silica. Figure 1.4 shows the proposed scheme for amine deposition, including formation of the micelles (Step 1), addition of an alkoxyaminosilane (Step 2), micelle adsorption onto silica (Step 3), removal of the surfactant, and capping of silica silanols (Step 4). Surfactant monomers, which are amphiphilic molecules containing a hydrophobic tail and a hydrophilic head group, aggregate in solution to form spherical micelles. The critical micelle concentration (CMC) is unique for each surfactant and solvent system, and this concentration provides the number of surfactant molecules required in solution to form only micellar structures (i.e., the monomer concentration remains constant).

A surfactant's CMC is associated with the variation of some property (e.g., conductivity or surface tension) as a function of concentration. Conductivity measurements and UV-visible spectroscopy are methods utilized in this chapter to monitor micelle formation and CMC in a TRIS buffer system; however, accurate CMC measurements can also be performed using light scattering, ultrasonic velocity, surface tension, and ionization equilibrium shift experiments (13, 14, 16, 18).

The proposed scheme involves encapsulating aminosilanes inside the hydrophobic interior of CTAB micelles, for these structures have been shown to stabilize intermediates and solubilize various organic groups (18). Specifically, amines tend to reside within the hydrophobic interior because the alkyl chains have a tendency to line up with the surfactant's alkyl region (18); such evidence has led our research group to explore using these micellar structures as templating agents for aminosiloxane deposition. CTAB micelles have been shown to spontaneously adsorb onto silica, and surfactant adsorption on silica has been used to prepare surfaces for use in paints, detergents, plastics, pharmaceuticals, cosmetics, pesticides, and separations (13). In addition, soft contact atomic force microscopy (AFM) has imaged adsorbed micelles on silica surfaces, and these micellar structures, containing approximately 95 monomers, appear to arrange themselves in a hexagonal lattice with equal separation (17).

The research of this chapter investigates the CTAB surfactant in solution for the purpose of forming micelles, encapsulating aminopropyltriethoxy silane, and depositing the micelle encapsulated aminosiloxanes onto a commercial high surface area silica. The evaluation of

these steps, the conditions for surfactant removal from silica, and characterization of the intermediate materials are explored in this chapter.

Experimental

Materials. 1,1,3,3-tetramethyldisilazane (Gelest) and cetyltrimethylammonium bromide (Aldrich Chemical Co.) were used without further purification. Davicat SI-1403 silica powder (245m^2/g) was supplied by Grace-Davison. Water was purified to a resistivity of 17-18 MΩ-cm with a Barnstead Nanopure system. TRIS buffer (5 mM) was prepared by using Tris-HCl and nanopure water, adjusting the buffer to pH 8.2 using dilute sodium hydroxide solution. Phosphate buffer (100 mM) was prepared by using NaH_2PO_4 and $Na_2HPO_4 \cdot 7H_2O$, adjusting the pH to 6.5. Solution UV-visible absorption spectra were collected by using a Jasco V-530 spectrophotometer.

Conductivity Studies of Micelle Solutions. The conductance of a 5 mM TRIS buffer solution (20 mL; pH 8.2) was measured by using a conductivity meter with stirring. Once the conductance value was constant, cetyltrimethylammonium bromide (CTAB; 1 mg) was added to the buffer solution. After five minutes of stirring, the conductivity meter was submerged in the solution and the conductance was recorded once constant. Conductance values were measured and recorded with each CTAB addition. Twelve CTAB additions were made, and a total of 25 mg of CTAB was added to the buffer solution. The conductance values were then plotted against the concentration of CTAB in solution for determination of the critical micelle concentration.

UV-visible Spectroscopy of Micelle Solutions Using Phenolsulfonphthalein. Phenolsulfonphthalein (Phenol Red; 0.1 mg) was added to 5 mM TRIS buffer solution (20 mL; pH 8.2), and the solution was stirred for 5 minutes. A UV-visible spectrum was collected from

300-900 nm by using a Jasco V-530 spectrophotometer. CTAB (25 mg) was then added to the phenol red/buffer solution, stirred for five minutes, and a second UV-visible spectrum was recorded.

Reflectance UV-visible Spectroscopy. Phenol Red (0.1 mg) was added to 5mM TRIS buffer solution (10 mL), and the solution was stirred. This solution was added drop wise to silica, which was prepared as previously described. After wetness impregnation, the silica was air-dried for 30 minutes; a reflectance probe was then used to obtain a UV-visible spectrum directly from the silica. Silica from the monolayer experiments, which had been stirred with a solution of phenol red and CTAB, was air-dried until visibly dry and also monitored using reflectance UV-visible spectroscopy.

APS Deposition and Removal of CTAB Surfactants. Cetyltrimethylammonium bromide (CTAB) was added to 5 mM TRIS buffer solution (40 mL; pH 8.2) in amounts that exceeded its calculated critical micelle concentration, and the solution was stirred at room temperature for one hour. Aminopropyltriethoxysilane (APS) was added to the solution and allowed to stir for 30 minutes. Prepared silica (200 mg) was added to the solution and stirred overnight at room temperature. Silica was filtered, washed with buffer three times, and vacuum dried overnight. CTAB surfactants were further removed by refluxing the silica material in ethanol (40 mL) overnight. The silica was then removed by vacuum filtration and washed with ethanol three times.

Encapsulation of the APS by the micelle was monitored by NMR by using a Varian Mercury 300 NMR Spectrometer (36). Two solutions were prepared with the same

concentration of CTAB, and APS solution was added to one of the solutions such that a 1:1 ratio of micelles:APS existed. The micelle solution was added to an NMR tube, and the APS solution with micelles was added to a capillary tube. The capillary tube was lowered into the NMR tube, and a spectrum was collected. Differences in the two solutions were determined based on the shifts in the spectrum.

Micelle Monolayer Capacity on Davicat Silica. CTAB (1.0296 g) and Phenol Red (0.1 mg) were added to 5mM TRIS buffer solution (500 mL; pH 8.2); this solution was stirred for 10 min until all CTAB and indicator dissolved. A UV-visible spectrum was collected from 500-700 nm, and the absorbance value at 571 nm was recorded. Ten runs were then set up in 100 mL beakers, each containing 40 mL of the prepared solution. Silica was pressed, sieved (40-80 mesh particles) and calcined at 500°C. Silica was weighed immediately after calcination and separated into ten equal samples of 100 mg each; each sample was added to a different beaker, and the solutions were stirred by using a magnetic stirrer. Nine of the ten runs were allowed to stir for 60 min, and the final solutions were monitored using UV-visible spectroscopy. The absorbance values at 571 nm were recorded. One run was monitored by UV-visible spectroscopy every 5 min for 90 min, and the absorbance values at 571 nm were recorded. Each of the ten runs was filtered to remove any fine silica particles before UV-visible spectra were obtained. A calibration curve was performed using the initial solution without silica, and an effective micelle monolayer value was calculated by using the data obtained.

Results

Critical Micelle Concentration Determination for CTAB. Cetyltrimethylammonium bromide (CTAB) is a surfactant capable of aggregating in solution to form spherical micelles, where the hydrophobic tail resides within the micelles and the hydrophilic, charged head groups are situated around the micelle's exterior (14). This surfactant is used commonly because the micellar formation and physical properties are well understood. For the CTAB micellar system, approximately 95 CTAB surfactant monomers aggregate in solution to form one micelle (17), and the scheme for this formation is provided in Scheme 3.1. In addition, an increase in the CTAB surfactant concentration leads to an increase in the monomer and micelle concentration in solution until the critical micelle concentration (CMC) is reached. Above the CMC, the concentration of free CTAB remains constant, and addition of more surfactant results only in subsequent micelle formation (Scheme 3.2). For our system, the formation of CTAB micelles and CMC values were monitored in solution by using conductivity and UV-visible spectroscopy.

The conductance values were monitored with increasing CTAB surfactant concentration in 5 mM TRIS buffer solution by using a conductivity meter; these conductance values are plotted against the concentration of CTAB in Figure 3.2. Two lines with different slopes were observed with increasing CTAB concentration. The critical micelle concentration (CMC) is the concentration at which the two lines intersect. The CMC for CTAB in 5mM TRIS buffer was found to be slightly greater than 0.5 mM. This value was supported by using UV-visible spectroscopy. In this experiment, phenolsulfonphthalein (Phenol Red) was added to a 5 mM TRIS buffer solution, and CTAB was added in varying increments. The solution's color changed

from pink to purple with the CTAB addition, and the maximum wavelength of absorbance shifted from 558 nm to 571 nm (Figure 3.4). With each increase in CTAB concentration, the absorbance values at 571 nm were measured and recorded; these absorbance values were plotted against CTAB concentration (Figure 3.1). The CMC was correlated to the CTAB concentration where the line's slope changed; its value was determined to be slightly less than 0.5 mM in 5 mM TRIS buffer.

Reflectance UV-visible Spectroscopy. Reflectance UV-visible spectroscopy was performed on silica that had been impregnated with a phenol red TRIS buffer solution; the silica immediately turned a bright yellow color. This spectrum showed the maximum absorbance of this material to be at 438 nm. Phenol red in a 5 mM TRIS buffer solution was stirred with CTAB micelles, and this solution was then stirred with the silica for 30 min to allow for adsorption of the micelles to the acidic surface. A reflectance spectrum was collected of the silica with the indicator encapsulated micelles, and the spectrum showed a strong peak at 561 nm (Figure 3.3). In addition, the silica color was a light pink instead of a bright yellow. The reflectance UV-visible peak at 438 nm, which was observed when the indicator resided alone on the silica, was not observed in the presence of CTAB micelles (Figure 3.3).

Micelle Monolayer Capacity of Davisil Silica. A solution of phenolsulfonphthalein, or phenol red, and CTAB (concentration > 0.5mM) in 5mM TRIS buffer was stirred with silica over a period of 90 min; every 5 to 10 min a portion of the solution was filtered and monitored by using UV-visible spectroscopy. A calibration curve was prepared by increasing the concentration of CTAB micelles in a 5mM TRIS buffer solution containing minimal amounts of

phenol red and monitoring the solution absorbance at 571 nm over the range of micelle concentration. It was assumed that the ratio of micelles to indicator remained constant for all standards due to random encapsulation of the indicator molecule by the solution micelles. Using the standard calibration curve, the absorbance values obtained from the filtered solutions were converted to concentration of CTAB micelles in solution. This concentration was determined based on the experimental CMC and 95 CTAB monomers/micelle literature value (17). It was determined that after 60 min the absorbance of the solution remained constant, indicating that adsorption of micelles onto silica was complete (Figure 3.5). Nine runs were performed over a period of 60 min, and the number of micelles capable of adsorbing to the surface was calculated for each run. Using these data, the average micelle monolayer capacity on Davisil silica calculated as 41.4 ± 5.5 nm^2/micelle. In addition, the average micelle diameter was calculated to be 3.62 ± 0.25 nm based on the monolayer capacity and the surface area of Davisil silica, 245 m^2/g.

Encapsulation of APS by CTAB Micelles. To evaluate the potential encapsulation of aminopropyltriethoxy silane (APS) inside the hydrophobic interior of the micelle, NMR studies were performed. Three NMR samples were prepared. In one sample, the silane solution was monitored in D_20. A second sample contained the silane solution in D_20 and a capillary tube containing the micelle and silane solution in D_20 was inserted into the NMR tube. A third sample, which served as an additional control, was prepared by inserting a capillary tube containing the silane solution into an NMR tube with only the silane solution in D_20.

The silane solution alone had a spectrum identical to that of the silane solution in the capillary tube, indicating that this method is appropriate for comparing the shifts. Conversely, the NMR spectrum of the silane solution differed from that of the silane solution with inserted capillary tube containing both micelles and silane in D_20. The shifts of the APS molecule are provided in Table 3.1, and the NMR spectrum can be found in the Appendix A. A shift is expected if the silane is encapsulated inside of the micelle due to its residence in a hydrophobic environment. However, the shifts are small, and such an effect may also be the result of the micelles changing the dielectric constant of the solution.

Removal of CTAB Surfactants and Surface Alkylation. The DRIFT cell analysis of amine-functionalized Davisil silica using micellar templates indicated that the ethanol refluxing conditions were sufficient to remove the surfactant monomers from the silica surface. In addition, the same spectrum (Appendix B) showed that there were amines present on the silica surface. The significant peaks of this spectrum included 3451, 2924, 2855, and 1584, which correlated well with literature values of an –NH stretch, two –CH aliphatic stretches, and an –NH scissor bend, respectively (37). It is significant that the aliphatic stretches are significantly smaller than material with adsorbed micelles, indicating that the 24 hour ethanol reflux was sufficient to remove the surfactants. The –CH aliphatic stretches increase in intensity when the surface is further modified with alkyl chains on the free hydroxyls.

Discussion

In an attempt to prepare site-isolated aminoalkyl-functionalized silicas, our research group employed micelles as templating molecules for depositing aminosiloxanes. Shown in Figure 1.4, this scheme involves the use of cetyltrimethylammonium bromide (CTAB), a surfactant that can aggregate in solution to form micelles. Although numerous surfactants form micelles, we chose CTAB because it is inexpensive, well-characterized in TRIS buffer, adsorbs to silica, and has been shown to encapsulate amine molecules.

Conductivity measurements and UV-visible spectroscopy of micellar solutions are useful in monitoring micelle formation and critical micelle concentration (CMC). Plots of solution conductivity against CTAB concentration provided a CMC value based on a break in the curve (Figure 2.1, arrow). This break corresponds to change in the variation of a physical property (15) and indicates the threshold concentration beyond which only micelles may form. Conductivity measurements can be used to monitor the presence of ion concentrations in solution, and when applied to CTAB systems, a solution containing monomeric surfactant molecules will increase its conductivity with an increase in ionic surfactants. Conversely, the formation of micelles may resemble that of a solution with lower ionic strength, for a single micelle (N=95) may affect a solution's ionic properties less than a solution of 95 individual ionic surfactants due to the localization of the micelles. Thus, the results of the conductivity studies were consistent with the expected changes in which the slope following the CMC break was expected to be less than the linear region approaching the CMC.

Addition of CTAB caused the solution's color to turn from a pink to a dark purple due to the phenol red's encapsulation by the micelle into the hydrophobic micelle interior; such a change in hydrophobicity can affect the indicator's acid-base equilibrium, affecting its UV-visible spectrum. The UV-visible spectroscopy experiments supported the CMC value determined by conductivity. In a 5mM TRIS buffer system, the maximum absorbance with an indicator, phenol red, is 558 nm. Increasing CTAB concentrations in a 5 mM TRIS solution containing phenol red resulted in an increase of absorbance at 571 nm, and the break in the curve was correlated to the CMC (Figure 3.1). The data showed the solution absorbance values remaining constant over the CMC. In this experiment, a small amount of indicator (<1 mg) was added to obtain suitable absorbance values, and at values above the CMC (i.e., when only micelle formation occurs) all indicator molecules were encapsulated. Under the CMC (i.e., when monomers and micelles were added), the micelles continued to form and the indicator was capable of entering the interior. As more micelles form, the absorbance at 571 nm was expected to increase, and the formation of only micelles would immediately exhaust the indicator supply, causing the absorbance at 571 nm to remain constant. The values of the conductivity and UV-visible spectroscopy experiments were both 0.5 mM, which was consistent with literature (38) and highlighted these two methods as sufficient for monitoring micelle formation.

The importance of monitoring micelle formation and determining the CMC value for our system is to obtain a reasonable value for the number of micelles in solution. When testing the proposed scheme (Figure 1.4), it is imperative that the ratio of amines to micelles is known.

An abundance of amines compared to micelles may result in multiple amines entering a single micelle, and deposition of these structures would likely result in greater amine-amine interactions on the surface since the surface density will be greater. Conversely, an abundance of micelles may result in creating a support with amine loadings too low to be useful catalysts. In addition, surfactants self-assemble in solution at high concentrations to form extended micelles, vesicles, and lamellar phases (20), and such a wide variation of templating structures and phases would not be ideal for our system.

Aqueous micelles are known to solubilize organic compounds that are slightly soluble or insoluble by encapsulating the molecules into their hydrophobic interior. NMR studies were performed to determine if aminopropyltriethoxy silane (APS) could be incorporated into CTAB micelles in aqueous environments. The NMR shifts of the APS proton in micellar conditions in comparison to D_2O were evident but minimal; these results are consistent with amine encapsulation. However, these shifts may have resulted from the solvent's shift in polarity with addition of surfactant, for the micelles may affect the relative static permittivity, or dielectric constant, of the solvent. Thus, it is unclear to us whether the amines are experiencing a different environment within the micelles or if the solvent is changed due to the micelles' presence.

If the NMR spectral data results from APS encapsulation, it is likely that the amino group is residing within the micelle interior, the alkyl chain is lining up with the surfactant's alkyl region, and the siloxane region is outside the micelle due to the large individual shifts of the B, C, and E protons in the spectrum (Appendix A). However, the D_20 used in this study remained

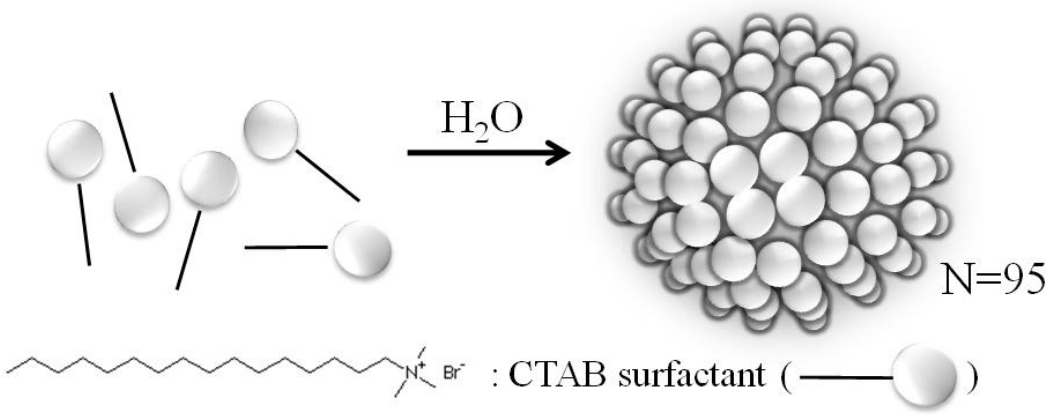
at a neutral pH, a value lower than the pI (approximately 9.0) of the amine; thus, the amine would be mostly in its protonated state. The protonated APS would be less likely than the non-charged molecule to enter the hydrophobic interior, and this result may elucidate the main problem in our micelle studies.

Further deposition studies should focus on this step to ensure that amines are entering the interior. In particular, increasing the pH of the aqueous solution (e.g., greater than 9) or alkylating the amine for increased hydrophobicity may increase the possibility for rapid amine encapsulation by CTAB micelles. Other proposed ideas for silane deposition involve the molecular recognition of APS in its protonated state by cucurbit[8]uril (Q8), a synthetic host capable of recognizing peptides in aqueous solution (39); the adsorption of this macromolecule to silica may be rapid and the amines would be less spaced than in the proposed micelle scheme, providing higher amine loadings.

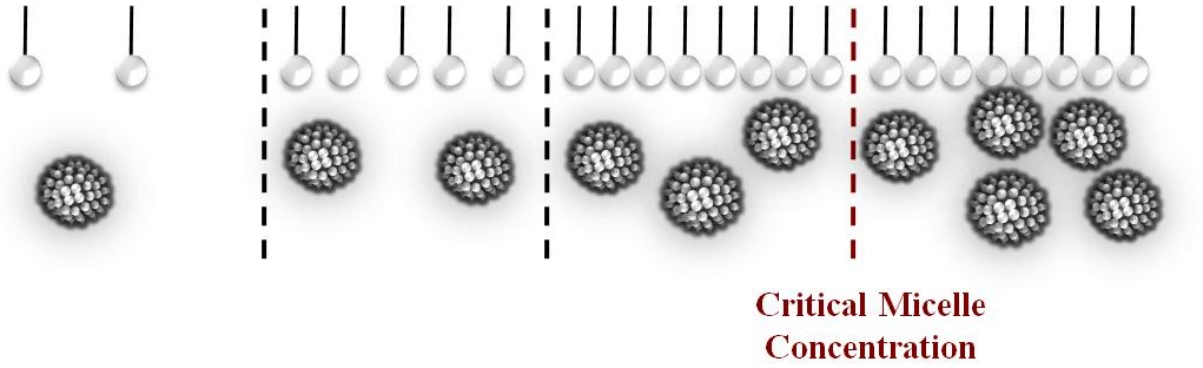
One study has shown micelles to arrange and separate themselves uniformly in a hexagonal lattice on silica surfaces, and AFM images show the micelles as spherical structures (17). The studies absorbing CTAB micelles onto Davisil silica were performed to quantify the micelle-surface interactions involved during the deposition scheme. Studies were performed by adsorbing indicator encapsulated micelles onto silica and monitoring the decreasing solution absorbance at 571 nm over time until the absorbance remained constant. The experimental diameter value was determined to be 3.62 ± 0.25 nm as compared to the literature value of 4.00 nm for free micelles in solution (40); this indicates that the monolayer determination study provided reasonable results for the micelle diameter. The good

correlation of the diameter value to literature and the incorporation of micelle-silica interactions provide evidence that the micelles are retaining a similar spherical shape on the surface as in solution.

The reflectance UV-visible spectroscopy study was performed to assess the structure of the adsorbed micelle. The phenol red molecules interact with the silica such that a reflectance UV-visible spectrum shows a maximum peak at 438 nm; however, deposited micelles with encapsulated phenol red on silica have a UV-visible maximum peak at 561 nm, which is similar to the phenol red in micelle solutions (571 nm). This data indicate, when adsorbed onto silica, phenol red encapsulated within micelles resides in an environment similar to that of the micelles in solution. In addition, the phenol red does not appear to substantially interact with the surface, suggesting that the micelles may retain their shape after deposition. It is evident from the reflectance spectra that micelles do not completely dissociate; however, it is possible that the micelle structure is modified by the support. For example, bilayer formation would also prevent interaction of the indicator with the surface (i.e., give similar spectral data).



Scheme 3.1. Surfactant Monomer Aggregation Mechanism for CTAB Micelle Formation in Aqueous Environments. This scheme shows the assembly of 95 CTAB surfactant monomers to form one micelle template.



Scheme 3.2. Depiction of the Critical Micelle Concentration (CMC). This mechanism indicates that an increase in surfactant concentration initially increases the concentration of both the surfactant monomers and micelles in solution (Steps 1-3); however, the monomer concentration will become constant (Step 4) after the CMC and any surfactant addition contributes to micelle formation in solution.

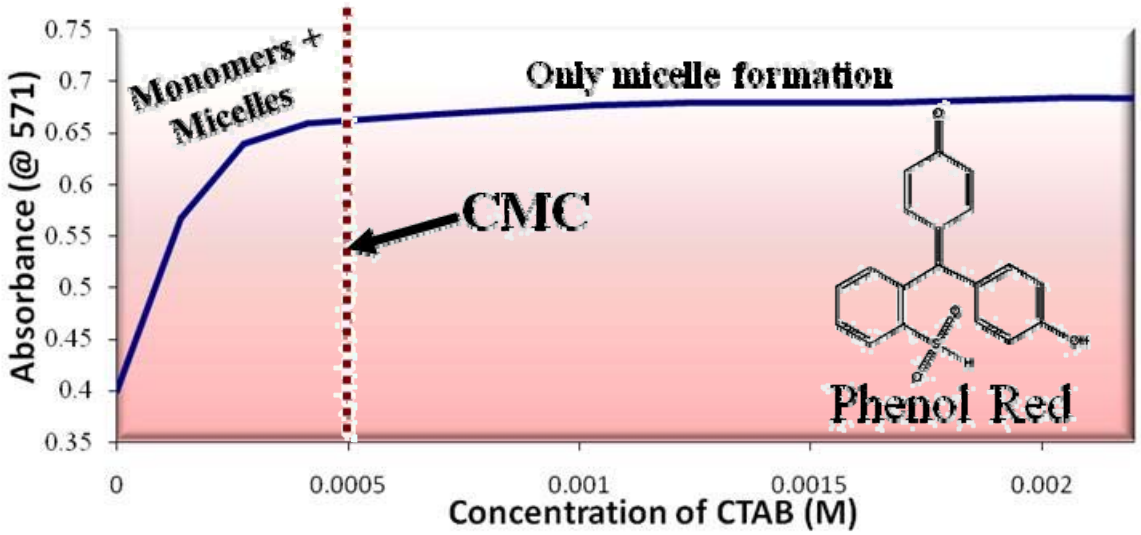


Figure 3.1. Determination of the Critical Micelle Concentration (CMC) Using UV-visible Spectroscopy. The absorbance (571 nm) of a 5mM TRIS buffer solution containing phenol red was monitored with increasing CTAB surfactant concentration. The CMC was determined as the concentration at which point the absorbance is no longer affected significantly by CTAB surfactant additions. The structure of the phenol red indicator is shown on the graph.

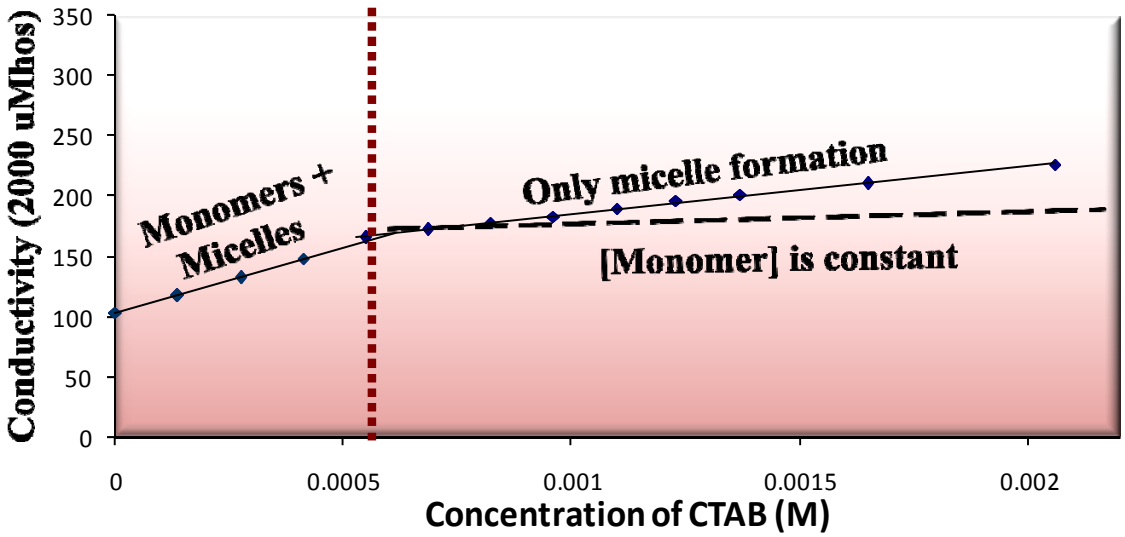


Figure 3.2. Determination of the Critical Micelle Concentration (CMC) Using Conductance Measurements. The condutivity of a 5mM TRIS buffer solution was monitored with increasing CTAB surfactant concentration. The CMC was determined as the concentration where the two linear regions of different slopes intersect.

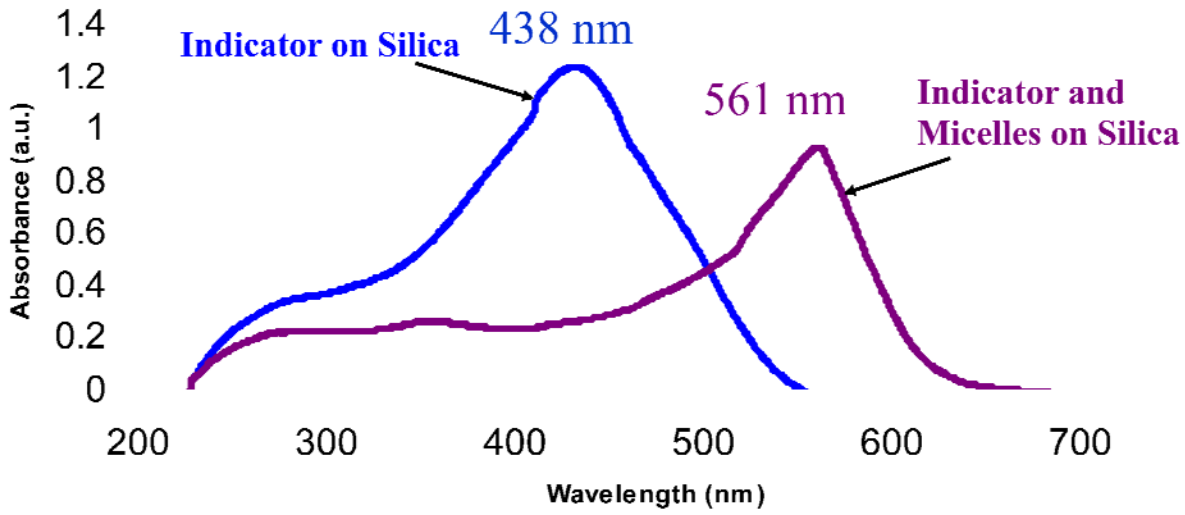


Figure 3.3. Reflectance UV-visible spectrum of silica wetness impregnated with an indicator, phenol red, (A) and with a solution of indicator stirred with micelles (B). A significant shift from 438 to 561 nm occurred with addition of micelles to the wetness impregnation solution containing phenol red.

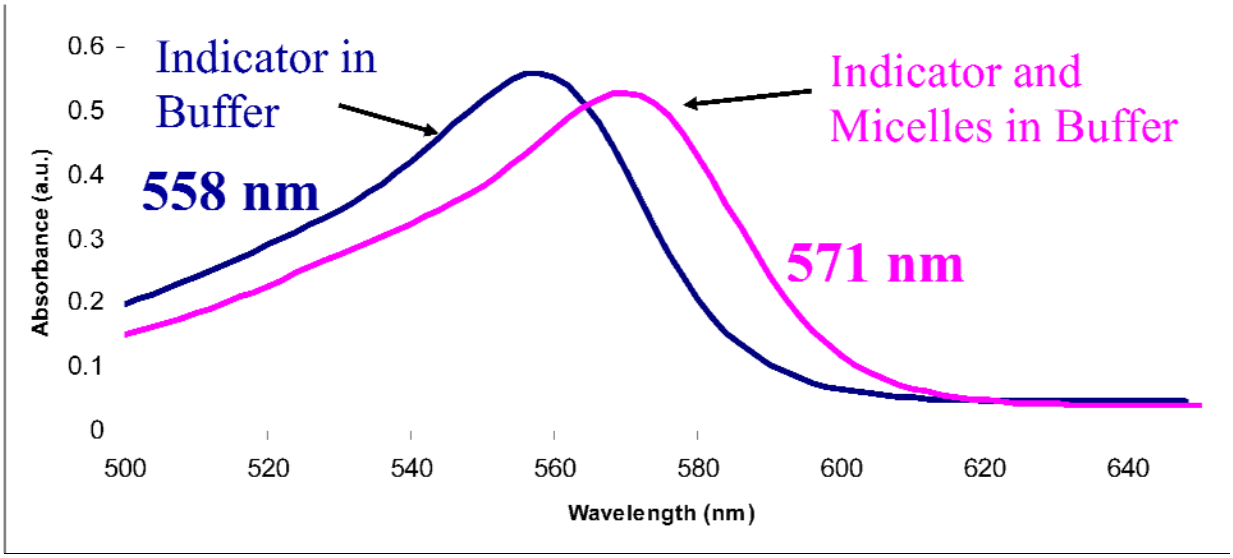


Figure 3.4. UV-visible spectrum of a 5mM TRIS buffer solution with Phenol Red in the absence (A) and in the presence of micelles (B).

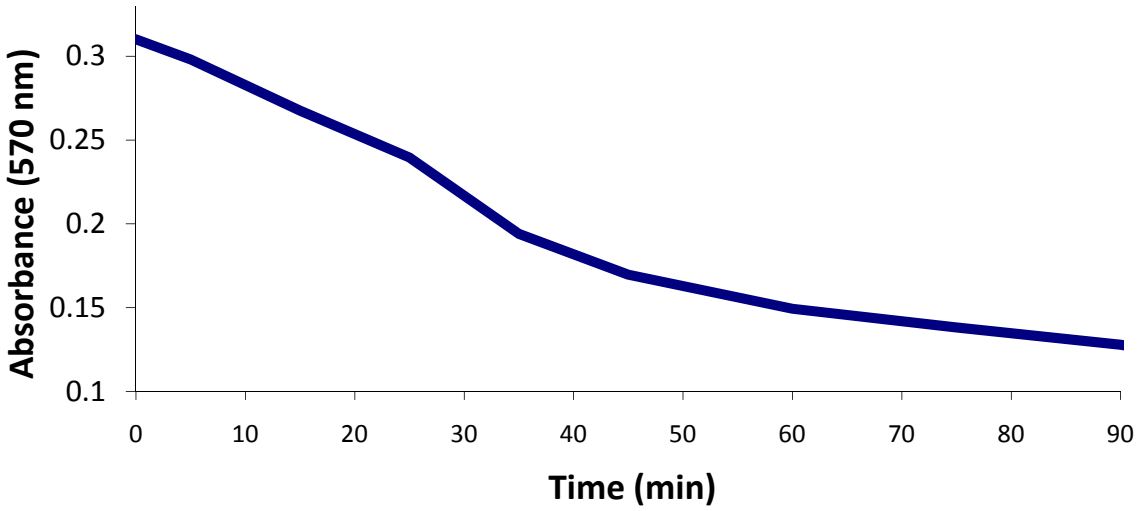


Figure 3.5. Micelle Monolayer Determination. The Absorbance at 570 nm of a solution containing phenol red indicator and micelles stirred with Davisil silica over time. The adsorption of indicator encapsulated micelles onto the silica resulted in the decrease of solution absorbance. The experiment was run for a sufficient time to allow for the micelles to adsorb completely, and the micelle monolayer capacity on the surface was determined based on the remaining number of micelles in solution.

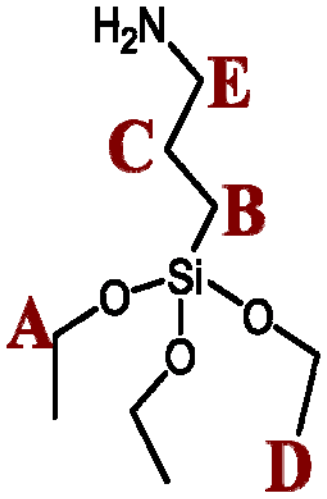
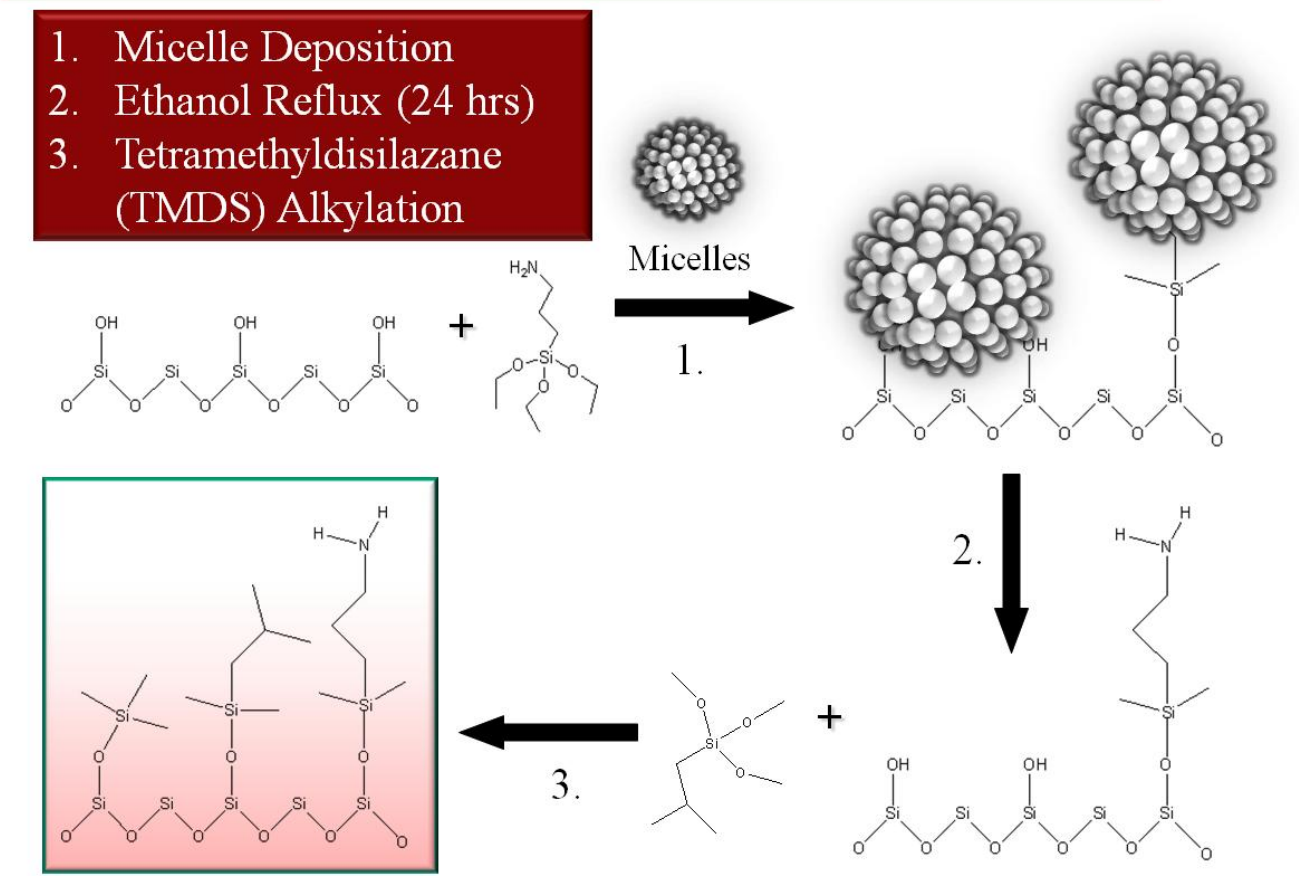
Peak	Shift Average (Hz)	Aminopropyltriethoxy silane Proton Assignments
A	1.125	
B	**6.381**	
C	**4.347**	
D	0.999	
E	**3.514**	

Table 3.1. NMR Spectroscopy Peak Shifts for APS Protons When in the Presence of CTAB Micelles. The NMR spectra for the APS molecules alone and in the presence of CTAB micelles are provided in the Appendix A. Peaks B, C, and E (bold) were of interest due to their larger shift relative to A and D. This study may provide evidence that the APS molecules are entering the micellar hydrophobic interior.



Scheme 3.3. Micelle Templating and Free Hydroxyl Alkylation for Elimination of Amine-Amine and Amine-Surface Interactions. The scheme proposed to create isolated sites involves silane encapsulation, micelle adsorption on Davisil silica, micelle removal, and surface alkylation with various alkylating reagents. Specifically, the APS deposition, micelle removal, and surface alkylation have all been verified using IR spectroscopy, and all spectra are provided in the Appendices B-C.

Fluorescence Resonance Energy Transfer (FRET) Development for APS Silica

Chapter 4

Background

The catalytic activity of supported amines can also be lowered by interactions between adjacent amines. Our research group is applying fluorescence resonance energy transfer (FRET) spectroscopy as a tool to characterize the amine-amine distances on APS materials. FRET is a characterization tool commonly utilized in biological systems to determine the distance between two regions on a certain protein. Specifically, one amino acid is labeled with a fluorophore (fluorescence donor), which is a molecule that can be excited at one wavelength and subsequently emit light over a range of wavelengths. A second amino acid residue is labeled with an acceptor molecule, which quenches the fluorescence of the donor. These molecules can be thought of as oscillating dipoles that resonate at the same frequency and transfer energy through a long range dipole-dipole coupling mechanism. A specific Förster radius exists for each donor and acceptor combination; this radius is the distance between the donor and acceptor molecules where 50% of the non-radiative energy is transferred from the donor to the acceptor. Based on Equation 1, the interatomic distance between the donor and acceptor molecules can be determined from the Förster radius and the experimentally determined transfer efficiency (23). To our knowledge, the application of FRET techniques to characterize silica surfaces has not been previously reported. At a minimum, this technique should allow comparing different materials and may allow for an evaluation of the distance between surface amine groups.

Experimental

Anchoring of FRET Fluorophores to APS Silica. A 50 mL round-bottom (RB) flask and a stir-bar were flame-dried and immediately capped and stored in a dessicator. 3-Indoleproprionic acid or levulinic acid was added to the cool RB flask, which was then sealed. Nitrogen was allowed to flow through the flask for 10 min, and distilled dichloromethane (DCM; 25 mL) was added with a syringe. This solution was stirred under nitrogen at 0°C. An aliquot of a 2 M solution of oxalic acid was added with a syringe to the reaction vessel; for all runs, the amount of oxalic acid added was 1.1 eq of the reagent to be anchored. Two drops of dimethylformamide (DMF) were added to initiate the reaction. The solution was allowed to stir 3.5 hours under nitrogen at 0°C. After the reaction was complete, amine-functionalized, protected silica (200 mg) was added quickly to the flask and stirred overnight under nitrogen at room temperature. The silica product was then filtered and washed three times with DCM.

Fluorescence Resonance Energy Transfer Studies. Amine-functionalized, protected silica was prepared as previously described. 3-Indoleproprionic acid (19 mg; 0.1 mmol), a fluorescent donor, was anchored to APS modified silica (0.1 g; 0.857 mmol APS/g SiO_2) as described above to achieve 100% coverage of amine sites. This sample was made into a slurry by sonicating a 3 mg portion of the sample in methanol (3 mL). The mixture was excited at 280 nm and monitored at 300-500 nm using a PTI.Model QM-7 Fluorescence Spectrometer. Similarly, 3-Indoleproprionic acid (8.1 mg; 0.05 mmol) was anchored to APS silica material (0.1 g; 0.857 mmol APS/g SiO_2) as described above to achieve 50% coverage of amine sites. Using the same excitation and emission wavelengths as the previous sample, the fluorescence of this

sample was measured after the sample was made into a slurry as described above. For this sample, the remaining free amine sites were reacted with levulinic acid, a fluorescence acceptor, as described previously. The fluorescent intensities were compared to the previously obtained data of APS silica with 50% donor coverage for transfer efficiency calculation. The same techniques for monitoring fluorescence were performed with unmodified silica without anchored fluorescent donors or acceptors. It should be noted that for all loadings of grafted and SBA-15 APS silica, the amount of donor and acceptor to be activated and subsequently anchored was dependent on the number of amines present on the surface.

Results

Anchoring FRET Molecules to Silica Materials. Conditions for anchoring a fluorophore, 3-Indolepropionic acid, and a fluorescence acceptor, levulinic acid, were determined. The synthetic scheme reaction conditions are shown in Figure 4.1. Carboxylic acid groups on the fluorophore (A) and the acceptor (B) were activated to reactive acid chlorides (Figure 4.1), which were subsequently reacted with the primary amines on APS silica materials. All amine-functionalized silica materials reacted with the donor fluorophore molecule to produce a visibly colored material that was characterized by using reflectance UV-visible spectroscopy. The presence of the acceptor on the surface was confirmed with FT-IR spectroscopy by identifying the levulinic acid carbonyl groups (Appendix D).

Fluorescence Resonance Energy Transfer Studies. Using the conditions previously mentioned to anchor the donor and acceptor molecules to APS silica materials, various FRET experiments and controls were performed to develop this characterization method for solid materials. To determine the relationship between fluorescence intensity and donor coverage on the silica surface, a 0.86 mmol/g sample of APS grafted silica was initially reacted with 3-Indolepropionic acid (donor) to cover 50% of the primary surface amines. The fluorescence intensity was monitored. The same material was then reacted with an excess of activated donor in order to fully react the surface amines with the fluorophore. The fluorescence intensity of the solid was monitored, and Figure 4.2 shows the relationship between fluorescence intensity and the donor coverage.

The APS silica without donor (Figure 4.2, sample C) had a small baseline spectrum due to light scattering by the silica particles in the sample. This peak was insignificant in comparison to the donor loaded materials. The silica with 50% donor coverage (Sample B, Figure 4.2) provided a fluorescence intensity of 30,600 a.u., and a full coverage of donor (Sample A, Figure 4.2) sample had a fluorescence intensity of 71,500 a.u.. This experiment showed that anchored donor molecules on APS silica could be monitored by using fluorescence spectroscopy. The intensity of the 50% covered material was approximately half of the fully covered material, although slightly lower than expected (35,800 a.u.)

It was essential to determine if the acceptor molecules were affecting the donor fluorescence via stacking or excessive buildup on the surface due to the excess amounts used in the reaction. To eliminate this possibility, a 0.51 mmol/g sample of APS grafted silica material was fully loaded with 3-Indolepropionic acid and its fluorescence properties were measured. The same material was then reacted with an excess of levulinic acid and tested via fluorescence spectroscopy. These spectra showed essentially no quenching (Figure 4.3).

In FRET studies, acceptor molecules can quench the fluorescence intensity of the donor if in close proximity (i.e., approaching the Förster radius). In a control experiment, the quenching, or energy transfer efficiency, between anchored donor and acceptor molecules was monitored for three samples that had approximately 50%, 30%, and 15% of the primary surface amines reacted with the donor. For all three samples, the donor was anchored to the APS silica and the fluorescence intensity was monitored. A slight excess of acceptor molecules was reacted with each sample and the intensity was reevaluated. The transfer efficiency between

the donor and acceptor was calculated by determining the percent decrease in fluorescence intensity when the acceptor was added. Figure 4.4 shows the trend in quenching for the three samples; the intensities were 55,000, 45,000, and 33,000 fluorescence units before acceptor addition and 24,000, 31,000, and 31,000 fluorescence units after acceptor addition for the materials with 50%, 30%, and 15% donor coverage, respectively. It was found that the greatest degree of fluorescence quenching occurred with materials that were densely covered with donor fluorophores, and low coverage of donor resulted in minimal quenching by the acceptor.

FRET was applied to various APS silica materials, including grafted Davisil and SBA-15 materials, and quenching was monitored to estimate the average inter-amine distance on the surface. Specifically, the 0.86 mmol/g material of Figure 4.2 was tested. A sample of this APS material was reacted with donor to cover 50% of the surface amines. After fluorescence measurements, the remaining amine sites were reacted with activated levulinic acid. Figure 4.5 shows the fluorescence intensity of the silica material before acceptor addition (A) and after the acceptor anchoring (B). A 23% energy transfer was observed based on the quenching of the donor's fluorescence. This transfer efficiency and the Förster radius (R_0) of the donor-acceptor pair, 0.626 nm, were evaluated with Equation 1 to determine an average distance (r) between the anchored donor and acceptor molecules. The calculated average distance was 0.77 nm, whereas the expected value for the distance between amine sites was 0.95 nm based on an idealized hexagonal arrangement of deposited amines over the entire surface of the material. Assuming such an arrangement based on the silica surface area provides a maximum average distance between sites.

This was a proof-of-concept experiment that showed FRET could be utilized as a spectroscopic ruler for evaluating relative amine-amine distances on solid supports. FRET analysis was applied to SBA-15 materials of various loadings to determine if this characterization tool could differentiate between slightly different loadings and distances between supported amines. The experiment was similar to the previous experiment, for 50% of the amines on various SBA-15 materials (1.3 and 1.6 mmol/g) were reacted with activated donor and subsequently with acceptor, fluorescence was measured, and quenching was monitored. Figure 4.6 provides a comparison of the expected average amine to amine distances based on surface area and the experimental distances calculated using Equation 1; the results again show that the idealized maximum distance is not reached. These values can be better compared in Table 4.1 where it is evident that an increase in SBA-15 APS loading from 1.3 to 1.6 mmol/g resulted in a large increase in the donor's fluorescence quenching. Specifically, the transfer efficiency between the acceptor and donor for the 1.3 mmol/g SBA-15 material was 29%, which is significantly less than the 51% quenching of the 1.6 mmol/g SBA-15 material.

The same FRET experiment was conducted for a highly loaded APS grafted Davisil silica material (4.5 mmol/g). It was determined that there was an estimated 0.59 nm average distance, which was much greater than the expected value of 0.30 nm. This distance is similar to the low loaded SBA-15 average distance of 0.62 (Table 4.1). Therefore, this experiment may indicate that there is a lower limit where the donor and acceptor molecules must maintain a certain distance due to steric hindrance of the molecules.

FRET Studies of Micelle Templated APS Silica Materials. Three micelle templated materials were prepared. The materials varied in their ratio of amine encapsulation, for there was either a 1:1 ratio of micelles to silane of a 1:0.6 ratio. In addition, the amount of micelles adsorbed to the surface of the silica was varied between 100% monolayer coverage to 60% monolayer coverage (Table 4.2). Micelle templated, alkylated APS Davisil silica (Figure 1.4) was reacted initially with 3-Indolepropionic acid (donor) to cover 50% of the primary surface amines. The fluorescence intensity of a 10mg/3mL slurry was then measured. The same material was then reacted with an excess amount of activated donor in order to fully react the remaining free surface amines with the acceptor. The fluorescence intensity of the solid was measured, and Table 4.2 provides the transfer efficiency data from the FRET experiment for the three micelle templated materials and one comparably grafted APS material.

The quenching results of the FRET experiment for the micelle materials indicated that there was comparable quenching with that of the grafted APS material even though it was expected that the amines would be significantly farther apart for the micelle templated material.

Discussion

Fluorescence resonance energy transfer (FRET) is a sensitive characterization tool commonly used to probe distances between donor fluorophores and acceptor molecules in biological systems. This technique involves nonradiative energy transfer from the excited donor to the acceptor and thus depends on the dipole-dipole interaction of the two molecules in close proximity. Furthermore, FRET is strongly dependent on the interatomic distance between the molecules (23). This chapter presents FRET as a characterization tool for anchored donor and acceptor molecules on silica surfaces for method development to determine an average interamine distance for amine-functionalized silicas.

3-Indolepropionic acid serves as the anchored donor used in all FRET studies for APS silicas because indole fluorescence quenching has been studied extensively for dynamic protein systems. In addition, the indole moiety is easily anchored and has a wide range of acceptor molecules compatible with the proposed FRET experiments on silica. The donor-acceptor pair involving indolepropionic acid and levulinic acid has a Förster radius (R_0) of 0.626 nm, which is the distance at which 50% energy-transfer occurs, and such a distance is ideal for comparing grafted and SBA-15 APS materials. Our research group has proposed that if the donor and acceptor are anchored appropriately, the average distance between the adjacent donor and acceptor molecules (i.e., between the adjacent surface amines) can be determined based on the experimental transfer efficiency (Equation 1).

The anchoring reaction of the donor was assessed by measuring the fluorescence of a silica sample where 50% of the surface amines were reacted with donor; the remaining amines were then reacted with acceptor and fluorescence was measured. The results indicate that the 50% amine reacted material fluoresced at an intensity slightly lower than 50% of the 100% reacted material's intensity. This might indicate that not all of the activated donor molecules reacted with the primary surface amines. Although precaution was taken to decrease the activity of the acid chloride donor and acceptor intermediates (e.g., by decreasing the temperature to 0˚C), the acid chlorides react rapidly in the presence of water. In FRET analysis, the actual amount of donor present is less important than the relative decrease in fluorescence intensity after addition of an acceptor, so this unavoidable deviation from the expected fluorescence intensity was insignificant (23). This experiment provided the evidence that the donor can be anchored to the surface and reasonable fluorescence measurements of the solid can be collected with minimal light scattering.

Experiments were designed to determine if fluorescence quenching results from phenomena other than the dipole-dipole interactions of the anchored donor and acceptor molecules. In particular, the study of which the donor was reacted completely with all available surface amines provided evidence that the donor is completely immobilized, for excess addition of levulinic acid to fully reacted material resulted in no fluorescence quenching, as shown in Figure 4.3. If hydrolysis of the anchored donor's amide bond occurred, it is expected that reaction with excess acceptor would result in significant quenching. This finding also eliminates the possibility that quenching occurs due to the anchoring of donor and acceptor molecules to

the same amine. Another experiment was designed to determine the effects of donor coverage on fluorescence quenching. The data, shown in Figure 4.4, indicate that there is a minimum limit of donor in order to observe quenching effects of an acceptor. This experiment highlights the importance of maintaining the same donor coverage for all APS silica materials when using FRET analysis as a comparison tool. In addition, this experiment may also suggest that energy transfer efficiencies can only be used to compare the isolation of various materials for differences in site isolation. However, the remaining FRET studies were all preformed at 50% donor coverage of APS silica, and all estimated distances were calculated as averages with the purpose of material comparison.

This FRET method was applied to grafted, SBA-15, and micelle-templated APS material in order to compare relative amine distances based on APS synthetic modification technique used. Table 4.1 provides two SBA-15 APS samples and two APS grafted samples with different amine loadings that were reacted similarly with donor (50%) and acceptor (excess); regardless of silica type, the experimental transfer efficiencies indicated that the lower loaded materials had amine groups that were better isolated than the materials with high loadings. This was a proof-of-concept experiment that provided strong evidence that FRET is capable of assessing the isolation of amine sites on different silica types based on fluorescence quenching.

Table 4.1 indicates that the SBA-15 material was generally well isolated in comparison to the grafted material. Specifically, an APS SBA-15 material with a loading of 1.3 mmol/g and an APS grafted silica with loadings of 0.87 mmol/g showed similar fluorescence quenching; thus, SBA-15 can achieve higher surface loadings with greater separation of groups than that of

grafted material. One result that may indicate FRET limitations is that of the 4.5 mmol/g loaded APS grafted material, for the transfer efficiency was comparable to that of the SBA-15 material. This was an unexpected result due to the large difference in expected distance between adjacent amines based on surface area measurements. Thus, it can be assumed that at small distances between the amines there is a limit of how close the donor and acceptor molecules can be anchored due to steric hindrance. In particular, for the grafted sample of interest, the expected distance was 0.3 nm, which is significantly smaller than the Förster radius of the donor-acceptor pair, and is smaller than the size of the individual indole moiety.

Micelle-templated materials were prepared by varying the amine:micelle ratio and micelle monolayer (ML) coverage on silica, and the FRET studies (Table 3.1) provided evidence that the lower loaded material had better site-isolation than the higher loaded material. However, for all micelle-templated material, the FRET studies show similar results to that of comparably loaded APS grafted material. This may suggest that the micelle templating scheme needs to be further characterized to determine confidently that the micelles are encapsulating the silane. It appears that either the deposition of the APS in the presence of micelles is similar to that of grafting techniques or that there are limitations to the FRET technique when working with loadings as low as seen in this experiment. In fact, the expected amine loadings were significantly less than the materials normally tested, as evidenced by the loading differences in Tables 4.1 and 4.2. In order to increase the probability of silane encapsulation, a silane molecule, such as iodopropyltriethoxy silane, could be deposited and subsequently converted to a primary amine via ammonia displacement of the active center. Other possible changes

include the use of a smaller surfactant; using a smaller surfactant would decrease the micelle diameter and increase the micelle monolayer capacity of silica. This effect would significantly increase the amount of silanes molecules capable of being deposited without increasing the ratio of silane:micelle.

A

B

Figure 4.1. Synthetic scheme and reaction conditions for activating a donor fluorophore, 3-Indolepropionic acid (A) and a fluorescence accepting molecule, levulinc acid (B) to acid chloiride species. These activated molecules were added to amine-functionalized silica for anchoring purposes.

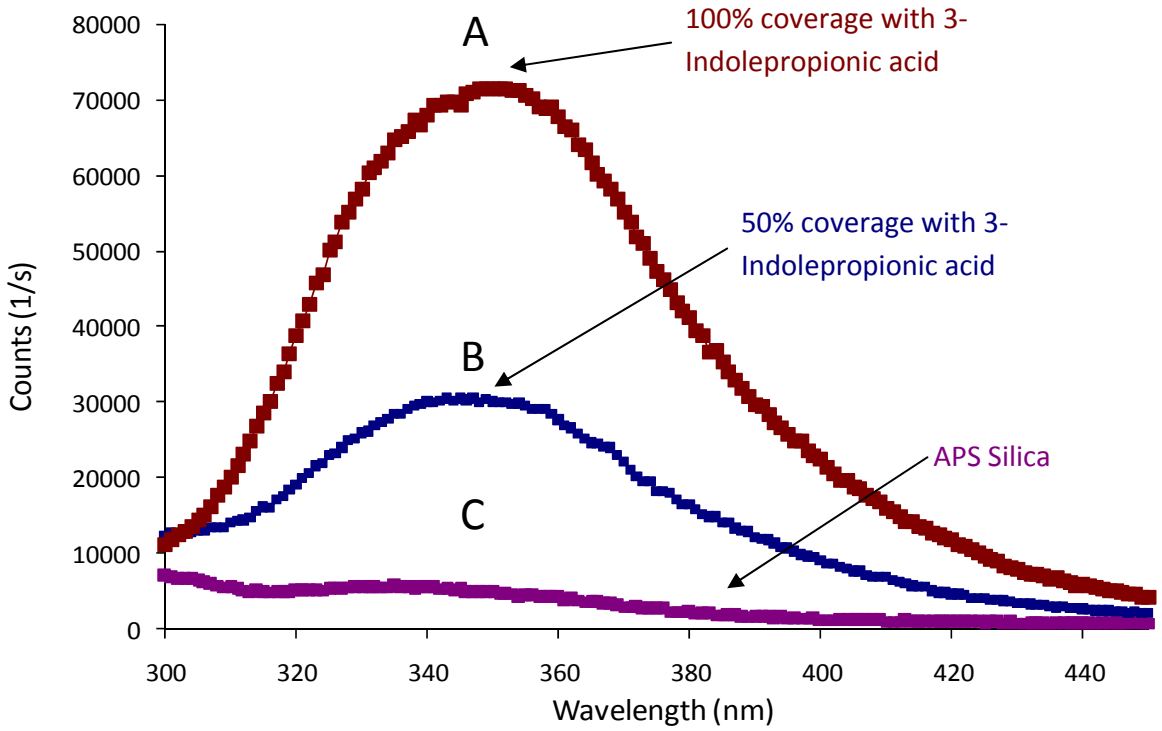


Figure 4.2. Fluorescence spectra of APS modified grafted Davisil silica of 0.857 mmol APS/g loading when 100% (A), 50% (B), and 0% (C) of the amine sites are reacted with the fluorescent donor, 3-Indolepropionic acid.

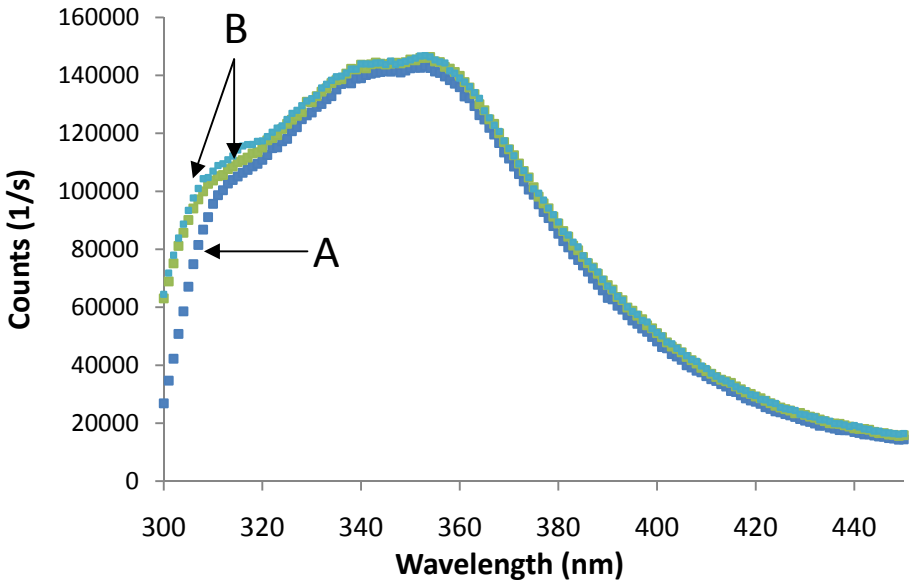


Figure 4.3. Fluorescence spectra of 100% donor covered APS grafted silica material (0.514 mmol/g) before (A) and after (B) the addition of excess activated acceptor (levulinic acid).

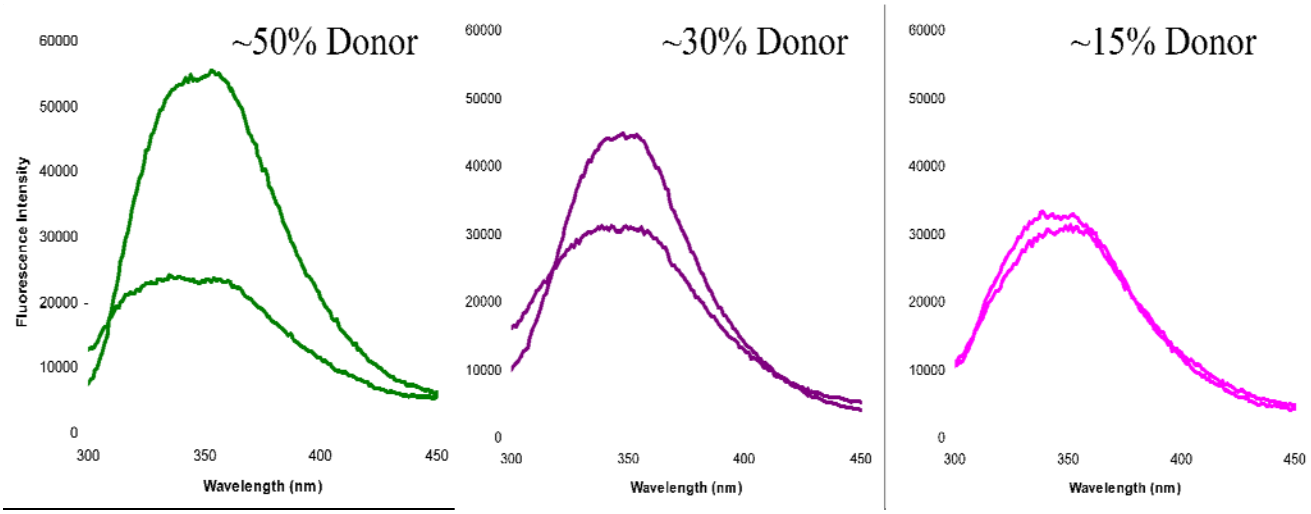


Figure 4.4. Quenching of different anchored 3-Indolepropionic acid loadings on APS silica materials by an acceptor, levulinic acid. The fluorescence intensities of three APS grafted silica materials with donor coverages of 50%, 30%, and 15% were monitored before and after the anchoring of levulinic acid. For all samples, the peak of lesser intensity represents the trial after acceptor addition.

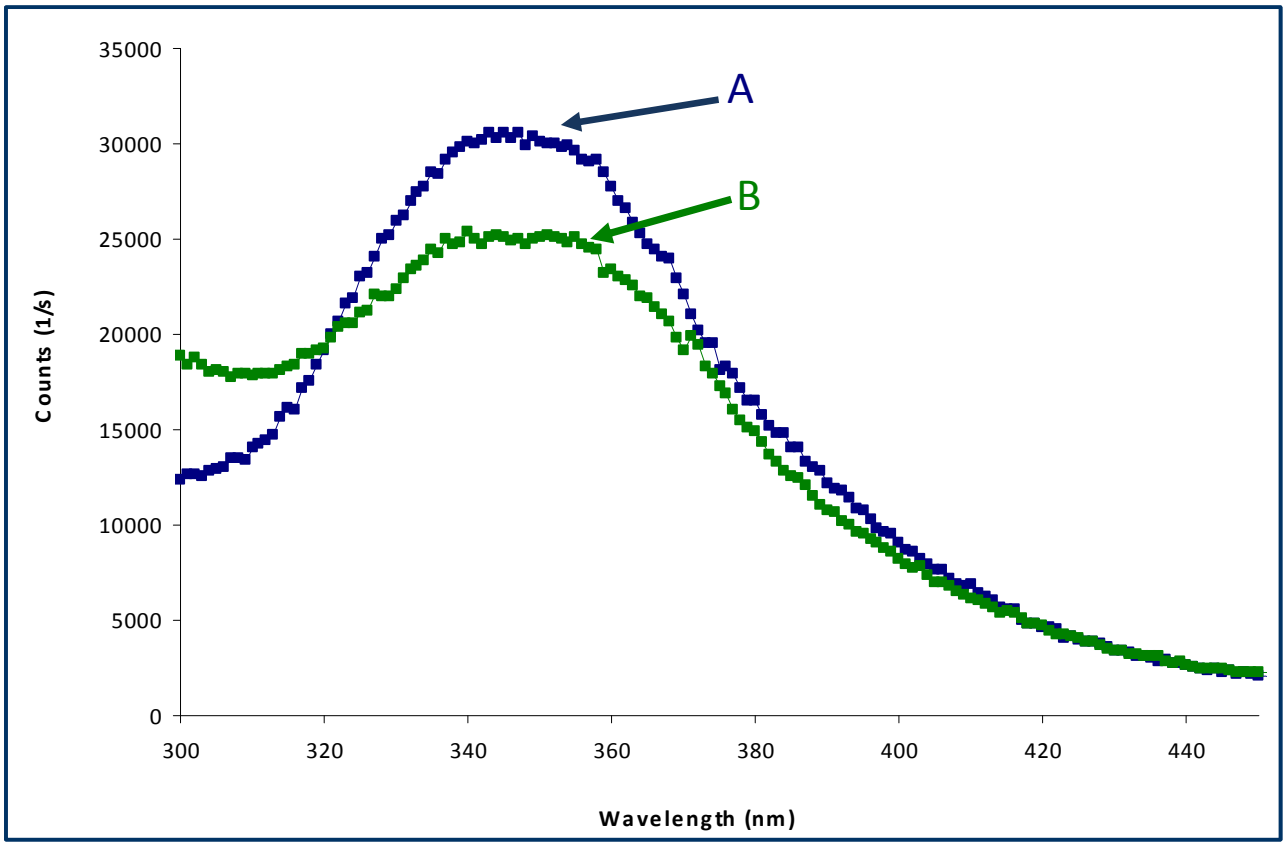


Figure 4.5. Fluorescence Resonance Energy Transfer Study. APS modified silica of 0.857 mmol APS/g SiO_2 loading was reacted with fluorescence donor, 3-indolepropionic acid, for 50% coverage of amine sites (A) and subsequently reacted with an excess of a fluorescence acceptor, levulinic acid (B). Transfer efficiency between the donor and acceptor molecules was determined based on fluorescence quenching and applied to Equation 1 for calculation of the donor to acceptor average interatomic distance (r).

	Amine Loading (mmol/g)	Transfer Efficiency (%)	Distance Estimate (nm)	Ideal / Expected Distance (nm)
SBA-15	**1.3**	**29%**	**0.73**	**0.96**
SBA-15	**1.6**	**51%**	**0.62**	**0.84**
Grafted Davisil	**4.5**	**58%**	**0.59**	**0.30**
Grafted Davisil	**0.87**	**23%**	**0.77**	**0.95**

Table 4.1. FRET Estimations of Average Amine to Amine Distances for Various Materials. All expected values were determined based on the material surface area.

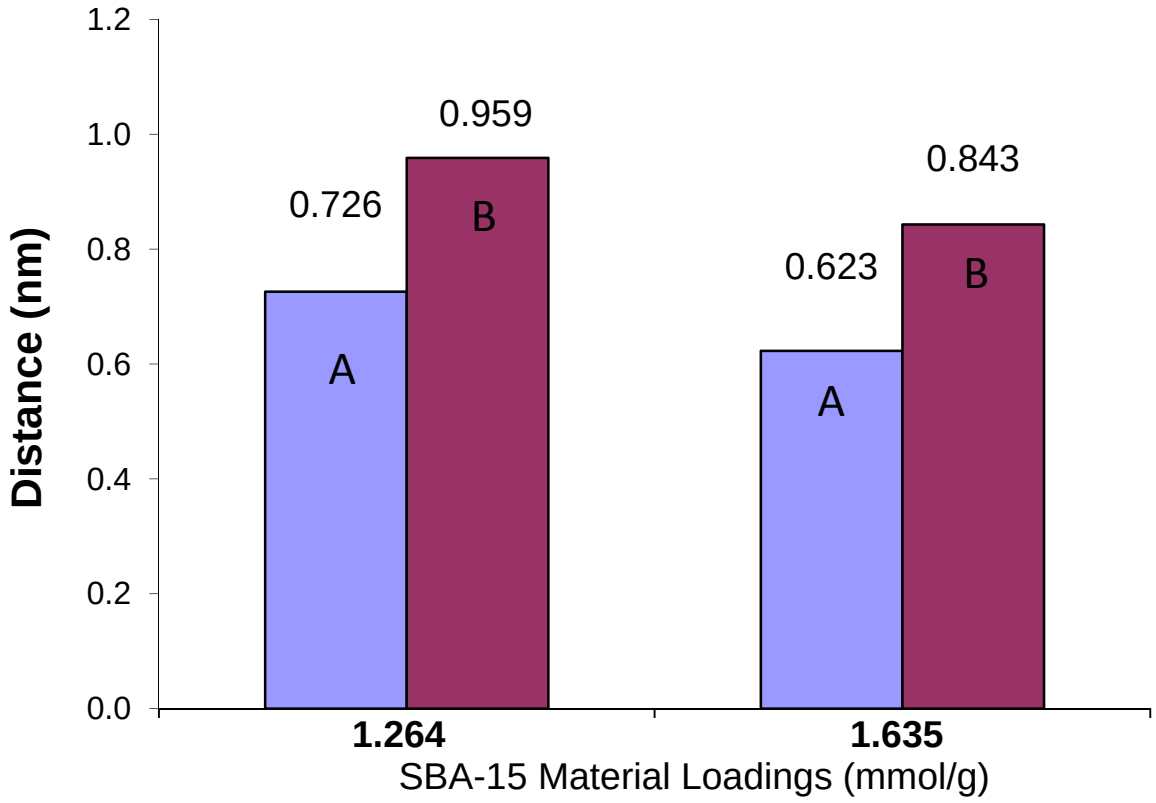


Figure 4.6. FRET Estimated Amine-Amine Average Distances (A) Compared to Maximum Expected Average Distances (B) of Various Loaded SBA-15 Materials (1.3 and 1.6 mmol/g). Expected distances are based on the surface area of the material.

Expected Loading[a] (μmol/g)	APS/micelle	Micelle ML Coverage	Trans. Efficiency[c] (%)
10	1.0/1.0	100%	66%
5.9	0.6/1.0	100%	57%
5.9	1.0/1.0	60%	59%
10[b]	--	--	57%

Table 4.2. Fluorescence Resonance Energy Transfer Studies on Micelle Templated APS Davisil Silica and Comparable Grafted APS Silica. Micelle templated APS silica materials are indicated by shading. [a]The expected loadings were determined from synthesis [b]Grafted material of comparable loading to the micelle material. [c]All quenching measurements were determined using fluorescence spectroscopy.

Applications in Heterogeneous Catalysis

Silicas have become widely used in the synthesis of organic—inorganic catalysts. Mesoporous SBA-15 is well suited to be a support for immobilized organized functional groups. The large uniform pore diameter provides sufficient room for reactant and product diffusion, thick walls provide hydrothermal stability, and rigidity of the silica matrix ensures that the bound functional groups do not change their positioning (27). Isolated sites on silica material could allow for the deposition of catalyst molecules.

Davis et al. have shown that there must be interaction between their anchored acid and base functional groups (e.g., between thiols and sulfonic acid) to achieve high activity and selectivity (27). Thus, there is a balance between spacing the two interacting functional groups a certain distance from each other. Our research group has proposed anchoring multiple functional groups on a single silane to achieve ideal interactions, and isolated sites on a silica support would ensure isolation of catalyst molecules, full cooperation between moieties on individual catalysts, and eliminate the disadvantageous interactions between adjacent catalyst molecules. Further studies include testing the catalytic activity of SBA-15, grafted, and micelle-templated APS material with anchored bifunctionalized catalyst molecules for determining the advantages of site-isolation.

Conclusion

A relatively simple, fast assay for accessible surface amines, which can be readily applied without scrupulously excluding water (e. g. with glove box or schlenk techniques), was developed by using ninhydrin to oxidize an anchored primary amine and cleave the C-N bond. The ninhydrin assay was generally in good agreement with known values for amines grafted onto commercial silicas and mesoporous SBA-15 materials that had amines incorporated into the mesopores during synthesis. Ninhydrin assays of the SBA-15 materials also distinguished between functional and unreactive amines and highlighted the importance of a base treatment after polymer removal if active amines are desired. This assay will aid in future characterization schemes and will provide a rapid means of evaluating catalytic activity on a per amine basis.

Fluorescence resonance energy transfer (FRET) method development for APS silica has been applied for the first time to grafted, SBA-15, and micelle-templated APS silica. SBA-15 material was determined to have isolated amine sites in comparison to grafted and micelle-templated APS silica. This novel characterization tool was determined to be capable of distinguishing between different materials based on amine-amine average distances.

References

1. McKittrick, M.W., Jones, C.W. *Chem. Mater.* 15 (2003): 1132-1139.
2. Hicks, J.C., Drese, J.H., Fauth, D.J., Gray, M.L., Qi, G., Jones, C.W. *J. Am. Chem. Soc. ASAP Communication*, 2008.
3. Taylor, I., Howard, A.G. *Analytica Chimica Acta* 271 (1993): 77.
4. McKittrick, M.W., Jones, C.W. *J. Am. Chem. Soc.* 126 (2004): 3052.
5. Yu, K., McKittrick, M.W., Jones, C.W. *Organometallics* 23 (2004): 4089.
6. McKittrick, M. W.; Jones, C. W. *Chem. Mater.* 17 (2005): 4758.
7. Dufaud, V., Davis, M.E. *J. Am. Chem. Soc.* 125 (2003): 9403.
8. Mbaraka, I., Shanks, B.H. *Journal of Catalysis* 2005 (2005): 365.
9. Hicks, J.C., Jones, C.W. *Langmuir* 22 (2006): 2676.
10. Katz, A., Davis, M.E. Nature 403 (2000): 286.
11. Walcarius, A., Etienne, M., Lebeau, B. *Chem. Mater.* 15 (2003): 2161.
12. McKittrick, M. W.; McClendon, S. D.; Jones, C. W. Chemical Industries, 104 *Catal. Org. React*. (2005): 267.
13. Paruchuri, V., Fa, K., Moudgil, B., Miller, J. *Applied Spectroscopy* 59 (2005): 668.
14. Harada, S., Fujita, N., Sano, T. *J. Am. Chem. Soc.* 110 (1988): 8710.
15. Treiner, C., Makayssi, A. *Langmuir* 8 (1992): 794.
16. Khamis, M., Bulos, B., Jumean, F., Manassra, A., Dakiky, M. *Dyes and Pigments* 66 (2005): 179.
17. Imae, T., Kamiya, R., Ikeda, S. *Journal of Colloid and Interface Science* 108 (1995): 215.
18. Jiang, B., Du, J., Cheng, S., Wang, Q., Zeng, X. *Journal of Dispersion Science and Technology* 24 (2003): 755.

19. Eskilsson, K., Yaminsky, V. *Langmuir* 14 (1998): 2444.

20. Phillies, G. *Structure and Flow in Surfactant Solutions* 578 (1994): 239.

21. Singh, H.. *Colloids and Surfaces* 50 (1990): 37.

22. Basurto, S., Torroba, T., Comes, M., Martinez-Manez, R., Sanceno, F., Villaescusa, L., Amoro, P. *Org. Lett.* 7 (2005).

23. Lakowicz, Joseph R. *Principles of Fluorescence Spectroscopy.* 3rd Edition. Springer, 2006.

24. Eftink, M., Ghiron, C. *Biochemistry* 23 (1984): 3891.

25. Clapp, A.R., Medintz, I.L., Mauro, J., Fisher, B. *J. Am. Chem. Soc.* 126 (2004): 301.

26. Han, L., Ruan, J., Li, Y., Terasaki, O., Che, S. *Chemical Materials*, 2007.

27. Zeidan, R., Davis, M.E. *Journal of Catalysis* 247 (2007): 379.

28. Fuerte, A.; Corma, A.; Sanchez, F. *Catal. Today.* 107 (2005): 404.

29. Gruttadauria, M.; Riela, S.; Lo Meo, P.; D'Anna, F.; Noto, R. *Tet. Lett.* 45 (2004): 6113.

30. Bass, J. D.; Anderson, S. L.; Katz, A. *Angew. Chem. Intl. Ed.* 42 (2003): 5219.

31. Motokura, K.; Tada, M.; Iwasawa, Y. *J. Am. Chem. Soc.* 129 (2007): 9540.

32. Zeidan, R. K.; Hwang, S.-J.; Davis, M. E. *Angew. Chem. Intl. Ed.* 45 (2006): 6332.

33. Hoang, V., Huang, Q., Eic, M., Do, T., Kaliaguine, S. *Langmuir* 21 (2005): 2051.

34. Wang, X.; Lin, K. S. K.; Chan, J. C. C.; Cheng, S. *J. Phys. Chem. B.* 109 (2005): 1763.

35. Chong, A. S. M.; Zhao, X. S. *J. Phys. Chem. B.* 107 (2003): 12650.

36. Smart, J. L.; McCammon, J. A. *J. Am. Chem. Soc.* 118 (1996): 2283.

37. Bass, J. D.; Solovyov, A.; Pascall, A. J.; Katz, A. Smart, J. L.; McCammon, J. A. *J. Am. Chem. Soc.* 128 (2006): 3737.

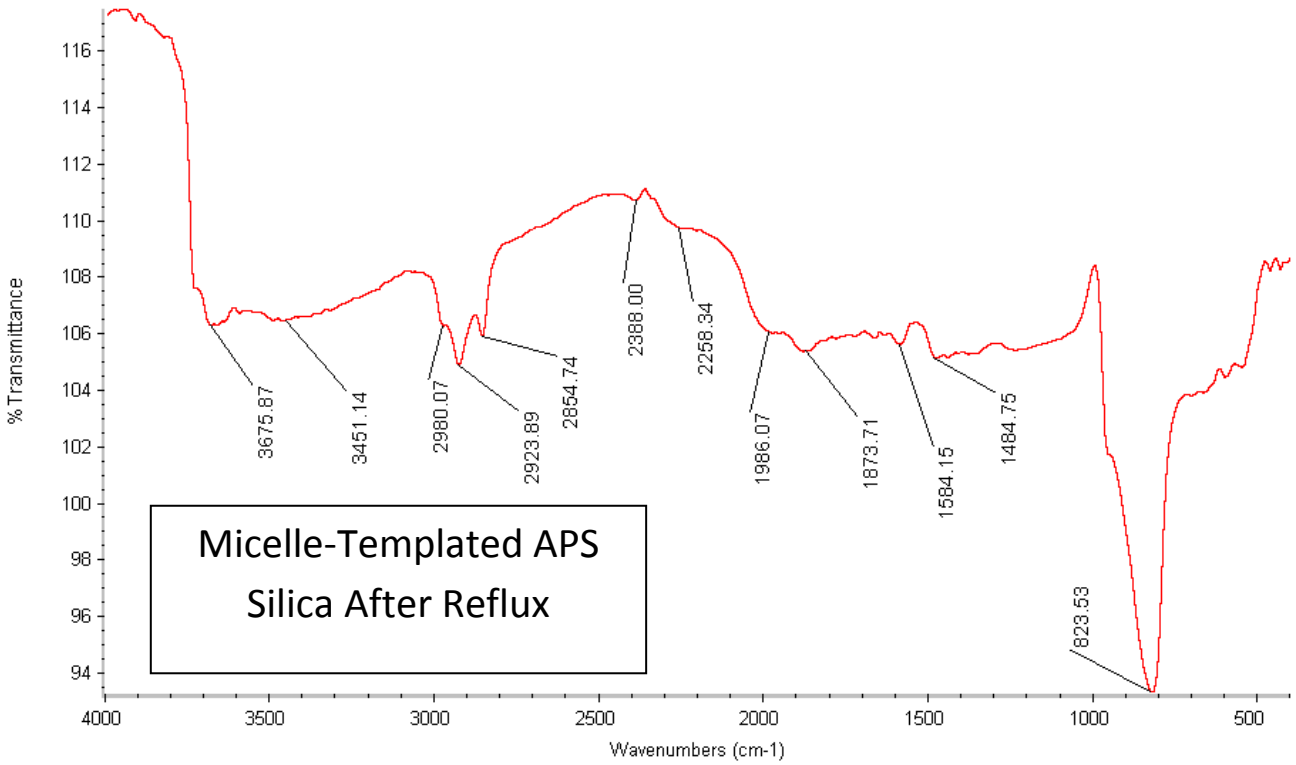
38. Eskilsson, K., Yaminsky, V.V. *Langmuir* 14 (1998): 2444.

39. Heitmann, L., Taylor, A., Hart, J., Urbach, A. *J. Am. Chem. Soc.* 128 (2006): 12574.

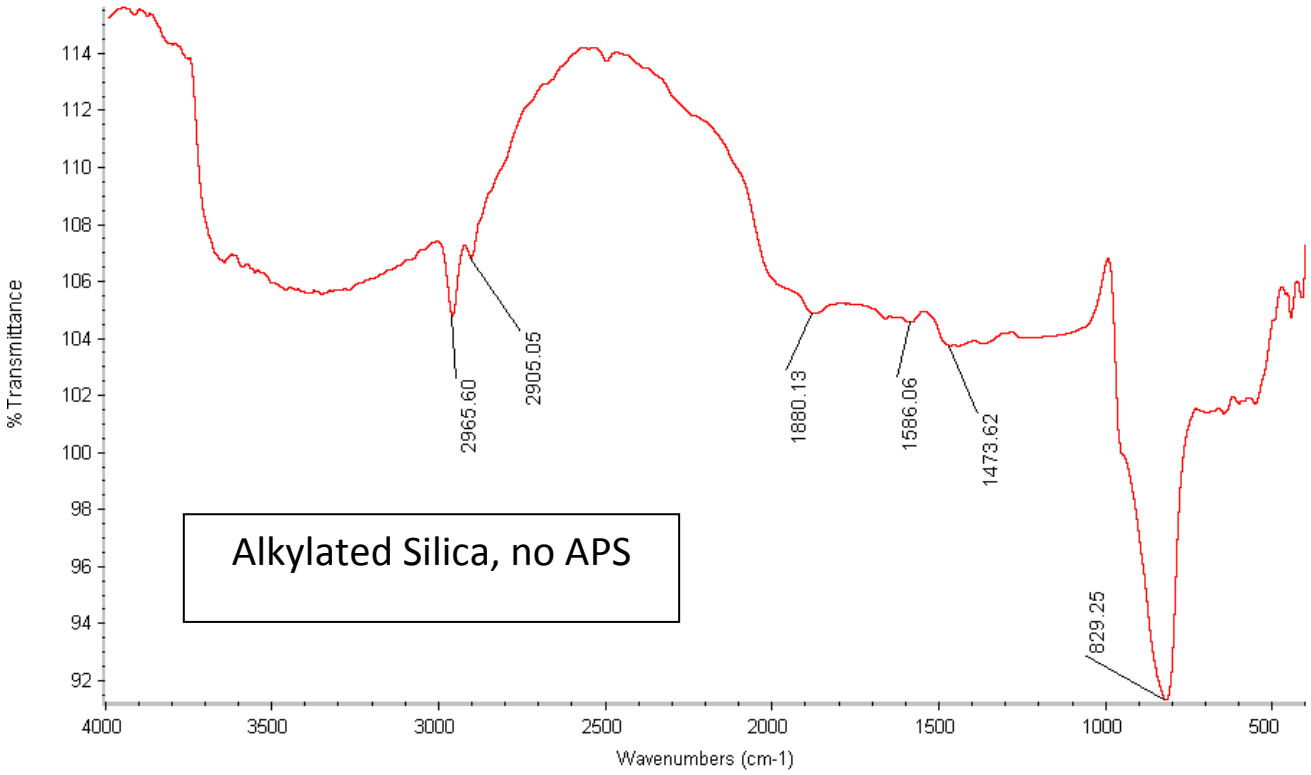
40. Goyal, P., Dasannacharya, B., Kelkar, V., Manohar, C., Srinivasa, K., Valaulikar, B. *Physica B*. 174 (1991): 196.

Appendix A

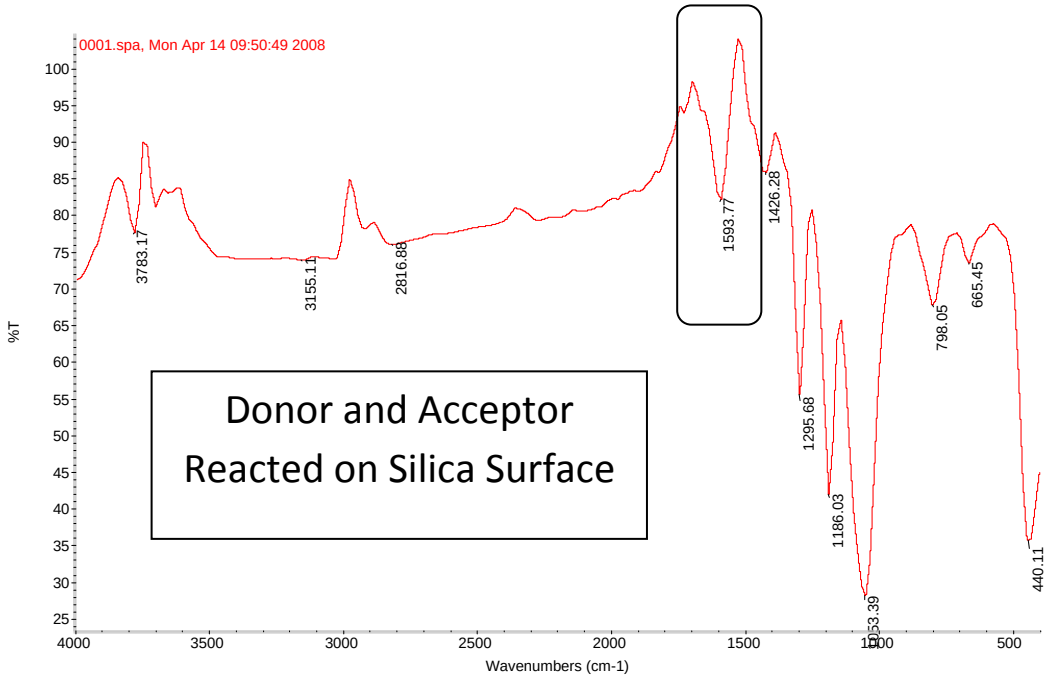
Appendix B

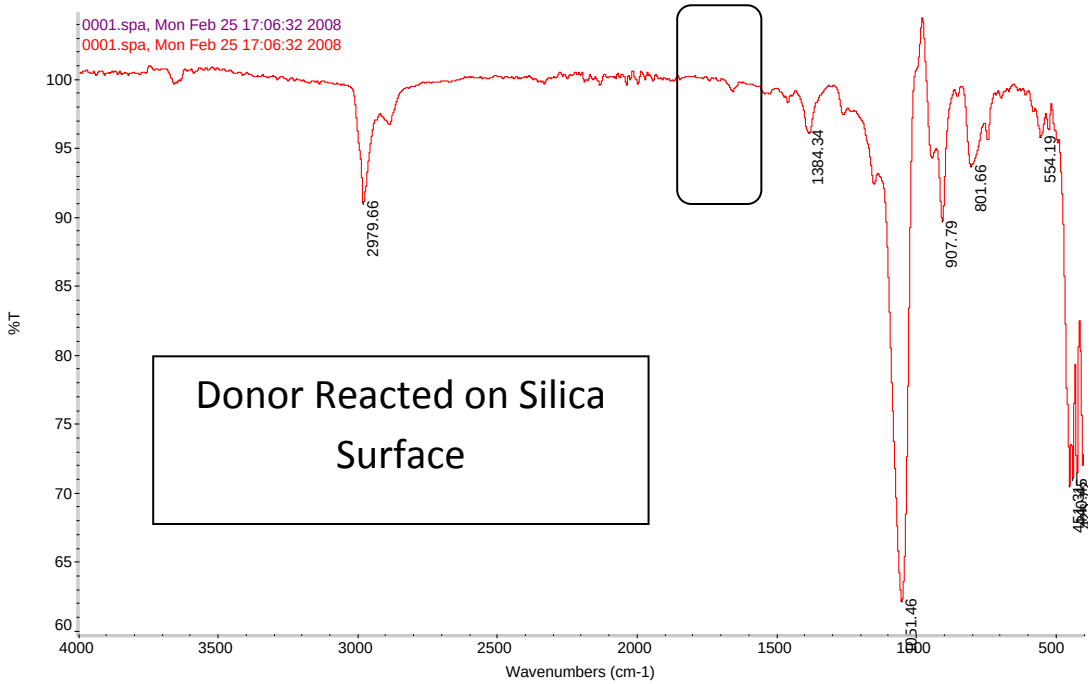

% Transmittance
116
114
112
110
108
106
104
102
100
98
96
94
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)
3675.87
3451.14
2980.07
2923.89
2854.74
2388.00
2258.34
1986.07
1873.71
1584.15
1484.75
823.53
Micelle-Templated APS
Silica After Reflux

Appendix C


% Transmittance
114
112
110
108
106
104
102
100
98
96
94
92
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)
2965.60
2905.05
1880.13
1586.06
1473.62
829.25
Alkylated Silica, no APS

Appendix D


0001.spa, Mon Apr 14 09:50:49 2008
%T
Wavenumbers (cm-1)
3783.17
3155.11
2816.88
1593.77
1426.28
1295.68
1186.03
1053.39
798.05
665.45
440.11
Donor and Acceptor
Reacted on Silica Surface


0001.spa, Mon Feb 25 17:06:32 2008
0001.spa, Mon Feb 25 17:06:32 2008
%T
Wavenumbers (cm-1)
2979.66
1384.34
1051.46
907.79
801.66
554.19
Donor Reacted on Silica
Surface