Title
Macromolecular coatings on porous silicon: applications in drug delivery, biosensing, and composites

Permalink
https://escholarship.org/uc/item/5rw07745

Author
Perelman, Loren Avery

Publication Date
2008

Peer reviewed|Thesis/dissertation
MACROMOLECULAR COATINGS ON POROUS SILICON:
APPLICATIONS IN DRUG DELIVERY, BIOSENSING, AND
COMPOSITES

A Dissertation submitted in partial satisfaction of the requirements for the degree
Doctor of Philosophy

in

Materials Science and Engineering

by

Loren Avery Perelman

Committee in charge:

Professor Michael J. Sailor, Chair
Professor Seth Cohen
Professor Sungho Jin
Professor Yu-Hwa Lo
Professor Jan Talbot

2008
The Dissertation of Loren Avery Perelman is approved, and it is acceptable in quality and form for publication on microfilm:

Chair

University of California, San Diego

2008
Dedication

For Elise and Cassius
# Table of Contents

Signature Page.................................................................................................iii

Dedication........................................................................................................iv

Table of Contents.............................................................................................v

List of Figures....................................................................................................x

List of Tables.....................................................................................................xiii

Acknowledgements..........................................................................................xiv

Vita....................................................................................................................xvi

Publications........................................................................................................xvii

Abstract of the Dissertation............................................................................xviii

## CHAPTER 1 INTRODUCTION TO APPLICATIONS OF POROUS SILICON……..1

1.1 INTRODUCTION TO POROUS SILICON................................................2

1.2 OVERVIEW OF MACROMOLECULE-POROUS SILICON
INTERACTIONS.................................................................................................5

1.3 DRUG RELEASE FROM POROUS SILICON FILMS..............................5

1.4 BIOLOGICAL AND CHEMICAL SENSING USING POROUS
SILICON-BASED OPTICAL INTERFEROMETERS.........................................7

1.5 POLYMER/POROUS SILICON COMPOSITES.......................................11

1.6 CRITERIA OF PREPARATION FOR POROUS SILICON......................12

1.7 REFERENCES.........................................................................................13

## CHAPTER 2 pH-TRIGGERED RELEASE OF VANCOMYCIN FROM PROTEIN-
CAPPED POROUS SILICON FILMS.................................................................16

2.1 ABSTRACT.................................................................................................17

2.2 INTRODUCTION.......................................................................................18

2.3 EXPERIMENTAL......................................................................................19

2.3.1 Porous Si sample preparation............................................................19

2.3.2 Characterization of porous Si films.................................................20

2.3.3 Drug loading in porous Si films.......................................................23

2.3.4 Protein coating..................................................................................23

2.3.5 Optimization of coating protocol....................................................24
2.3.6 Collection of drug release samples..............................................25  
2.3.7 High pressure liquid chromatography (HPLC) analysis..............26  
2.3.8 Light scattering analysis.....................................................26  

2.4 RESULTS AND DISCUSSION...........................................................26  
2.4.1 FTIR analysis of vancomycin loaded into freshly-etched porous Si .................................................................26  
2.4.2 Characterization of porous silicon film thickness and porosity and drug capacity.........................................................26  
2.4.3 Drug capacity of porous films..................................................29  
2.4.4 Characterization of vancomycin release from uncoated porous silicon films..............................................................36  
2.4.5 Placement of a protein capping layer on drug-loaded films........37  
2.4.6 Flow cell experiments............................................................38  
2.4.7 pH-triggered release of vancomycin from protein-coated Si-H films.............................................................................38  
2.4.8 Measurement of removal of the protein coating by light scattering..............................................................................44  
2.4.9 Mechanism of drug release from protein-coated porous Si-H Films..................................................................................46  

2.5 CONCLUSIONS..................................................................................48  

2.6 REFERENCES...................................................................................49  

CHAPTER 3 QUANTITATIVE BIOSENSING OF VANCOMYCIN USING A BSA-COATED POROUS SILICON OXIDE INTERFEROMETER FUNCTIONALIZED WITH Ac-L-Lys-D-Alanine-D-Alanine.................................................................54  

3.1 ABSTRACT...........................................................................................55  
3.2 INTRODUCTION..................................................................................55  
3.3 EXPERIMENTAL..................................................................................57  
3.3.1 Preparation of porous Si..............................................................57  
3.3.2 Oxidation of porous Si.................................................................57  
3.3.3 BSA loading..................................................................................58  
3.3.4 Measurement of refractive indices............................................58  
3.3.5 Gravimetric determination of porosity......................................58  
3.3.6 Optical measurement of thickness and porosity......................59  
3.3.7 Determination of BSA content....................................................59  
3.3.8 Synthesis of Ac-L-Lysine-D-Alanine-D-Alanine-OH.................60  
3.3.9 Synthesis of Ac-L-Lysine-D-Alanine-D-Lactone-OH...............63  
3.3.10 Functionalization of BSA-coated porous SiO$_2$ surfaces..........63  
3.3.11 Flow cell experiments..............................................................63  
3.3.12 Reflectometric spectroscopy.....................................................64  
3.3.13 Obtaining optical thickness....................................................64  
3.3.14 Determination of equilibrium binding constants...................65  

3.4 RESULTS AND DISCUSSION.............................................................66
3.4.1 Porous SiO₂ interferometric biosensor preparation and characterization…………………………………………………………66
3.4.2 Porous SiO₂ preparation…………………………………………66
3.4.3 BSA adsorption to porous SiO₂ surface…………………………66
3.4.4 Characterization of porous SiO₂ and BSA-loaded porous SiO₂ porosity and thickness………………………………………………….70
   3.4.4.1 Gravimetric determination of porosity…………………70
   3.4.4.2 Spectroscopic liquid infiltration method (SLIM) analysis………………………………………………...71
3.4.5 Functionalization of BSA adsorbed onto the porous SiO₂ surface…………………………………………………………71
3.4.6 Theoretical consideration of vancomycin loading in BSA-KAA samples…………………………………………………………72
3.4.7 Optical analysis of binding events within porous SiO₂ samples…74
3.4.8 Binding of vancomycin to functionalized BSA-coated porous SiO₂ surfaces……………………………………………………75
3.4.9 Optical response of BSA-coated porous SiO₂ interferometer functionalized with KAA……………………………………………79
3.5 CONCLUSIONS…………………………………………………………...83
3.6 REFERENCES……………………………………………………………..84

CHAPTER 4 CONFINEMENT OF THERMORESPONSIVE HYDROGELS IN NANOSTRUCTURED POROUS SILICON DIOXIDE TEMPLATES……………89

4.1 ABSTRACT………………………………………………………………..90
4.2 INTRODUCTION………………………………………………………….90
4.3 EXPERIMENTAL…………………………………………………………92
   4.3.1 Materials………………………………………………………….92
   4.3.2 Etching procedure………………………………………………..93
   4.3.3 Thermal oxidation………………………………………………..93
   4.3.4 Preparation of poly(NIPAM) and porous SiO₂/poly(NIPAM) hybrids…………………………………………………………94
   4.3.5 Scanning electron microscopy……………………………………94
   4.3.6 Gravimetric determination of porosity…………………………95
   4.3.7 Determination of surface area and pore dimensions using the BET method…………………………………………………………95
   4.3.8 Measurement of interferometric reflectance spectra…………………96
   4.3.9 Determination of porosity and film thickness by spectroscopic liquid infiltration method (SLIM).……………………………………97
   4.3.10 Determination of optical thickness……………………………...98
4.4 RESULTS AND DISCUSSION………………………………………………98
   4.4.1 Preparation of porous SiO₂/hydrogel hybrids……………………98
   4.4.2 Characterization of porosity and thickness of porous SiO₂ templates……………………………………………………………………101
   4.4.3 Spectroscopic liquid infiltration method (SLIM) for
determination of thickness and porosity……………………………………..103
4.4.4 Comparison of gravimetric measurement with SLIM for porosity determination…………………………………………………..103
4.4.5 BET measurements of porous SiO$_2$ templates………………………….104
4.4.6 In-situ synthesis of poly(NIPAM) in porous SiO$_2$ templates………..106
4.4.7 Optical properties of oxidized porous SiO$_2$/poly(NIPAM) hybrids during thermal cycling………………………………………………….109
4.4.8 Effect of porosity and pore size of the porous SiO$_2$/poly(NIPAM) hybrid on observed optical thickness changes………………….112
4.4.9 Interpretation of optical thickness changes observed on thermal cycling of porous SiO$_2$/poly(NIPAM) hybrids……………………117
4.5 CONCLUSIONS………………………………………………………121
4.6 REFERENCES………………………………………………………….122

CHAPTER 5 PREPARATION AND CHARACTERIZATION OF A MULTIFUNCTIONAL POLY(N-ISOPropylACRYLAMIDE-CO-ACRYLIC ACID)/POROUS SiO$_2$ NANOHYBRID………………………………………………..127

5.1 ABSTRACT………………………………………………………………..128
5.2 INTRODUCTION…………………………………………………………129
5.3 EXPERIMENTAL…………………………………………………………130
  5.3.1 Materials…………………………………………………………………130
  5.3.2 Etching procedure……………………………………………………131
  5.3.3 Preparation of poly(NIPAM-co-AAc) and poly(NIPAM-co-
  AAc)/porous SiO$_2$ hybrids……………………………………………….131
  5.3.4 Oxidation of porous Si films…………………………………………132
  5.3.5 Scanning electron microscopy………………………………………132
  5.3.6 Gravimetric determination of porosity……………………………..132
  5.3.7 Measurement of pre-gel solution and buffer refractive indices….133
  5.3.8 Measurement of interferometric reflectance spectra………………133
  5.3.9 Measurement of template porosity and thickness by the 
spectroscopic liquid infiltration method (SLIM)…………………………134
  5.3.10 Determination of optical thickness……………………………..135
5.4 RESULTS AND DISCUSSION……………………………………………135
  5.4.1 Preparation of poly(NIPAM-co-AAc)/porous SiO$_2$ hybrids……135
  5.4.2 Preparation of porous SiO$_2$ template………………………………137
  5.4.3 Characterization of the porous SiO$_2$ thickness and porosity…..137
    5.4.3.1 SEM characterization of porous SiO$_2$ template……………….137
    5.4.3.2 Spectroscopic liquid infiltration method (SLIM)
    analysis of porous SiO$_2$ template thickness and porosity………..137
    5.4.3.3 Gravimetric measurement of porosity…………………………..139
  5.4.4 In-situ synthesis of poly(NIPAM-co-AAc) in porous SiO$_2$
  templates…………………………………………………………………….140
  5.4.5 Optical analysis of poly(NIPAM-co-AAc)/porous SiO$_2$ hybrids..143
  5.4.6 Changes in EOT during pH cycling experiments…………………..143
5.4.6.1 pH cycling.........................................................143
5.4.6.2 Conditioning of the hybrids under flow conditions.....144
5.4.6.3 Effect of acrylic acid content on the optical response...146
5.4.6.4 Rapid cycling of pH.........................................149
5.4.7 Interpretation of EOT changes during pH cycling...........149
5.4.8 Changes in the EOT of poly(NIPAM-co-AAc)/Porous SiO$_2$
hybrids during thermocycling.......................................155
5.4.9 Explanation of EOT changes in poly(NIPAM-co-AAc)/
porous SiO$_2$ hybrids during thermocycling experiments.........156
5.5 CONCLUSIONS.......................................................158
5.6 REFERENCES.........................................................159
List of Figures

Figure 1.1  Schematic of electrochemical porous Si preparation

Figure 1.2  Schematic of conditions for generation of Fabry-Pérot fringes in porous Si films.

Figure 1.3  Fabry-Pérot reflectance spectrum from porous Si thin film

Figure 2.1  Diffuse reflectance infrared spectra of a freshly-etched (Si-H) film before (a) and after (b) vancomycin-loading

Figure 2.2  Thickness of porous Si films as a function of the duration of the electrochemical etch used in preparation.

Figure 2.3  Total mass of vancomycin released from a porous Si layer (Si-H surface chemistry) as a function of the layer thickness

Figure 2.4  Percent of vancomycin released into PBS solution vs. time from chemically modified porous Si films

Figure 2.5  Experimental setup for pH-triggered drug release measurements

Figure 2.6  pH-triggered release of the BSA protein capping layer and the vancomycin payload, measured by HPLC

Figure 2.7  pH-triggered release of vancomycin from protein-coated porous Si layers of varying thickness as a function of time

Figure 2.8  Intensity of light scattered from a BSA capping layer as a function of time exposed to flowing pH 7.4 buffer, monitoring the disappearance of the protein layer from the porous Si sample

Figure 3.1  Porous SiO₂ interferometric biosensor preparation and operation

Figure 3.2  FTIR spectrum of BSA-coated porous SiO₂

Figure 3.3  Schematic of functionalized biosensor surfaces

Figure 3.4  Optical response of various surfaces to dosing with a 16.8 μM solution of vancomycin

Figure 3.5  Optical response of KAA functionalized BSA coated porous SiO₂ to various dosing concentration of vancomycin
Figure 3.6  Change in optical thickness of a KAA functionalized BSA coated porous SiO$_2$ interferometer vs. concentration of vancomycin……..82

Figure 4.1  Synthesis of porous SiO$_2$/hydrogel hybrids…………………………..99

Figure 4.2  Cross-sectional scanning electron micrographs (secondary electron images) of a thermally oxidized porous Si layer……………….102

Figure 4.3  Cross-sectional scanning electron micrograph (back-scattered electron image) of porous SiO$_2$/poly(NIPAM) hybrid……………..108

Figure 4.4  Optical thickness changes of a porous SiO$_2$/poly(NIPAM) hybrid upon three consecutive heating/cooling cycles…………………..110

Figure 4.5  Optical thickness changes of porous SiO$_2$/poly(NIPAM) hybrids observed upon thermal cycling……………………………………..113

Figure 4.6  Effect of the volume:surface area ratio of the porous SiO$_2$ template……………………………………………………………………116

Figure 4.7  A Schematic illustrating the structural changes proposed to occur in the porous SiO$_2$/poly(NIPAM) hybrid in a heating-cooling cycle…………………………………………………………118

Figure 5.1  Preparation scheme of poly(NIPAM-co-AAc)/SiO$_2$ hybrids………136

Figure 5.2  Cross-sectional scanning electron micrographs (secondary electron) of a thermally oxidized porous Si layer…………………..138

Figure 5.3  Cross-sectional scanning electron micrograph (back-scattered electron) of poly(NIPAM-co-AAc)/porous SiO$_2$ hybrid……………142

Figure 5.4  EOT changes of a 15% AAc hybrid during a single pH cycle……..145

Figure 5.5  Effective optical thickness response of hybrids with different AAc content during pH 4-7 cycles……………………………………..147

Figure 5.6  Changes in effective optical thickness (EOT) as a function of AAc content in poly(NIPAM-co-AAc)/porous SiO$_2$ hybrids………..148

Figure 5.7  Changes in EOT during rapid pH cycling of a 25% poly(NIPAM-co-AAc)/Porous SiO$_2$ hybrid……………………………………………150
Figure 5.8  Thermal cycling of a 10% AAc poly(NIPAM-co-AAc)/porous SiO$_2$ hybrid at pH 7. 

157
List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Thickness and porosity of porous silicon films</td>
<td>30</td>
</tr>
<tr>
<td>2.2</td>
<td>Loading capacity for vancomycin as a function of surface chemistry</td>
<td>34</td>
</tr>
<tr>
<td>3.1</td>
<td>Refractive index values of solutions used in biosensor study</td>
<td>76</td>
</tr>
<tr>
<td>4.1</td>
<td>Thickness and porosity of thermally oxidized porous Si layers</td>
<td>100</td>
</tr>
<tr>
<td>4.2</td>
<td>Physical characteristics of porous SiO$_2$ layers measured by nitrogen adsorption measurements</td>
<td>105</td>
</tr>
<tr>
<td>5.1</td>
<td>Composition of pre-gel solutions</td>
<td>141</td>
</tr>
</tbody>
</table>
Acknowledgements

As nearly anyone can attest, the process of getting a Ph.D. is a long and arduous one, to say the least. Fortunately, I have been lucky enough to be surrounded by an excellent group of people from start to finish, and I would be remiss if I did not thank each person for their contribution to my degree, whether it be entertainment, actual work, or (in most cases) a combination of the two.

First off, I’d like to thank Mike Sailor for taking me into his lab and teaching me several invaluable lessons. While the road hasn’t always been an easy one, I am a significantly better scientist because of taking this trip. Mike has been very supportive and allowed me to study several different facets of porous silicon, even if we weren’t always clear what the outcome would be.

Next up are the post-docs. If Mike is the kingpin of the lab, these are his (not-so) evil henchmen (and henchwomen, if such a word exists). In all seriousness, I had the great fortune of working with a phenomenal team of post-docs, including Frederique Cunin (our French representative), Michael P. Schwartz (loudest voice this side of Green Bay, WI. I’m certain you still owe me roughly 8.2 billion papers somehow), Claudia Pacholski (the German-est German ever, and a damn good researcher to boot. Also, maker of the best weinerschnitzel on this continent), J. Christopher Thomas (Pinky’s side-kick; a.k.a “the brain”), and Ester Segal (lab mom, my fellow Jewish compatriot, and giver of too many toys for Cassius, chocolates for the lab, and good spirits for us all). At times, each of you really helped push me to keep going, and thankfully, believed in me along the way. All of you had a significant impact on me achieving this degree, and for that, I am grateful to each of you.
Finally, my coworkers. We suffered together and achieved more than we ever knew we could. I am a lucky guy to have ended up in a lab with each of you. Beyond the Saki bombs, the butt dances, the limo rides, the US weekly journal club, the extensive discussions, and many, many more things, I have been intellectually enriched to have worked with you. So, thank you to Haohao Lin, Yangyang Li, Jamie Link, Ronnie Cheung, Emily Anglin, Ken McGillivray, Sara Alvarez, Anne Ruminski, Luo Gu, Brian King, Joe Park, Manual Orosco, Jennifer Park, Jennifer Singelyn, Liz Wu, Adrian Garcia Sega, Dan Blackwood, Jason Dorvee, Shawn Meade, Matt Moore, Troy Moore, Tina Lee, Willa M., and Nate Trujillo. Phew…that’s a lot of people.

In particular, I’d like to thank Aaron Wohlrhab for all the custom synthesis presented in this work. Thank you to Yang Yang Li and Claudia Pacholski for assistance and discussions on the work contained in chapter 2. Thank you to Mike Schwartz for thoughtful discussions concerning chapter 3. Finally, thank you to Ester Segal, Jennifer Singelyn, and Troy Moore for discussion and assistance with measurements made in chapters 4 and 5.
Vita

1979

1979

June 2000

June 2000-Aug. 2002

May 2002

September 2002

Sept. 2002 - Aug. 2007


March 2008

Born, Cleveland, Ohio

Arnold and Mabel Beckman Scholarship

Research Asst., Dept. of Chem., Harvey Mudd College

B.S. Chemistry, Harvey Mudd College

Joan and Irwin Jacobs Fellowship

Research Asst., Materials Science and Engineering, University of California, San Diego

Teach Asst., Dept. of Chemistry and Biochemistry, University of California, San Diego

Ph.D., Materials Science and Engineering, University of California, San Diego
Publications

Perelman, Loren A.; Singelyn, Jennifer; Segal, Ester; Sailor, Michael J. Preparation and Characterization of a pH- and Thermoresponsive Hydrogel/Porous Silica Nanocomposite. Manuscript in Preparation

Perelman, Loren A.; Wohlrab, Aaron S.; Schwartz, Michael P.; VanNieuwenhze, Michael S.; Sailor, Michael J. Quantitative Biosensing of Vancomycin Using a BSA-Coated Porous Silicon Oxide Interferometer. Manuscript in Preparation

Perelman, Loren A.; Pacholski, Claudia; Li, Yang Yang; VanNieuwenhze, Michael S.; Sailor, Michael J. pH-Triggered Release of Vancomycin from Protein-Capped Porous Silicon Films. Nanomedicine (2008) Accepted for publication

Segal, Ester; Perelman, Loren A.; Cunin, Frederique; Di Renzo, Francesco; Devoissselle, Jean-Marie; Li, Yang Yang; Sailor, Michael J. Confinement of Thermoresponsive Hydrogels in Nanostructured Porous Silicon Dioxide Templates. Adv. Funct. Mater. (2007), 17, 1153-1162.


Anglin, Emily J.; Schwartz, Michael P.; Ng, Valerie P.; Perelman, Loren A.; Sailor, Michael J. Engineering the chemistry and nanostructure of porous silicon Fabry-Perot films for loading and release of a steroid. Langmuir (2004), 20(25), 11264-11269.

ABSTRACT OF THE DISSERTATION

Macromolecular Coatings on Porous Silicon: Applications in Drug Delivery, Biosensing, and Composites

by

Loren Avery Perelman

Doctor of Philosophy in Materials Science and Engineering

University of California, San Diego, 2008

Professor Michael J. Sailor, Chair

Two classes of macromolecules, proteins and polymers, are coated onto porous Si films in a variety of geometries in order to study fundamental behaviors of these coatings and their potential device applications. The unique preparation control that porous Si allows in both nano-morphology and surface functionalization provides the means for the coatings.

In chapter two, a drug delivery platform using bovine serum albumin (BSA) protein as a stimuli-responsive capping layer on porous Si is described and characterized. It was found that the surface chemistry of the porous Si film has a
profound influence on both drug loading capacity and drug release kinetics, providing for control over these drug release variables. The BSA is observed to act as a pH-responsive trigger for the release of vancomycin from the porous Si film. The drug is safely stored in the porous matrix at pH 4 and is released after triggering with pH 7.4 phosphate buffered saline.

Chapter three discusses a porous SiO$_2$-based biosensor that is prepared by oxidizing a porous Si film, adsorbing BSA to the surface as a coating, and functionalizing the protein with specific target probes for vancomycin. The BSA was observed to adsorb strongly to the surface, resisting desorption in both phosphate buffered saline and triton-X buffer solutions. Quantitative binding information for the tripeptide Ac-L-Lysine-D-Alanine-D-Alanine and vancomycin is determined using the optical properties of the porous Si as a transduction methodology.

Chapters four and five describe the fabrication of thermoresponsive and multifunctional nanohybrids, respectively, using stimuli-responsive hydrogels to infiltrate and coat oxidized porous Si films. The optical properties of the porous Si films are used to study the response of the hydrogel phase of the hybrids to a variety of stimuli. The optical changes correspond to previously-described physical changes in the hydrogel phase, and it was determined that this platform provides a unique opportunity to study fundamental material properties of hydrogels and hybrids, while also having potential applications in many diverse fields, including microfluidics, drug delivery, and biosensing.
CHAPTER 1

INTRODUCTION TO POROUS SILICON AND SELECTED APPLICATIONS
1.1 INTRODUCTION TO POROUS SILICON

Since its discovery in the 1950s, porous Si has been studied extensively in a wide variety of applications ranging from semiconductor processing techniques, photoluminescent device fabrication, and organic vapor sensors to biomaterials, polymer templates, and biosensors. The key feature of porous Si that allows it to be utilized in so wide an array of technologies is the controllability of its fabrication parameters. As such, porous Si provides an exciting opportunity for the development of new materials and devices.

Porous Si may be prepared through an electrochemical etch in an appropriate electrolyte and may require light, depending on the dopant contained within the Si. The porous Si utilized throughout this work is based on anodic etching of highly doped p-type Si in a hydrofluoric acid-based electrolyte solution in the dark. A scheme for the etching mechanism is presented in Figure 1.1. A current is applied to the Si wafer, and Si atoms at the Si/electrolyte interface are polarized. These atoms become subject to attack by fluoride ions in the electrolyte solution, causing the Si to be released in the form of SiF$_6$ and also leading to the evolution of H$_2$ gas. The overall chemical equation of the process is as follows:

$$\text{Si} + 2\text{h}^+ + 6\text{HF} \text{(aq)} \rightarrow \text{SiF}_6^{2-} \text{(aq)} + \text{H}_2 \text{(g)} + 4 \text{H}^+ \text{(aq)}$$

Key features of porous Si, namely pore diameter, pore volume-to-surface area ratio, and pore depth are dictated by the variables of the etching process. By carefully controlling facets of the process, such as electrolyte concentration, dopant concentration, etch
Figure 1.1 Schematic of electrochemical porous Si preparation. A current is applied to the Si wafer, and Si atoms at the Si/electrolyte interface are polarized. These atoms become subject to attack by fluoride ions in the electrolyte solution, causing the Si to be released in the form of SiF$_6$ and also leading to the evolution of H$_2$ gas. The overall reaction is presented in Eq. 1.1.
current density, and etch duration, one can control the morphology of the resulting porous Si.

Immediately after etching porous Si is terminated in a highly reactive hydride species, which provides a convenient means for subsequent attachment of functional molecules, but also leads to environmental oxidation of the material by oxygen and water. An excellent review by Buriak provides an overview of several different methods for attachment of molecules to the porous Si surface\(^2\). Two of the more commonly employed techniques for surface functionalization involve either hydrosilylation of the surface in solutions of alkene-terminated molecules or electrochemical addition of alkyl chains using alkyl halides\(^3\). Alternatively, the surface can be passivated through controlled oxidation, whether chemically- or thermally-driven. The resulting surface can be a mixture of nanocrystalline Si and SiO\(_2\) (low temp. or chemical oxidation) \(^4\) or purely SiO\(_2\) (high temp. oxidation for sufficient time) \(^5\). Attempts to fully oxidize the surface via chemical means typically result in extensive degradation of the porous Si film.

There are few materials that exhibit the preparative behavior that porous Si does. The exceptional degree of control over the chemical and physical features of porous Si provides for the use of this material in a wide variety of applications. As will be demonstrated in the following chapters, the coupling of porous Si with macromolecules can lead to a number of device applications.
1.2 OVERVIEW OF MACROMOLECULE-POROUS SILICON INTERACTIONS

The work detailed in this thesis involves the coating of porous Si with macromolecules, in particular, proteins and hydrogels. There are certainly a number of potential configurations of these two major classes of molecules to interact with the porous Si morphology, and this work is not intended as a complete analysis of these. Rather, this work was approached from a materials science perspective. That is, by controlling the geometric interaction between porous Si and macromolecules, what kind of problems can be solved? As will be demonstrated, careful control of these interactions can lead to a diverse collection of technologies, all unified by using large molecules to control the desired behavior and response of a given device.

1.3 DRUG RELEASE FROM POROUS SILICON FILMS

Porous Si has recently received some attention as a platform for the loading and release of drug substances. Enhanced paracellular delivery of insulin across intestinal Caco-2 cell monolayers from porous Si microparticles has been studied\textsuperscript{6}. Sustained release of dexamethasone from alkyl-functionalized porous Si films has also been demonstrated\textsuperscript{7}. The bulk of porous Si-based drug release materials are based on two principles; 1. tuning of the surface chemistry to enhance the affinity of the drug for the surface, thus improving loading efficiency and controlling the release profile; and 2. passive release of the drug through a combined mechanism of diffusion and degradation.
of the porous Si host into surrounding matrices. An investigation of multiple model drug compounds from mesoporous Si particles explains these principles in greater detail.

Perhaps one aspect that is lacking from these porous Si-based passive diffusion devices is precise control over the release of drug. Other nanomaterials have been functionalized in order to impart a triggering control over the release of drug. For instance, release of model compounds from polyphosphazene-based dendrimers has been triggered by addition of sodium chloride solutions. Liposomes have been used for phospholipase A2 triggered siRNA release. Perhaps most analogous to porous Si drug release platforms are release systems based on MCM-41 porous microparticles. An overview of this technology is contained in the review by Giri and coworkers. Of particular interest are stimuli-sensitive capping moieties, such as quantum dots and dendrimers, used to control drug release under very specific conditions. The current limit of this technology is the pore morphology of the MCM-41 material—pores can range between 2-20 nm. This size regime could potentially be limiting for delivery of oligonucleotide- or protein-based drug release platforms, as these molecules can be much larger in size than typical small drug molecules. Porous Si provides much greater control over pore morphology, with pore sizes ranging from 2 nm to several microns. Since the loading of drug molecules is so heavily dictated by surface chemistry, and the surface area to volume ratio can be controlled for porous Si, it is possible to prepare materials with optimal packing densities using porous Si as opposed to other, less flexible materials.
Processing of porous Si provides both convenient control over morphology and resulting surface chemistry. The capping technologies used in other works coupled with the remarkable control over porous Si should provide a number of exciting means to control drug release. In Chapter 2 of this work, a detailed study of the influence of porous Si surface chemistry on drug loading and release, as well as triggered release using protein macromolecules as stimuli-responsive caps is presented.

1.4 BIOLOGICAL AND CHEMICAL SENSING USING POROUS SILICON-BASED OPTICAL INTERFEROMETERS

Under appropriate conditions, porous Si will display Fabry-Pérot interferometric fringes, which can be utilized as a transduction element in sensing applications. The fringes are governed by the following relationship:

\[ m\lambda = 2nL \]  

(1.2)

where \( m \) is fringe order, \( n \) is refractive index, and \( L \) is the porous Si film thickness. The quantity \( nL \) is referred to as the optical thickness (OT) of the film. It is convenient to express transduction in terms of changes in OT, as this enables precise determination of changes in refractive index or film thickness. As refractive index increases, the fringes shift accordingly, and the OT of the film increases. Figure 1.2 displays a schematic representation of the optical interference that occurs in porous Si films. Figure 1.3 displays a typical Fabry-Pérot interferogram. It should be noted that in all the studies contained herein, the white light source was applied to the porous Si sample at a 90°
Figure 1.2 Schematic of conditions for generation of Fabry-Pérot fringes in porous Si films. A white light source is used to illuminate the surface, and reflections occur at the air-porous Si interface (a) and the porous Si-bulk Si interface (b). The constructive and destructive interference patterns lead to an interferogram. The interference spectrum is highly sensitive to changes in refractive index within the porous matrix and to changes in overall film thickness, allowing for several sensing-based applications.
Figure 1.3 Fabry-Pérot reflectance spectrum from porous Si thin film. The location of fringe maxima is governed by the Fabry-Pérot condition, given in equation 1.1.
angle, thus eliminating any consideration of angular dependence of the interference spectrum.

Porous Si has been studied extensively as an optical chemical and biological sensor. One particular advantage to porous Si-based optical sensors (beyond the fabrication) is that they are label-free, allowing for detection of analyte without permanent and potentially expensive alteration of the analyte. The earliest works were based on rote changes in refractive index due to replacement of air with organic vapor/condensate within the pores. Even in these works, the effect of surface chemistry on the sensor response was investigated and noted to have a substantial influence over the sensor response. In short, the surface can be tuned to improve adsorption of one particular analyte over another.

The ease of surface functionalization also provides a convenient methodology for fabrication of highly specific and sensitive biosensors. A series of papers detailing the evolution of one of the earliest porous Si-based optical biosensors provides a convenient overview of the principles of this technology. In Dancil’s work, the investigators were able to selectively detect IgG binding to protein A that had been immobilized onto an appropriately functionalized porous Si surface. Furthermore, this work demonstrated the utility of bovine serum albumin (BSA) in acting as an agent to inhibit non-specific binding (NSB).

Over time the sophistication of porous Si-based biosensors has improved. Two major advances are of particular note; 1. the simplification of attachment chemistry from 5 steps to 1 or 2 steps; and 2. the development of reference channels into the porous Si film, allowing for suppression of background noise or changes in matrix
composition\textsuperscript{4,19}. By using thermally oxidized porous Si (porous SiO$_2$), Schwartz and coworkers were able to directly adsorb their target probe, protein A, to the interferometer’s surface. As a result, the sensor displayed excellent robustness from dissolution while still providing for specific sensing of IgGs.

In Chapter 3 of this work the developments used by Schwartz are taken a step further to sense the small molecule vancomycin. BSA is adsorbed to the porous SiO$_2$ surface and used as an “anchor” to attach target probe molecules. The BSA, as demonstrated in Dancil’s work, acts to inhibit NSB and provides a convenient means for target probe attachment.

**1.5 POLYMER/POROUS SILICON COMPOSITES**

A number of studies have been conducted to improve the stability and mechanical properties of porous Si by incorporating a polymer into the film\textsuperscript{20-24}. However, the polymers in these works have not been used actively in any other process. Li and coworkers made a polymer replica of the porous Si film, essentially a negative of the porous film, and used it for monitored drug release\textsuperscript{20}. In this case, the porous Si is used as a template, and the final product is made completely of polymer. The advantages to using porous Si as template are that an optical code is imprinted into the polymer, but the stability issues that affect porous Si are negated through careful selection of the polymer. Given the exceptionally wide variety of polymers in existence, it is feasible that polymer imprints with any number of desirable qualities could be prepared.
Some notable applications of polymer impregnation into porous Si have been studied. By placing a fluorescent polymer into a porous Si microcavity, Levitsky and coworkers were able to develop a TNT sensor\textsuperscript{25}. Both the optical properties of the porous Si microcavity and the fluorescence of the polymer led to a multiplexed response of the sensor to dosing with TNT. Such multimodal capability is desirable as devices become increasingly complex. Another application involves the placement of a porous Si microcavity film between two sheets of commercially available hydrogel wound dressings\textsuperscript{26}. In this work, the authors use the microcavity to sense the flux of glucose through the hydrogel matrix.

In chapters 4 and 5 of this work, thermosensitive and multifunctional hydrogels are synthesized in situ within porous SiO\textsubscript{2} templates. The fundamental properties of the hydrogels were studied, and it was determined that the changes in OT as a result of external stimuli corresponded to physical changes in the porous SiO\textsubscript{2}/hydrogel hybrids. These hybrids have potential implications in a wide number of areas, including microfluidics, biosensing, and drug delivery.

\textbf{1.6 CRITERIA OF PREPARATION CONDITIONS FOR POROUS SILICON}

In the world of porous Si research, function dictates form. In other words, the various applications explored in this thesis required a significant amount of forethought and tuning to achieve the desired response from each system. In chapter 2, porous Si is used to both trap and release drug. The preparation conditions desirable in this case are such that allow for significant capture of the drug molecule within the pores and large
enough pores so that release of the compound would not be inhibited by diffusion through the pore openings. In chapter 3, the pores needed to be large enough to accommodate significant loading of BSA and still provide room for the analyte molecule, Vancomycin, to be sensed for. The porous Si used to prepare the composites in chapter 4 ranged in pore size and preparation conditions in order to study what affect the size and morphology had on the response of the composite. Finally, in chapter 5, conditions were tuned to provide for the largest optical response from the composite.

1.7 REFERENCES


CHAPTER 2

pH-TRIGGERED RELEASE OF VANCOMYCIN FROM PROTEIN-CAPPED POROUS SILICON FILMS
2.1 ABSTRACT

An *in-vitro* model system for pH-triggered release of the antibiotic vancomycin from porous Si films is studied. Vancomycin is infused into a mesoporous Si film from a mixed aqueous/acetonitrile solution and trapped by means of a capping layer containing the protein bovine serum albumin (BSA). The protein effectively traps vancomycin in the porous nanostructure at pH 4.0; the protein dissolves and vancomycin is released into solution when the pH increases to 7.4. The surface chemistry of porous Si exerts a substantial effect on the efficacy of drug loading. The amount of drug loading is largest in freshly-etched (hydrophobic, hydrogen-terminated) porous Si, and smaller in methyl-modified, undecylenic acid-modified, and thermally oxidized samples. The quantity of drug loaded in a freshly-etched porous Si chip is proportional to the thickness of the porous layer, which exhibits a constant volume loading efficiency of 31% (v/v). Flow-cell experiments designed to mimic the transition from pH 4 to 7 that occurs when material moves from the stomach to the upper intestinal tract were performed on the freshly-etched films, and vancomycin and BSA release rates were quantified by HPLC analysis of the effluent from the flow cell. There is a small, constant rate of vancomycin release at pH 4 that is independent of the amount of drug loaded in the pores. This is attributed to diffusion of vancomycin from the BSA capping layer. The release rate increases 5-10 fold when the pH of the solution in the flow cell increases to 7.4; 100% of the drug is released within three hours of this increase.
2.2 INTRODUCTION

Vancomycin is a naturally occurring glycopeptide capable of treating Gram-positive Staphylococcus Aureus infections\textsuperscript{1, 2}. It is often used to treat severe staphylococcal infections, and it is becoming increasingly important as the number of bacterial strains resistant to commonly-used β-lactam therapeutics grows. Drug release platforms that can target hard-to-reach locations in the body are of interest for the treatment of such infections. A number of approaches have been proposed to achieve release in bone tissue\textsuperscript{3-6}, vitreous media\textsuperscript{7}, or in the gastrointestinal tract\textsuperscript{8, 9, 10, 11}. Vancomycin is rapidly degraded by acid and proteases present in the stomach, reducing the concentration available for absorption in the lower gastrointestinal tract. High doses of vancomycin are undesirable because they are nephrotoxic\textsuperscript{12}, they cause inner-ear damage\textsuperscript{13}, and they can enhance the evolution of drug-resistant bacterial strains. Here we study a model system for the delivery of vancomycin to the lower intestines based on pH changes that occur naturally along the gastrointestinal tract. Vancomycin is trapped in a porous Si host by means of a bovine serum albumin (BSA) capping layer. It is found that the protein effectively traps the drug at pH 4, but at pH 7.4 the protein is removed from the porous host, releasing the drug payload.

Porous Si exhibits a number of properties that make it an attractive material for controlled release drug delivery. It is biocompatible\textsuperscript{14-17} and bioresorbable\textsuperscript{10, 18}. Its tunable pore sizes and volumes\textsuperscript{19, 20} allow for considerable control over quantity of drug loaded and the rate that drug is released. A number of convenient chemistries exist for the modification of porous Si surfaces\textsuperscript{21}. Porous Si or SiO\textsubscript{2} host matrices have been
used for in-vitro release of the steroid dexamethasone (from dodecyl-terminated porous Si)\textsuperscript{10}, ibuprofen (from templated mesoporous silica particles)\textsuperscript{22} and cis-platin (from calcium phosphate-doped porous Si films)\textsuperscript{23}. In addition, porous Si microparticles have been shown to deliver insulin across Caco-2 monolayers\textsuperscript{24}.

2.3 EXPERIMENTAL

2.3.1 Porous Si sample preparation

Porous Si samples were prepared from single-crystalline p-type Si (boron-doped, 8x10\textsuperscript{-4} Ω·cm resistivity, polished on the (100) face, obtained from Siltronix, inc.) by anodic etch in a 3:1 (v:v) solution of 48% aqueous HF (Fisher Scientific) in ethanol with a constant current density of 533 mA/cm\textsuperscript{2}. Samples were etched for various times, depending on the film thickness desired. A two-electrode configuration was utilized, employing a platinum mesh counter electrode. The etch cell exposed 1.33 cm\textsuperscript{2} of the Si wafer to the etchant. All samples were rinsed with ethanol and dried under a stream of nitrogen after etching. These samples are referred to as “freshly etched” or Si-H in this work.

Thermally oxidized porous Si samples (SiO\textsubscript{2}) were prepared by placing the freshly etched film into a Lindberg/Blue tube furnace for one hour at 800 °C, after which the sample was allowed to slowly cool to room temperature.

Electrochemically-methylated porous Si films (Si-CH\textsubscript{3}) were prepared according to the published procedure\textsuperscript{25}. Briefly, a freshly-etched porous Si sample was left in the etch cell, which was sealed with a custom glass cap fitted with a platinum electrode.
The sealed cell was purged with nitrogen and an acetonitrile solution 0.2 M in methyl iodide and 0.2 M in lithium iodide was introduced. A negative potential of 1.7 V was applied to the porous Si sample for a total of 15 minutes under a white light source. The sample was then rinsed with acetic acid, acetonitrile, and ethanol.

Undecylenic acid-grafted samples (Si-C_{10}H_{20}-COOH) were prepared by heating freshly-etched porous Si samples in neat undecylenic acid as previously described.\textsuperscript{23} Air was removed from the system by subjecting the samples to 3 freeze-pump-thaw cycles on a Schlenk line system. The sample was maintained under a weak nitrogen purge and heated to 150°C for 2 hours. The sample was then rinsed with ethanol and acetone to remove unreacted undecylenic acid.

2.3.2 Characterization of porous Si films

Diffuse-reflectance mode Fourier transform infrared (FTIR) spectra were obtained with a Nicolet-Magna 550 spectrometer. Scanning electron microscope (SEM) images were obtained with an FEI Company Quanta 600 operating at an accelerating voltage of 20 kV and a nominal spot size of 3 nm.

Reflectance spectra for determination of sample thickness, porosity, and rate of oxidation/dissolution were obtained with an Ocean Optics S2000 CCD spectrometer fitted to an optical microscope via fiber optics. The method for collecting reflectivity spectra from porous Si and porous SiO\textsubscript{2} Fabry-Pérot films has been described.\textsuperscript{26} White light from a tungsten lamp (Ocean Optics) is fed through one arm of a bifurcated fiber optic cable and focused through a lens onto the porous Si substrate at normal incidence. Reflected light is collected through the same optics, and the distal end of the second arm
of the bifurcated fiber optic cable is input to the CCD spectrometer. Spectra were not corrected for spectral response of the instrument or the lamp. The quantity \( nL \), referred to as the optical thickness (OT) in this work, is determined from the Fabry-Pérot relationship:

\[
m\lambda = 2nL
\]  

(2.1)

where \( \lambda \) is the wavelength of maximum constructive interference for spectral fringe of order \( m \), \( n \) is the index of refraction of the porous layer and its contents, and \( L \) is the thickness of the porous layer. The value of OT was determined by Fourier transformation of the reflectivity spectrum. The wavelength axis of the spectrum from the Ocean Optics spectrometer was inverted and a linear interpolation applied such that the data were spaced evenly in units proportional to frequency (nm\(^{-1}\)). A Hanning window was applied to the spectrum, it was redimensioned to 4096 data points and then zero padded to the power of two. A discrete Fourier transform using a multidimensional fast prime factor decomposition algorithm from the Wavemetrics, inc (www.wavemetrics.com) IGOR program library (FFT) was applied. Fourier transformation results in a peak whose position along the x-axis is equal to the quantity \( (2nL) \) of eq. 2.1.

The quantity OT is related to the amount of protein in the pores.\(^{21, 27, 28}\) The refractive index of the porous layer is an average of the refractive index of the as-prepared Si oxide and the material in the pores (PBS, media, and/or protein). Replacement of
aqueous media (n ~ 1.3) by protein (n ~ 1.5) leads to a larger value of the optical thickness.

Reflectance spectra of freshly-etched porous Si samples in air and immersed in ethanol were collected prior to the drug loading and release experiments to determine the thickness and porosity of the sample. Thickness and porosity of the samples is determined from this data using the spectroscopic liquid infiltration method (SLIM) and application of the Bruggeman effective medium model, described previously \(^{19, 29}\). Gravimetric determination of thickness and porosity was also performed by weighing the Si wafer before etching, after etching, and after total dissolution of the porous Si film in 1 M sodium hydroxide solution\(^{29}\).

Measurement of the optical reflectivity spectra of the porous Si samples was performed in flowing aqueous phosphate buffered saline (PBS) solutions in order to determine to what extent drug release might be attributed to dissolution of the porous host matrix. Either oxidation of porous Si to SiO\(_2\), or dissolution of the film results in a decrease in refractive index of the film that is detected as a shift of nL to smaller values.

After completion of a complete flow cell experiment (triggering and release, see below), the samples were rinsed with 30% aqueous acetonitrile solution to remove any remaining materials (protein coating or vancomycin) from the pores. The sample is completely dried under a stream of nitrogen. The reflectivity spectrum was obtained in air and ethanol and the thickness and porosity were determined.
2.3.3 Drug loading in porous Si films

Vancomycin hydrochloride hydrate was purchased from Aldrich Chemicals. Buffer solutions (pH 4) were purchased from Fisher Scientific. Bovine serum albumin (BSA) and Dulbecco’s phosphate buffered saline (PBS) were purchased from Sigma Chemicals. Drug loading was accomplished by spin-coating. 50 µL of a 1.35x10^{-2} M solution of vancomycin in 33% (v/v) acetonitrile in pH 4 phthalate-based aqueous buffer was placed on the surface of the porous Si film. After 30 s, the excess solution was removed by spinning at 3000 RPM (Laurell WS400B). This process was repeated two more times and then the sample was rinsed quickly with PBS solution to remove any excess drug on the surface that had not infiltrated the pores. The total quantity of drug loaded by this procedure was determined spectrophotometrically by placing the sample in a PBS solution and monitoring the absorbance peak at 282 nm (attributed to vancomycin) on a Hewlett-Packard 8452A diode array UV-Vis spectrophotometer. The drug-loaded sample (planar area = 1.3 cm²) was placed in 10 mL of PBS buffer and a 3 mL aliquot was temporarily removed for spectrophotometric analysis every five minutes for 1 h. A subsequent measurement was made after 24 h for comparative purposes.

2.3.4 Protein coating

A solution of 20:1 BSA:vancomycin (by mass) was prepared (with 20 mg/mL BSA and 1 mg/mL vancomycin) and allowed to cool at ~4°C for approximately one hour prior to use. A mixture of precipitated BSA and vancomycin formed at the bottom of the test tube. The precipitate mixed with supernatant was introduced into the porous
Si film using a previously-described\textsuperscript{21} flow cell at a rate of \(~1\) mL/min for a total of 3 minutes, after which the surface appeared to be completely coated with the BSA-vancomycin mixture. The flow cell has a dead volume of \(~1.8\) mL. The flow rate was reduced to \(~250\) \(\mu\)L/min, and the sample was rinsed for 15 minutes with pH 4 buffer to remove any excess coating mixture prior to drug release measurements.

\textbf{2.3.5 Optimization of coating protocol}

The original concept behind capping drug in porous silica films was based on the observation that certain proteins adsorbed to silica quite strongly at lower pHs (\(~4\)) and partially desorbed at higher pHs (6-10) during flow cell experiments. As such, it was believed that small molecules, such as a drug, could be effectively capped by placing a small amount of BSA onto the surface, effectively “corking” the pores closed. Unfortunately, as will be discussed later, vancomycin does not load very well into silica films due to the surface chemistry. It was determined that a freshly-etched surface was optimal for drug loading, but this surface was poor at adsorbing BSA, and a new capping methodology was required. The new capping scheme was developed based on the necessary conditions: low solubility at pH 4, effective trapping of the vancomycin contained within the porous Si film, dissolution at pH 7.4, and does not interfere with vancomycin.

Besides the BSA-vancomycin mixture that was found to be an effective capping mixture, several other efforts were made to trap drug in the porous Si matrix. The first effort was to “salt-out” the protein from solution. This method has been utilized in the past to promote protein precipitation\textsuperscript{30}. Solutions of ammonium sulfate or sodium
chloride and BSA were prepared and tested as capping materials. The salts did cause precipitation of BSA, and a mixture of insoluble salt and protein were left at the bottom of the mixing vessel. Unfortunately, when flow cell experiments were conducted on samples utilizing the salt-BSA coating, vancomycin was observed to begin flowing from the pores immediately. Similarly, a mixture of BSA and cellulose was prepared. It was believed that the presence of cellulose would decrease the overall solubility of the mixture leading to deposition of a thick coating layer on the porous Si when introduced into the flow cell. As was previously observed with salt solutions, the vancomycin began flowing from the porous Si immediately, indicating the inability of this material to act as an effective capping layer. Finally, a mixture of BSA and sucrose was prepared by grinding the two powders together in a mortar and pestle. The mixture was placed in a furnace at 120 °C for 2 hours in order to covalently couple the sugar and protein together via the Maillard reaction\(^{31}\). The mixture proved to retain a high degree of solubility in pH 4 buffer, yielding it ineffective for capping purposes.

### 2.3.6 Collection of drug release samples

The drug-loaded, protein-coated porous Si films in the above flow-cell were subjected to constant flow (1 mL/min) of buffer solution. Eluent samples were collected every 3 min at the outlet of the flow cell and placed into HPLC vials. The pH 4 buffer was allowed to flow over the sample for 30 min (10 samples), and then the solution was changed to PBS (pH 7.4) and flow was maintained for an additional 60 min (20 samples).
2.3.7 High pressure liquid chromatography (HPLC) analysis

Samples were analyzed for vancomycin and BSA content using a Phenomex reverse-phase C18 column on a Beckman Coulter System Gold HPLC. A water-acetonitrile solvent system was used. Vancomycin eluted at 38% acetonitrile, and BSA eluted at 65.5% acetonitrile. The column was flushed with a 5 minute flow of 100% acetonitrile after every sample to avoid contamination.

2.3.8 Light scattering analysis

The dissolution of the protein coating layer was monitored by light scattering. A tungsten light source coupled into microscope optics using a fiber optic bundle was used to illuminate the surface. A second set of microscope optics collected the scattered light, which was analyzed with an Ocean Optics S2000 spectrometer as previously described. The scattered light was collected at an angle nominally set 20 degrees off of the specular reflection axis. Spectra were collected every 30 seconds, and intensity of scattered light was quantified as the total integrated area of the spectrum, corrected for dark counts.

2.4 Results and Discussion

2.4.1 FTIR analysis of vancomycin loaded into freshly-etched porous Si

Vancomycin was loaded into the hydrophobic porous Si matrix immediately after sample etching. The diffuse reflectance FTIR spectrum provides a convenient means to monitor sample chemistry and confirm drug loading. Prior to drug loading, the
FTIR spectrum displays bands characteristic of freshly-etched porous Si (Fig. 2.1a): a strong band at 2110 cm\(^{-1}\) associated with \(\nu_{\text{Si-H}}\) stretching vibrations, a \(\delta_{\text{SiH}_2}\) scissors mode at 915 cm\(^{-1}\), bands assigned to Si-H deformation (666 and 627 cm\(^{-1}\)) and Si-Si lattice modes (627 cm\(^{-1}\), overlapping with Si-H wag\(^{33,34}\)). Furthermore, a band at 1065 cm\(^{-1}\), attributed to \(\nu_{\text{Si-O}}\) stretching vibrations, indicates that a slight amount of oxidation has occurred to the sample during handling or preparation of the film for FTIR analysis. Vibrational bands in the FTIR spectrum characteristic of vancomycin confirm drug loading (Fig. 2.1b): a band at 1683 cm\(^{-1}\) is associated with aromatic ring modes and the amide I stretch. Strong bands at 1503 cm\(^{-1}\) correspond to amide II, and a C-C stretching mode is observed at 1158 cm\(^{-1}\)^{34,35}.

2.4.2 Characterization of porous silicon film thickness and porosity and drug capacity

Table 2.1 summarizes the measured thickness and porosity of the porous Si samples used in this study. Film thickness was directly determined by cross-sectional scanning electron microscope (SEM), and confirmed by gravimetric and optical analysis^{19}. The nominal pore diameter for these samples is 10-100 nm. The thickness of a porous Si sample is directly proportional to the duration of etch used in its preparation (Fig 2.2). Porosity is related to the current density used in the etch, which was fixed at 533 mA/cm\(^2\) in this study. Porosity was determined by gravimetric measurement and by optical measurement. The optical measurement uses a technique known as SLIM, for spectroscopic liquid infiltration method. SLIM involves acquisition of reflectivity spectra upon infiltration of different liquids of known refractive indices and application
Figure 2.1 Diffuse reflectance infrared spectra of a freshly-etched (Si-H) film before (a) and after (b) vancomycin-loading. Group frequency assignments characteristic of the porous Si substrate and of vancomycin are indicated. The samples were prepared by scraping the porous Si film from the Si substrate prior to spectral acquisition, and the y-scale (Kubelka-Munk units) is normalized and offset between the two spectra to allow comparison. Porous Si samples used in this figure were etched at a constant current density of 533 mA/cm² for 30 s.
of the Bruggeman effective medium model\textsuperscript{29}. It measures the porosity accessible to infiltration by the liquid (open porosity)\textsuperscript{36}, and thus represents an under-estimate of the total (open and closed) porosity of the material provided by the gravimetric measurement. In the present case, the optical and the gravimetric measurements agree within the error limits of the measurements. The porosity of the films used in this study is $\sim 72 \pm 1\%$.

\textbf{2.4.3 Drug capacity of porous films}

The amount of vancomycin that can be loaded into a porous Si film scales with film thickness in the expected manner. Vancomycin was loaded into several films of differing thickness without the addition of a protein capping layer to trap the drug. The films were then placed in pH 7.4 PBS solution and the amount of drug released into solution was determined by measurement of the UV absorbance at $\lambda = 282$ nm (Fig. 2.3). The initial rate of release is dependent on the total amount of drug loaded; however, for all samples $\geq 70\%$ of the drug is removed from the pores within the first hour. A plot of total drug loaded as a function of film thickness (Fig. 2.3) shows that the amount of drug that can be loaded scales with the thickness of the film, indicating that the volume capacity of a film is not dependent on film thickness and suggesting that the distribution of drug in the porous film is relatively homogeneous. Given a density of vancomycin of 1.203 g/cm$^3$\textsuperscript{37}, the volume loading efficiency of vancomycin in the freshly-etched porous Si films used in this study is $31 \pm 2\%$. 
### Table 2.1 Thickness and porosity of porous Si films

<table>
<thead>
<tr>
<th>Etch time</th>
<th>Thickness (microns)</th>
<th>Porosity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SEM</td>
<td>Optical</td>
</tr>
<tr>
<td>15 s</td>
<td>4.13 ± 0.22</td>
<td>3.83 ± 0.11</td>
</tr>
<tr>
<td>30 s</td>
<td>7.87 ± 0.35</td>
<td>7.30 ± 0.18</td>
</tr>
<tr>
<td>45 s</td>
<td>10.87 ± 0.75</td>
<td>10.64 ± 0.55</td>
</tr>
</tbody>
</table>

*a* Values derived from cross-sectional electron microscopy (“SEM”), fit of the reflectivity spectrum to the SLIM model (“Optical”), and gravimetric measurement (“Gravimetry”). All samples were etched at a constant current density of 533 mA/cm².

*b* Optical measurements performed by spectroscopic measurement of the samples upon infiltration of liquids with known refractive indices, as described in the text.
Figure 2.2 Thickness of porous Si films as a function of the duration of the electrochemical etch used in preparation. Thickness values obtained from three methods are compared in this figure: cross-sectional electron microscopy (“SEM”); fit of the optical constants derived from the reflectivity spectra to the Bruggeman effective medium approximation (“Bruggeman”); and gravimetric measurement (“Gravimetry”). All samples were etched at a constant current density of 533 mA/cm$^2$. 
Figure 2.3 Total mass of vancomycin released from a porous Si layer (Si-H surface chemistry) as a function of the layer thickness. Vancomycin was quantified by monitoring the UV absorbance at 282 nm. The amount of vancomycin that can be loaded into a film scales with film thickness. All samples were etched at a constant current density of 533 mA/cm². Sample thickness was determined by SEM measurement.
The volume loading capacity was found to be significantly affected by the surface chemistry of the porous Si film, as has previously been observed for loading of dexamethasone in porous Si films\textsuperscript{19}. A number of surface chemistries were examined to maximize vancomycin loading, including freshly etched porous Si (Si-H), thermally-oxidized porous Si (SiO\textsubscript{2}), electrochemically-methylated porous Si (Si-CH\textsubscript{3}), and undecylenic acid-grafted porous Si (Si-C\textsubscript{10}H\textsubscript{20}-COOH).

Table 2.2 compares the amount of vancomycin that can be loaded into the various sample types. As might be expected, the addition of functional groups to the surface tends to reduce the open porosity of the samples, and it also tends to reduce the amount of drug that can be loaded\textsuperscript{19}. There is a volume expansion that occurs on oxidation of porous Si to porous SiO\textsubscript{2} that leads to shrinkage of the pore diameters\textsuperscript{38,39}, and oxidized material has roughly one-third of the drug loading capacity of the freshly etched material.

Methylated porous Si samples (Si-CH\textsubscript{3}) also show a relatively low drug capacity, which may be the result of the hydrophobicity of this particular surface chemistry\textsuperscript{40}. Based on the mixed solvent elution profiles in the HPLC experiments, vancomycin displays the highest affinity for amphipophilic solvent systems. Whereas freshly etched porous Si is quite hydrophobic, the FTIR data (Fig. 2.1) indicate that the Si-H samples undergo some oxidation during the drug loading process. This is expected to impart some amphiphilic character to the Si-H surface, improving the drug loading capacity. Drug loading results for the undecylenic acid-terminated samples (Si-C\textsubscript{10}H\textsubscript{20}-COOH) further support this hypothesis. The capacity of the acid-terminated film for vancomycin is less than half of that of the freshly etched film (Table 2.2). This may
Table 2.2 Loading capacity for vancomycin as a function of surface chemistry

<table>
<thead>
<tr>
<th>Surface species</th>
<th>Open porosity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Volume loaded&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Mass loaded&lt;sup&gt;d&lt;/sup&gt; (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si-H</td>
<td>72 ± 1%</td>
<td>31 ± 2%</td>
<td>283 ± 20</td>
</tr>
<tr>
<td>SiO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>62 ± 4%</td>
<td>11 ± 2%</td>
<td>76 ± 10</td>
</tr>
<tr>
<td>Si-C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;20&lt;/sub&gt;-COOH</td>
<td>62 ± 3%</td>
<td>13 ± 1%</td>
<td>95 ± 7</td>
</tr>
<tr>
<td>Si-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>65 ± 4%</td>
<td>8 ± 1%</td>
<td>60 ± 8</td>
</tr>
</tbody>
</table>

<sup>a</sup> All samples were prepared using the same etching conditions (533 mA/cm<sup>2</sup> for 30 s).

<sup>b</sup> Open porosity determined by optical measurement of the liquid-infiltrated films.

<sup>c</sup> Vancomycin loaded using the same protocol from a 1.35 x 10<sup>-2</sup> M solution of vancomycin in 33% (v/v) acetonitrile in pH 4 phthalate-based buffer. Volume fraction loaded determined by UV absorbance measurement of the amount released at pH 7.4.

<sup>d</sup> Mass of vancomycin loaded in the film. Mass of a porous film (prior to surface modification and/or drug loading) is nominally 0.7 mg.
Figure 2.4 Percent of vancomycin released into PBS solution vs. time from chemically modified porous Si films. The SiO\textsubscript{2} and Si-CH\textsubscript{3} samples display rapid release, while both Si-C\textsubscript{10}H\textsubscript{20}-COOH and Si-H samples exhibit slower release profiles. The observed differences in the profiles are attributed to differences in surface chemistry. Traces represent percent of total loaded drug released from each type of film into PBS solution. Vancomycin concentrations measured by UV/Vis spectrometry. All films were prepared at 533 mA/cm\textsuperscript{2} for 30 s.
be due in part to steric hindrance of the Si-C_{10}H_{20}-COOH species; this rather bulky surface species occupies a significant portion of the available pore volume. The Si-C_{10}H_{20}-COOH samples display a somewhat higher loading capacity relative to the Si-CH_{3} or SiO_{2} samples.

2.4.4 Characteristics of vancomycin release from uncoated porous silicon films

In addition to affecting the drug capacity, surface chemistry has a pronounced influence on the temporal release characteristics of vancomycin of the porous films. Fig. 2.4 presents release profiles for the series of surface chemistries studied. All sample types display a burst release in the first few minutes of immersion in PBS. For the SiO_{2} and Si-CH_{3} samples, essentially all of the loaded drug is released during this period. The Si-CH_{3} sample is the most hydrophobic, and the rapid release observed is attributed to a low affinity of the drug for the sample surface. The SiO_{2} samples also display a rapid burst release of vancomycin, presumably due to a similarly low surface binding affinity. Drug release from the Si-C_{10}H_{20}-COOH samples is the slowest of the sample types measured. We hypothesize that the amphiphilic nature of this surface leads to an increased binding affinity for vancomycin. The aliphatic chain of the acid provides a hydrophobic local environment, while the carboxylate terminus supplies a hydrogen bonding moiety. As mentioned above, the Si-H sample is also expected to display amphiphilic behavior due to partial oxidation of the hydrophobic Si-H surface species. The rate of release of drug from the Si-H samples is comparable to that of the Si-C_{10}H_{20}-COOH samples.
The total amount of drug that can be loaded into each film type is consistent with the interpretation of the observed release rates, reflecting the relative affinity of vancomycin for each surface chemistry. Thus the Si-H and Si-C\textsubscript{10}H\textsubscript{20}-COOH samples display the slowest release and the largest amount of drug loading (Table 2). The hydrophilic SiO\textsubscript{2} surface and the hydrophobic Si-CH\textsubscript{3} surface both display a burst release characteristic and a lesser degree of loading.

The Si-H samples display a larger initial burst of drug compared with the Si-C\textsubscript{10}H\textsubscript{20}-COOH samples, likely a result of degradation and dissolution of the porous matrix in the PBS solution. Porous Si films with chemical species tethered to the surface via Si-C bonds, such as the C\textsubscript{10}H\textsubscript{20}-COOH sample, have been shown to be much more stable in aqueous media than the freshly etched Si-H material\textsuperscript{40}. After the initial burst, there is a linear increase in the concentration of drug in solution. Roughly 75\% of the loaded drug is released in the first hour. After 24 h in PBS solution, no additional changes in drug concentration are observed. The freshly etched samples were selected for the pH-triggered drug release experiments due to their high loading capacity. These were prepared at an etch current density of 533 mA/cm\textsuperscript{2} for a duration of either 15, 30, or 45 s, producing films of thickness 4.1, 7.9, or 10.9 \textmu m, respectively (SEM measurement).

2.4.5 Placement of a protein capping layer on drug-loaded films

The drug-loaded Si-H samples were placed in a custom-made flow-cell assembly attached to a peristaltic pump. The pores were sealed by introduction of a concentrated bovine serum albumin (BSA) solution (20:1 w/w BSA:vancomycin) at pH
4 (phthalate buffer). The BSA protein and vancomycin have low solubility in the buffer solution, and the sealing solution appeared milky-white due to precipitates. After a few minutes under flowing conditions, the surface of the porous Si film appeared white due to the protein deposit. Control experiments indicate that this coating retains a 20:1 ratio of BSA:vancomycin, with a total average of ~1500 µg of BSA and ~70 µg of vancomycin being deposited per 1.33 cm$^2$ sample.

### 2.4.6 Flow cell experiments

A schematic representation of the apparatus used in the flow cell experiments is presented in Fig. 2.5. After coating the drug-loaded porous Si sample with the BSA/vancomycin composite, pH 4 buffer is passed through the cell for 15 min to remove any residual protein coating material. Aliquots of the effluent are then collected every 3 min (Fig. 2.5a). After 10 aliquots are collected (30 min), the elution solution is changed from a pH 4.0 (phthalate) to a pH 7.4 buffer (PBS) and 20 additional aliquots are collected (60 min, Fig 2.5b-2.5c). All aliquots collected were analyzed for BSA and vancomycin content by HPLC.

### 2.4.7 pH-triggered release of vancomycin from protein-coated Si-H films

The protein-coated systems display a marked increase in BSA and vancomycin release rates when the pH of the flowing eluent is changed from 4 to 7.4, Fig 2.6. The increase is attributed to dissolution of the BSA capping layer, as BSA is known to have a higher solubility at pH 7.4 relative to pH 4.41 A number of other factors may contribute to the increased release of vancomycin into solution under these conditions.
Figure 2.5 Experimental setup for pH-triggered drug release measurements. a) pH 4.0 buffer solution is introduced into sample chamber. b) pH 7.4 (PBS) buffer is introduced, dissolving the protein coating and initiating release of vancomycin from the pores. c) Protein coating is completely removed and residual vancomycin is released from the pore reservoirs.
BSA undergoes a conformational change from its F form to its N form in this pH range,\textsuperscript{41} which may lead to a higher diffusivity of vancomycin through the BSA/vancomycin capping layer. It is also possible that vancomycin is carried into solution by soluble BSA: vancomycin is known to bind to Human Serum Albumin (HSA) in serum filtrates\textsuperscript{42}.

The free vancomycin observed during the initial stages of release (at pH 4 and immediately after changing to pH 7.4, Fig. 2.6) is thought to originate from the BSA protein coating layer. Initial vancomycin release rates measured from a control system (a similar BSA/vancomycin composite film deposited on an unloaded, freshly-etched porous Si sample) are comparable (Fig. 2.7). Approximately 70 µg of vancomycin is contained in the protein coating layer and 278 µg of vancomycin is contained in the pore reservoirs for the device measured in Fig. 2.6.

The effect of changing the reservoir size in the porous Si matrix on drug loading capacity and release kinetics was studied. Three samples of different thickness but similar porosity and pore dimensions were prepared by varying the duration of the electrochemical etch. The etchant solution, current density (533 mA/cm\textsuperscript{2}), and drug loading/capping protocol were the same for all samples. The amount of drug that can be loaded displays a linear relationship with porous film thickness, corresponding to a loading capacity of 230 mg of vancomycin per cm\textsuperscript{3} of porous Si. A control sample consisting of a protein-coated porous film (7 µm thick) with no drug loaded in the reservoir is shown in Fig. 2.7. As mentioned above, the procedure used to prepare the protein coatings leaves a small amount of vancomycin in the coating layer, and release of this drug quantity is observed in the control sample trace. At pH 4.0 the leaching of
Figure 2.6 pH-triggered release of the BSA protein capping layer and the vancomycin payload, measured by HPLC. (a) Percent of total vancomycin and BSA contained in the device released into solution as a function of time. (b) Rate of appearance of BSA protein capping layer and vancomycin in the eluent solution as a function of time. Solid lines indicate the time at which the pH 4 buffer flowing through the cell was changed to pH 7.4. Samples prepared by etching at 533 mA/cm² for 30 s, porous layer is nominally 7.9 µm thick (measured by SEM).
Figure 2.7 pH-triggered release of vancomycin from protein-coated porous Si layers of varying thickness as a function of time. The total amount of vancomycin contained by and released from the film increases with the thickness of the porous Si reservoir. (a) Percent of total vancomycin contained in the device released into the eluent solution as a function of time. Solid circles indicate a 4.0 µm-thick film containing 185 µg vancomycin; Open squares, 7.5 µm-thick film containing 325 µg vancomycin; Solid squares, 11 µm-thick film containing 395 µg vancomycin. The solid vertical line at t = 30 min indicates the time at which the pH 4 buffer flowing through the cell is changed to pH 7.4. (b) Rate of appearance of vancomycin in the eluent solution as a function of time. The thickness of each porous film is attached to the trace. Thickness is controlled by the duration of the electrochemical etch used in preparation as follows: “control,” protein coating only, no drug loaded into the porous Si layer (which is 7.5 µm thick), etch time 30 s; “4.0 µm,” 185 µg vancomycin loaded, protein-coated Si-H film, etch time 15 s; “7.5 µm,” 325 µg vancomycin loaded, protein-coated Si-H film, etch time 30 s; “11 µm,” 395 µg vancomycin loaded, protein-coated Si-H film, etch time 45 s. The protein coating in all samples contains an additional 70 µg vancomycin. Vancomycin was quantified by HPLC analysis of the flow cell effluent. All samples prepared by etching at 533 mA/cm².
Figure 2.7 continued.
vancomycin from the capping layer is observed in the release profiles for all three loaded films and for the control. When the pH of the elution solution is changed to 7.4, all three loaded films display the marked increase in release rate characteristic of release of drug from the reservoirs upon dissolution and removal of the BSA capping layer (Fig. 2.7).

### 2.4.8 Measurement of removal of the protein coating by light scattering

White light scattering measurements were performed in order to test the relationship between the removal of the protein coating layer and the onset of rapid drug release observed in Figs 2.6 and 2.7. Light scattering has been used to quantify morphological and population changes in mammalian and bacterial cells on porous Si surfaces previously. In the present case, the BSA protein coating layer possesses a micron-scale roughness that scatters light efficiently, and the intensity of light scattered from the coated porous Si surface is a measure of this surface roughness. The underlying porous Si film is optically smooth and contributes little to the scattered light spectrum. During the initial stages of dissolution of the protein coating there is no change observed in the intensity of the scattered light, Fig. 2.8. As dissolution of the coating nears completion, there is a rapid decrease in the intensity of scattered light as the protein scattering domains disappear. There is a 50 min delay between the introduction of the pH 7.4 buffer and complete disappearance of light scattering associated with the protein coating layer. This agrees well with the HPLC data monitoring BSA release from the films; there is an abrupt decrease in the intensity of scattered light at the point where free BSA is no longer observed in solution.
Figure 2.8 Intensity of light scattered from a BSA capping layer as a function of time exposed to flowing pH 7.4 buffer, monitoring the disappearance of the protein layer from the porous Si sample. The sharp drop in scattering intensity that occurs ~50 minutes after introduction of pH 7.4 buffer correlates with the disappearance of the BSA protein from solution, monitored by HPLC (Fig. 2.6). Data from two separate samples are presented to demonstrate reproducibility.
2.4.9 Mechanism of drug release from protein-coated Si-H films

Release of vancomycin from the composite films is proposed to be determined by four processes: diffusion of drug from the BSA layer, dissolution of the BSA layer, diffusion of drug from the porous Si reservoir layer, and dissolution of the porous Si layer. Dissolution of the porous Si reservoir layer can be thought of as involving two steps, oxidation and hydrolysis, represented by eqs. 2.2-2.3. Oxidation of porous Si can occur with either water or \( \text{O}_2 \) acting as an oxidant, eq. 2.2. Dissolution of the oxide then takes place by hydrolysis, eq. 2.3. The extent of oxidation and dissolution of the porous Si reservoir layer was determined by optical reflectivity spectroscopy. The composite refractive index of the film (porous Si, SiO\(_2\) and air in the pores) decreased from the original (before drug loading and protein coating) value of 1.547 to a value of 1.365 after drug release. Assuming that (a) no measurable changes in the metric thickness of the film had occurred and (b) the protein coating and drug were completely removed from the porous Si layer, the change in composite refractive index can be ascribed to oxidation of Si (eq. 2.2) and the subsequent dissolution of a fraction of the resulting SiO\(_2\) (eq. 2.3). With this measurement it is not possible to distinguish Si oxidation from SiO\(_2\) hydrolysis, but two limiting cases can be determined. If it is assumed that only oxidation (eq. 2.2) is taking place, then 60% of the porous Si film was oxidized over the course of the experiment. Alternatively, if it is assumed that both eq. 2.2 and eq. 2.3 occur, 25% of the porous Si film dissolved, corresponding to an increase in porosity from 72 ± 1% to 79 ± 1%.
A control experiment was conducted to determine the effect of the protein coating on porous Si film degradation. A freshly-etched porous Si (Si-H) sample was prepared and placed directly in the flow cell with no drug loaded or protein coating. The degradation of this film was measured by optical reflectivity spectroscopy, and the composite refractive index was observed to decrease from 1.547 to 1.305. Relative to the two limiting cases described above, this decrease corresponds to oxidation of 80% of the porous Si layer (eq. 2.2, oxidation-only case) or dissolution of 36% of the porous Si film (eqs. 2.2 and 2.3, oxidation followed by dissolution resulting in an increase in porosity of the film from 72 ± 1% to 82 ± 1%). It can be concluded that the drug-loaded, protein coated Si-H films are more stable than the bare Si-H film at pH 7.4.

The kinetics of drug release are consistent with a rate limiting step involving drug desorption from the surface of the porous matrix. A marked increase in the rate of release is observed when the pH increases from 4 to 7.4 and the capping layer is removed (Fig. 2.7). Once triggered by the jump in pH, the amount of time needed to release the drug payload is ~1 hr, independent of film thickness (Fig. 2.7a). The rate of drug release is proportional to the reservoir volume (Fig. 2.7b), displaying a first order dependence on the total amount of drug loaded in the reservoir. Vancomycin is a small molecule (~1-2 nm) relative to the diameter of the pores in the reservoir (10-100 nm), and apparently the pores are large enough such that they do not limit the release.
kinetics. If drug release were limited by diffusion in the pores, the time required to release all of the drug payload (% loaded drug released vs time, Fig. 2.7a) should be proportional to film thickness. The temporal profiles observed in Fig. 2.7 are consistent with a mechanism in which the kinetics of vancomycin removal from the pore wall surface is rate limiting. Presumably this desorption process involves a combination of simple desorption of vancomycin and dissolution of the porous matrix. Since the amount of vancomycin adsorbed the pore walls scales with film thickness, the rate of drug delivery into solution should also scale with film thickness if drug desorption/surface dissolution are the rate limiting processes.

2.5 CONCLUSIONS

pH-triggered release of the antibiotic vancomycin from porous Si films containing a BSA protein capping layer was demonstrated. The total amount of drug that can be loaded into the porous Si reservoirs is controlled by the thickness and by the surface chemistry of the porous Si film. Surface chemistry also plays a role in determining the release profile. Surfaces with a low affinity for vancomycin have a small capacity and tend to release the drug in a burst, while amphiphilic surface chemistry increases the loading capacity and releases vancomycin over a more extended period.

The ability of a capping layer to allow pH-triggered release was demonstrated using the protein bovine serum albumin (BSA) capping a reactive, freshly etched porous Si film containing vancomycin. Release of the drug into aqueous solution was
induced by changing the buffer pH from 4 to 7.4, simulating the transition that occurs when material travels from the stomach to the lower gastrointestinal tract. The protein capping layer is relatively insoluble at pH 4 but soluble at pH 7.4. Drug release from the porous Si reservoir occurs concomitant with dissolution of the capping layer. The mechanism of drug release is ascribed to a combination of drug desorption and matrix dissolution.

2.6 References


7. Gavini, E.; Chetoni, P.; Cossu, M.; Alvarez, M. G.; Saettone, M. F.; Giunchedi, P., PLGA microspheres for the ocular delivery of a peptide drug, vancomycin using


CHAPTER 3

QUANTITATIVE BIOSENSING OF VANCOMYCIN USING A
BSA-COATED POROUS SILICON OXIDE INTERFEROMETER

FUNCTIONALIZED WITH Ac-L-Lys-D-Alanine-D-Alanine
3.1 ABSTRACT

A porous Si oxide interferometric biosensor was constructed by coating the surface of the interferometer with bovine serum albumin (BSA). BSA adsorption strength was measured in phosphate buffered saline (PBS) and Triton-X buffer solutions. BSA was observed to remain almost completely bound to the surface in both solutions. Custom-synthesized Ac-L-Lysine-D-Alanine-D-Alanine (Ac-KAA) was attached to the biosensor surface via activation of the free carboxylates of the glutamic and aspartic acid peptide residues found within BSA. A thermodynamic dissociation binding constant, \( K_d \), of \( 17.9 \pm 3.8 \mu M \) was measured for the binding of vancomycin to the surface-bound Ac-KAA, well within the previously reported range of 1-100 \( \mu M \). The BSA-coated surface was found to inhibit the non-specific binding of vancomycin. Furthermore, studies indicated that vancomycin did not bind to ethanolamine- or Ac-L-Lysine-D-Alanine-D-Lactone-functionalized surfaces, demonstrating a high degree of specificity required for sensing of the target analyte.

3.2 INTRODUCTION

Label-free sensor technologies provide for the rapid acquisition of fundamental binding constants. These devices have been extensively utilized as environmental sensors, for the determination of pharmaceutical behaviors, and for fundamental understanding of biological processes. A wide array of technologies has emerged that is
especially robust in achieving these goals, including SPR, reflectance spectroscopy, and porous Si-based interferometers.

Porous Si is an attractive material in the development of sensors, owing to the ability to precisely control the pore dimensions over a wide range of biologically relevant length scales and its facile surface functionalization. Recent research efforts in porous Si-based interferometers have centered on the simplification of target probe attachment schemes and the ever-increasing sophistication of signal transduction methodology, with particular focus on the inclusion of reference channels. In some cases, these two concepts have been combined to create especially sensitive biosensor platforms in porous Si.

In this present work, a general method for testing peptide/drug interactions is investigated. The need for simple methods to measure such interactions, particularly on a high-throughput scale, is especially important in pharmaceutical research. The ability to process Si for use in array-based technologies makes it a potential candidate for achieving such goals. A simplified attachment scheme has been developed for the sensing of vancomycin, an important glycopeptide antibiotic capable of treating Staphylococcus aureus infections. An acetylated lysine-alanine-alanine tripeptide residue is used as a target probe, as it has been used in the past to mimic bacterial wall precursors as a means for understanding the mechanism of vancomycin activity. The tripeptide is attached to oxidized porous Si (porous SiO$_2$) via the aspartic acid and glutamic acid carboxylate moieties on bovine serum albumin (BSA) that has been non-specifically adsorbed to the porous SiO$_2$ surface prior to target probe attachment. In our previous work we demonstrated excellent specificity for vancomycin, while resisting
non-specific adsorption using the same methodology \cite{11}. For the work described here, we extend the utility of the technique by demonstrating quantitative determination of a thermodynamic binding constant using flow cell experiments.

### 3.3 Experimental

#### 3.3.1 Preparation of porous Si

All porous Si samples were prepared from polished, single crystalline Si (ρ = 1.1 mΩ·cm; Siltronix Inc.) and etched using a two-electrode setup with a platinum mesh counter-electrode. Si wafers were placed into a Teflon etch cell, and 1.33 cm$^2$ of Si was exposed to the etching solution (3:1 49\% HF:EtOH v/v). CAUTION: HF is highly toxic and should be handled with great care. If exposed to HF directly on the skin or by inhalation, seek medical attention immediately. HF and EtOH were acquired from Fisher and Aaper, respectively. All samples were etched at a constant current of 385 mA/cm$^2$ for 30 s. The freshly-etched porous Si was rinsed with copious amounts of EtOH and dried under a stream of nitrogen.

#### 3.3.2 Oxidation of porous Si

Porous Si samples were placed in a tube furnace (Lindberg Blue) for 1 hr at 800 °C under ambient conditions. Samples were allowed to cool to room temperature before further handling.
3.3.3 BSA loading

A solution of 5 mg/mL BSA (Aldrich) in pH 4.0 buffer (Fisher) was prepared. Porous SiO$_2$ samples were placed in 5 mL of BSA solution and allowed to incubate for 24 hr at room temperature. Samples were then rinsed in DI water and placed in a vial containing PBS for 24 hr to remove any weakly bound protein. Samples were analyzed on a Nicolet Magna-IR 550 Fourier transform infrared spectrometer for confirmation of BSA adsorption.

3.3.4 Measurement of refractive indices

All refractive indices of solutions were measured on a Milton Roy refractometer.

3.3.5 Gravimetric determination of porosity

Porous SiO$_2$ samples were weighed, completely degraded in a 3:1 49% aqueous HF:EtOH solution, and weighed for a final weight. The porosity was determined using the following equation:

$$P = \frac{V_{\text{total}} - V_{\text{SiO}_2}}{V_{\text{total}}}$$

(3.1)

where $V_{\text{total}}$ is the total volume of material removed from the bulk Si substrate, as determined by:

$$V_{\text{total}} = A \times L$$

(3.2)

where A is the etched area, and L is the total thickness of the porous film as measured spectroscopically (see below). The total volume of SiO$_2$ removed from the porous layer, $V_{\text{SiO}_2}$, was calculated by:
\[ V_{SiO_2} = \frac{m_1 - m_2}{d} \] (3.3)

Where \( d \) is the density of bulk SiO\(_2\), taken as 2.6 g cm\(^{-3}\).

### 3.3.6 Optical Measurement of Porosity and Thickness

Porosity and thickness of porous SiO\(_2\) and BSA-loaded porous SiO\(_2\) samples were measured using the previously-described spectroscopic liquid infiltration method (SLIM).\(^9,^{10,15}\) Briefly, porous SiO\(_2\) samples were filled with solutions of differing indices of refraction and spectroscopic measurement of the optical thickness (OT) were made (see below for thorough discussion on determination of OT). Changes in OT were monitored, and a mathematical solution to the Bruggeman medium approximation was obtained. In this study we used DI water, PBS, ethanol, hexane, and acetone, and the 15% pre gel solution, with refractive indices of 1.333, 1.336, 1.359, 1.372, and 1.357, respectively, to measure changes in OT.

### 3.3.7 Determination of BSA content

BSA content was directly measured by placing the samples in a 5% solution of aqueous HF for 1 hr, thereby dissolving all of the SiO\(_2\). The BSA was quantified using a coomassie-type Bradford Assay (Pierce). Control solutions of 5% aqueous HF and varying amounts of BSA were tested to ensure that the coomassie dyes would be stable in acidic conditions, as well as to generate a standard curve.
3.3.8 Synthesis of Ac-L-Lysine-D-Alanine-D-Alanine-OH

All reactions were carried out in flame-dried glassware under an atmosphere of dry nitrogen or argon. Unless otherwise mentioned, all solvents were dried using a Seca Solvent System (Glass Contour). All other commercially available reagents were used as received.

\( ^1\text{H NMR spectra were measured at 300 MHz on a Varian Mercury instrument, at 400 MHz on a Varian Gemini-400, or at 500 MHz on a Varian VXR-500 instrument.} \)

Analytical thin layer chromatography (TLC) was performed using Whatman glass plates coated with a 0.25 mm thickness of silica gel containing PF 254 indicator, and compounds were visualized with UV light, potassium permanganate stain, cerium molybdate stain or ninhydrin stain. Flash chromatography purifications were performed using Silicycle 60 Å, 35-75 μm silica gel or Biotage purification system (SP1 HPFC system). All compounds purified by chromatography were sufficiently pure for use in further experiments, unless otherwise noted.

\[
\text{Boc-DAla-DAla-OBn} : \text{To a solution of Boc-DAla-OH (3.85 g, 20.37 mmol) and H}_2\text{N-DAla-OBn (3.65 g, 20.37 mmol) in THF (61 mL) at 0°C was added HOBt (5.50 g, 40.7 mmol), DIEA (3.55 mL, 20.37 mmol), and EDC (7.81g, 40.7 mmol). The reaction was slowly warmed to room temperature and stirred for 8 hours. The reaction was quenched with EtOAc (10 mL) and the solvent was removed. The residue was redissolved in EtOAc (50 mL) and washed with 1N HCl (1 x 30 mL), Saturated aqueous}
\]
NaHCO₃ (1 x 30 mL), and Brine (1 x 30 mL). The EtOAc was dried over Mg₂SO₄, filtered and condensed. The crude product was purified by column chromatography (Hexanes-EtOAc 7:3) to afford the Boc-Dala-DAla-OBn (6.92 g, 19.76 mmol, 97%) as a hydroscopic foam.

**Boc-Lys(Z)-DAla-DAla-OBn**: Boc-DAla-DAla-OBn (5.73 g, 16.35 mmol) was treated with 4N HCl-Dioxane (15 mL) and the resulting mixture was stirred at room temperature for 90 min. The volatiles were removed in vacuo. The residual HCl was further removed by adding Et₂O (30 mL) to the hydrochloride salt followed by its removal in vacuo. This residue and Boc-Lys(Z)-OH (6.220, 16.35 mmol) were dissolved in DMF (45 mL). The mixture was then treated sequentially at 0°C with DIEA (5.70mL, 32.7mmol), HOBT (4.86, 36.0 mmol), and EDC (6.27 g, 32.7 mmol). The reaction mixture was stirred for 8hrs slowly warming to room temperature. The reaction was quenched with H₂O (2 mL) and the solvent was condensed. The residue was redissolved in EtOAc (100 ml), washed with 1N HCl (1 x 100 mL), Saturated NaHCO₃ (1 x100 mL), and Brine (1x 100 ml), dried over Mg₂SO₄, filtered and concentrated in vacuo. Chromatography (CHCl₃/MeOH, 20:1) gave Boc-Lys(Z)-DAla-DAla-OBn (9.47 g, 15.46 mmol, 95%) as colorless foam.
Boc-Lys(Z)-DAla-DAla-OBn (4.47 g, 7.29 mmol) was treated with 4N HCl/dioxanes (10 mL) and the resulting mixture was stirred at room temperature for 90 min. The volatiles were removed in vacuo. The residual HCl was further removed by adding Et₂O (30 mL) to the hydrochloride salt followed by its removal in vacuo. This residue was dissolved in CH₂Cl₂ (25 ml) followed by the addition of DIEA (7.6 ml, 43.7 mmol) and Acetic anhydride (2.75 mL, 29.1 mmol). The mixture was stirred at room temperature for one hour. The solvent was removed and the residue was dissolved in 100 ml CH₂Cl₂/IPA (3:1) and washed with 1N HCl (1 x 100 mL), Saturated NaHCO₃ (1 x 100 mL), and Brine (1 x 100 mL), dried over Na₂SO₄, filtered and concentrated in vacuo. Chromatography (CHCl₃/MeOH, 20:1) afforded Ac-Lys(Z)-DAla-DAla-OBn (3.97 g, 7.23 mmol, 97%) as a colorless foam.

Ac-Lys(Z)-DAla-DAla-OH: Ac-Lys(Z)-DAla-DAla-OBn (2.15 g, 3.88 mmol) was dissolved in MeOH (100 ml) and 5% palladium on carbon (0.500 g) was added. The mixture was purged with H₂ and stirred under an atmosphere of H₂ for 8 hours. The mixture was filtered through pad of celite and rinsed with H₂O (30 mL). The solvent
was removed to dryness affording Ac-Lys-DAla-DAla-OH (1.25 g, 3.78 mmol, 98%) as a white solid.

3.3.9 Synthesis of Ac-L-Lysine-D-Alanine-D-Lactone-OH

KALac samples were prepared according to literature\textsuperscript{21}. All samples were tested for purity using NMR, HPLC, IR, and MS techniques.

3.3.10 Functionalization of BSA-coated porous SiO\textsubscript{2} surfaces

The scheme for functionalizing BSA was adapted from Hermanson\textsuperscript{47}. The carboxylic acids on the surface-adsorbed BSA were activated using a solution 15 mM in N-hydroxysulfosuccinimide sodium salt (Fluka) and 75 mM in N-(3-Dimethylaminopropyl)-N’-ethylcarbodiimide (Aldrich) in pH 6.0 buffer (Fisher). Samples were then placed in a solution of 2 mg/mL of peptide in PBS for 12 hours. Samples were rinsed with PBS and placed in a 0.1 M solution of ethanolamine (Aldrich) in DI water for 30 min to functionalize any unreacted carboxylic acids. Samples were then rinsed with PBS, DI water, and placed under vacuum until they were used.

3.3.11 Flow cell experiments

Samples were placed in a custom flow cell assembly attached to a VICI m50 liquid handling pump system. Solutions of vancomycin were flowed over the sample at a fixed rate of 250 μL/min. The flow cell has a dead volume of approximately 2 mL.
3.3.12 Reflectometric spectroscopy

Interferometric reflectance spectra of the porous SiO$_2$ interferometers was measured using an Ocean Optics CCD S-2000 spectrometer. A bifurcated fiber optic cable was used to attach microscope optics to the spectrometer. The other end of the fiber optic cable was attached to a tungsten light source that was focused through the optics to a spot size of ca. 1-2 mm$^2$. Spectra were collected in the wavelength range of 400-1000 nm, with a 20 ms spectral acquisition time. 100 individual spectra were averaged together for each spectral acquisition. Data was collected every 30 s. Illumination of the sample and detection of reflected light were both done at 0º relative to surface normal.

3.3.13 Obtaining optical thickness

The value of optical thickness (OT) was determined from the Fabry-Pérot relationship given in eq. 3.7 (see Results and Discussion section). The value of OT was derived from a Fourier transformation of the reflectance spectrum. The wavelength axis of the spectrum from the Ocean Optics spectrometer was calibrated using a least-squares fit of five spectral lines observed from a neon lamp, at 585.3, 614.3, 640.2, 703.2, and 811.5 nm. The data spacing of the spectrometer is approximately 0.4 nm. The x-axis was inverted and a linear interpolation was applied such that the data were spaced evenly in units of nm$^{-1}$. A Hanning window was applied to the spectrum, it was redimensioned to 4096 data points and zero padded to the power of two. A discrete Fourier transform using a multidimensional fast prime factor decomposition algorithm from the Wavemetrics, inc (www.wavemetrics.com) IGOR program library (FFT) was
applied. The Fourier transform of the spectrum yields a peak whose position on the x-axis corresponds to the value of $2nL$ in eq. 1 (see Results and Conclusions section).

### 3.3.14 Determination of equilibrium binding constants

The reaction of surface functionalized KAA with vancomycin was modeled by the expression

$$V + KAA \xleftrightarrow{k_d} VKAA$$

(3.4)

where KAA represents the available KAA binding sites, V represents free vancomycin in solution, VKAA represents the surface-bound KAA/vancomycin complex, and $k_a$ and $k_d$ are the kinetic rate constants for KAA/vancomycin binding and dissociation, respectively. The equilibrium dissociation constant ($K_d$) is defined as

$$K_d = \frac{KAA[V]}{VKAA}$$

(3.5)

and can be represented by the following equation (Schwartz):

$$\Delta OT_f = \frac{\Delta OT_{max}[V]}{K_d + [V]}$$

(3.6)

where $\Delta OT_{max}$ represents the signal obtained when all KAA binding sites are occupied and $\Delta OT_f$ is the steady-state value of $\Delta OT$ for a given [V]. $K_d$ is obtained from a numerical fit to the plot of [V] versus $\Delta OT_f$ (see Results and Discussion section, Figure 3.6). A thorough discussion of the derivation of the aforementioned is well-established by Schwartz et al $^{12}$. 
3.4 RESULTS AND DISCUSSION

3.4.1 Porous SiO$_2$ interferometric biosensor preparation and characterization

Figure 3.1 displays an overview of the biosensor preparation and its principle of operation. Detailed description of preparation is contained in the experimental section.

3.4.2 Porous SiO$_2$ preparation

Freshly prepared porous Si is known to be somewhat susceptible to degradation via oxidation and dissolution in aqueous environments. The samples in this study have been thermally oxidized in order to convert the porous Si film in SiO$_2$. Oxidation is known to improve the chemical robustness of porous Si. Furthermore, the SiO$_2$ surface provides a convenient means for adsorbing proteins to the surface. For example, protein A has been successfully adsorbed to the surface and utilized as a binding probe in the quantitative detection of various IgG molecules. Porous SiO$_2$ has been used to optically monitor the adsorption of BSA, and it has been demonstrated that a significant amount of BSA will stick to the porous SiO$_2$ surface in aqueous conditions.

3.4.3 BSA adsorption to porous SiO$_2$ surface

It has been observed that certain proteins, including BSA, adsorb strongly to glass, porous Si, and porous SiO$_2$ surfaces. We thought that strongly-adsorbed BSA would provide convenient features for the fabrication of a biosensor.
Figure 3.1 Porous SiO$_2$ interferometric biosensor preparation and operation. (a) A PSiO$_2$ film is prepared. (b) The sample is submerged in a solution of BSA in pH 4.0 buffer, and the pore walls become coated. (c) Excess BSA is rinsed off in PBS buffer (pH 7.4) and the peptide KAA is attached to the adsorbed protein. (d) Vancomycin is introduced and binds to the attached KAA.
First, BSA is commonly used in ELISA-type biosensors in order to inhibit non-specific binding of analytes to the sensor surface\(^8\),\(^38\),\(^39\). As such, a BSA-coated surface should act to prevent other biomolecules from non-specifically adsorbing. Second, the BSA provides a convenient means of robust functionalization of the surface that could be used for further modifications.

BSA is adsorbed to porous SiO\(_2\) by placing the sample into a solution of BSA for 24 hrs. While the sensor is constructed to operate in phosphate buffer saline (PBS, pH = 7.4), the adsorption of the initial BSA layer was determined to be far more efficient at pH 4. BSA is positively charged below its pI of 4.7\(^40\), and thus will interact more strongly with the electronegative porous SiO\(_2\) surface at the lower pH. One side effect of this loading scheme is that some BSA is weakly adsorbed at this pH. To remove the weakly-bound BSA the samples were soaked in PBS for 24 hours. BSA content was qualitatively confirmed by FTIR measurement, shown in figure 3.2. Particularly noted are the strong amide I and II stretches at 1650 and 1550, respectively and \(v_{\text{SiO}_2}\) stretching over the range 1080-1150\(^41\). An attempt to remove the BSA from the surface for quantification was made using surfactant-based Triton-X buffer, which is generally used to clean proteins from surfaces\(^38\),\(^39\). A Bradford coomassie-type assay was used to determine the extent of BSA removal from the surface. After soaking the BSA-coated chip in the buffer for 24 hours, only roughly 3-5 \(\mu\)g of material was observed to have come off the surface. FTIR of this sample (not shown) appeared essentially the same as the unsoaked sample, with strong amide I and II bands present, indicating that substantial amounts of BSA remained adsorbed to the surface. Clearly,
Figure 3.2 FTIR spectrum of BSA-coated porous SiO$_2$. Notable bands at 1550 and 1650 cm$^{-1}$ are attributed to amide II and I, respectively. $\nu_\text{SiO}_2$ is observed over the range 1080-1150 cm$^{-1}$. The sample was analyzed in diffuse reflectance mode.
the BSA remaining in the porous SiO₂ interferometer after soaking in PBS is quite strongly adsorbed to surface and capable of resisting removal with surfactant.

The adsorption of BSA onto porous SiO₂¹¹,³⁷ and Si³⁴-³⁶ has been studied extensively in the past. It has been determined that the interaction of BSA with porous SiO₂, and generally, glass, leads to a loss of tertiary structure, effectively denaturing the protein. Native BSA is known to undergo a pH-induced conformational change⁴⁰, and a potential consequence of denaturing the protein is that it loses its pH sensitivity. Since the biosensor is operated in pH 7 aqueous solution, it is especially important that the BSA remain inert under these conditions. Ultimately, the BSA coating on porous SiO₂ adsorbs strongly enough to reliably act as a non-specific binding inhibitor and a substrate for the attachment of molecules. The BSA is expected to remain inert under the conditions utilized in this study, thereby completely eliminating spurious signals due to events other than the specific binding of vancomycin.

3.4.4 Characterization of porous SiO₂ and BSA-loaded porous SiO₂ porosity and thickness

3.4.4.1 Gravimetric determination of porosity

The total porosity of unloaded porous SiO₂ samples was determined by gravimetric means. Details of the measurement are included in the experimental section. The porosity was measured to be 80 ± 4%. 
3.4.4.2 Spectroscopic liquid infiltration method (SLIM) analysis

SLIM has been used extensively in the past to measure the open porosity of porous Si and SiO$_2$ films$^{9,10,15}$. In short, the porous samples are filled with fluids of varying refractive indices, leading to changes in the optical thickness (OT) of the sample. The Bruggeman effective medium model is then utilized to solve for the thickness and porosity of the sample. Unloaded porous SiO$_2$ samples were measured to have an open porosity of 79 ± 2% and a thickness of 7920 ± 130 nm. SLIM was used to measure the porosity of the BSA loaded samples using only aqueous-based fluids (e.g., PBS, deionized water) in order to not substantially affect the adsorption condition of BSA during the measurement. A porosity of 73 ± 3% and thickness of 7800 ± 110 nm was obtained. The reduction of porosity indicates that some of the pore volume was occupied by adsorbed BSA.

3.4.5 Functionalization of BSA adsorbed onto the porous SiO$_2$ surface

The free carboxylic acid groups contained within the BSA molecule provided a convenient means towards attaching the specific target probe and relevant control molecules. The tripeptides used in this study were custom synthesized, and the details of their synthesis are contained in the experimental section. Three different sensor surfaces were prepared. The first utilized acetylated-L-Lysine-D-Alanine-D-Alanine peptide chains (Ac-KAA), which acted as a highly specific probe for vancomycin. Acetylated-L-Lysine-D-Alanine-D-Lactone (Ac-KALac) was used as a negative control. The presence of the lactone derivative acted to disrupt the hydrogen bonding scheme that allows vancomycin to strongly bind to Ac-KAA, thereby strongly reducing
or eliminating the capability for this binding to occur. Finally, the surface was functionalized with ethanolamine in order to test for specific effects of the chemical attachment process itself. Figure 3.3 provides an overview of the resulting surface chemistries after attachment of the various target probes.

3.4.6 Theoretical consideration of vancomycin loading in BSA-KAA samples

In order to better understand the potential vancomycin loading capacity of the sample, we calculated the total capacity of the porous SiO$_2$ interferometers. A coomassie-type Bradford assay was used to quantitatively measure the total BSA loading in the films. Samples were placed in a 5% sample of HF, thereby completely dissolving the SiO$_2$ and liberating the BSA. A total of 115 ± 10 µg was contained within the films. The Bradford assay was tested for compatibility with HF via controls and determined to accurately determine concentration of BSA in HF solutions. As previously reported, only a few micrograms of BSA came off of the surface in solutions of buffered surfactant, indicating that the vast majority of the BSA stays bound to the surface.

Based on the BSA content in the porous SiO$_2$ intereferometers, the total potential amount of vancomycin that can bind can be calculated. As mentioned before, the SiO$_2$-BSA films contain an average of 115 ± 10 µg of BSA. Given a molecular weight of roughly 66,000 g/mol for BSA, the total number of BSA molecules contained in a single interferometer is roughly 1.74 nmol. There is a total of 100 carboxylic acids available for the binding of Ac-KAA on a single BSA molecule, with 41 coming from aspartic acid residues, 58 coming from glutamic acid residues, and 1 coming from the
Figure 3.3 Schematic of functionalized biosensor surfaces. (a) BSA-KAA; (b) BSA-KALac; (c) BSA-ethanolamine; (d) detailed reference of Ac-KAA; and (e) detailed reference structure of Ac-KALac.
terminal end of the primary amino acid chain\textsuperscript{42-44}. As such, there are a potential of 174 nmol sites for attachment of Ac-KAA or control molecules. In the attachment scheme used (detailed in experimental), there is roughly a 30-fold excess of Ac-KAA in the solution used for coupling, allowing for complete coverage of the BSA-coated surface with Ac-KAA. If complete coverage were achieved, there would be 174 nmol of vancomycin binding sites on the interferometer surface. At a molecular weight of 1485 g/mol, one could expect a total adsorption of approximately 260 \(\mu\)g of vancomycin into the interferometer film. It should be noted that this is the theoretical upper limit of binding availability. It is unlikely that all of the carboxylic acids are functionalized or that all the sites functionalized with KAA are accessible to vancomycin. Furthermore, it is known that vancomycin does not bind quantitatively to this receptor. However, given the SLIM-measured open porosity and thickness of BSA-coated films, a total open volume of 0.001 cm\(^3\) is accessible within the interferometer after coating with BSA. If the volume of Ac-KAA is ignored, and complete binding is observed, then a local concentration of 252 mM of vancomycin would be contained within the pores.

### 3.4.7 Optical analysis of binding events within porous SiO\(_2\) samples

Adsorption of vancomycin can be monitored via changes in the optical thickness of the sample. Details of the optical setup are included in the experimental section. Briefly, a reflectivity spectrum arises from Fabry-Pérot interference occurring from reflections at the top and bottom interfaces of the porous SiO\(_2\) interferometer. The maxima of this interferogram is governed by the following relationship:

\[ m\lambda = 2nL \quad (3.7) \]
where \( m \) is fringe order, \( \lambda \) is wavelength of incident light, \( n \) is refractive index, and \( L \) is the physical thickness of the hybrid film. The quantity “\( nL \)” is referred to as the optical thickness (OT) of the interferometer. Changes in either \( n \) or \( L \) lead to a shift in the interference maxima, resulting in a change in the OT\(^8,12,45\). As vancomycin binds to the Ac-KAA receptors within the porous SiO\(_2\) film, the composite refractive index of the sensor increases, leading to an increase in the OT.

### 3.4.8 Binding of Vancomycin to functionalized BSA-coated porous SiO\(_2\) surfaces

Vancomycin is known to bind to Ac-KAA with a dissociation constant in the range of 1-100 \( \mu \text{M} \)^{21,26}. It was expected that dosing with vancomycin on Ac-KAA functionalized surfaces would lead to an increase in OT; however, it is important to quantify, if possible, the response of porous SiO\(_2\) interferometric biosensors that were functionalized with other molecules in order to test for specificity and inhibition of non-specific binding (NSB). It is especially important that any general attachment scheme prevents NSB, as this will lead to false positives. One advantageous aspect of the BSA coating is that it simultaneously provides a surface that is resistant to NSB while still providing functionality as a linker molecule. The porous SiO\(_2\) surface was functionalized with a number of different species in order to test the behavior of biosensing on these surfaces. Because the concentration of vancomycin solutions used in this study was so low, the refractive indices of these solutions did not differ greatly from the background refractive index of PBS. Table 3.1 contains the measured refractive indices of solutions used in the biosensor study. As such, a change in OT was
Table 3.1 Refractive index values of solutions used in biosensor study

<table>
<thead>
<tr>
<th>Solution</th>
<th>[vancomycin] (µg/mL)</th>
<th>[vancomycin] (µM)</th>
<th>Refractive Index&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>1.3356 ± 0.0002</td>
</tr>
<tr>
<td>1</td>
<td>3.13</td>
<td>2.1</td>
<td>1.3356 ± 0.0002</td>
</tr>
<tr>
<td>2</td>
<td>6.25</td>
<td>4.2</td>
<td>1.3357 ± 0.0002</td>
</tr>
<tr>
<td>3</td>
<td>12.5</td>
<td>8.4</td>
<td>1.3359 ± 0.0002</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>16.8</td>
<td>1.3360 ± 0.0002</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>33.6</td>
<td>1.3362 ± 0.0002</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>67.2</td>
<td>1.3364 ± 0.0002</td>
</tr>
</tbody>
</table>

<sup>a.</sup> Refractive index values as measured
<sup>b.</sup> phosphate buffered saline
expected only when there was a concentration of vancomycin in the porous SiO$_2$ film via either specific or non-specific adsorption. Figure 3.4 demonstrates the optical response of the porous SiO$_2$ biosensor to dosing with solutions of phosphate buffered saline (PBS) 16.8 µM in vancomycin. The biosensor chips were placed in a custom flow cell, and a constant flow of 250 µL/min. was established. The sophistication of the sensor functionalization increases along the y-axis. Sample (a) is a blank porous SiO$_2$ sample (porous SiO$_2$). The sensor exhibited no optical response to the vancomycin solution, indicating that vancomycin did not concentrate appreciably within the pores. Similar trends were observed for samples functionalized with BSA (b; SiO$_2$-BSA), Ethanolamine functionalized BSA (c; BSA-eth), and Ac-KALAc functionalized BSA (d; BSA-KALac). Trace e demonstrates the response of a BSA-coated porous SiO$_2$ sample functionalized with Ac-KAA (BSA-KAA). Upon dosing with a 16.8 mM solution of vancomycin, an increase on the order of 6 nm was observed, indicating that vancomycin had adsorbed appreciably to the surface leading to increase in the OT (see eq. 3.7).

For samples a-c the lack of sensor response is attributed to a lack of interaction between vancomycin and the respective surfaces. In previous work involving loading of vancomycin into porous Si films, it was observed that the surface chemistry has a substantial effect on the adsorption and trapping of the molecule within the nanostructure $^{46}$. Vancomycin was found to interact most appreciably with surfaces exhibiting an ambiphilic behavior. The hydrophilic porous SiO$_2$, SiO$_2$-BSA, and BSA-eth surfaces did not provide the appropriate environment for the appreciable concentration of vancomycin. Furthermore, the fact that vancomycin did not bind to
Figure 3.4 Optical response of various surfaces to dosing with a 16.8 µM solution of vancomycin. (a) neat porous SiO₂; (b) BSA-coated porous SiO₂ (SiO₂-BSA); (c) ethanolamine functionalized BSA coated porous SiO₂ (BSA-eth); (d) KALac functionalized BSA coated porous SiO₂ (BSA-KALac); and (e) KAA-functionalized BSA coated porous SiO₂ (BSA-KAA). All samples were analyzed under a constant flow of 250 µL/min in a custom flow cell.
BSA-eth surfaces indicates that the covalent modification of the adsorbed BSA itself did not alter the sensor surface in a fashion that promotes the non-specific binding of vancomycin. For BSA-KALac samples, the lack of sensor response is theorized to be due to the disruption of the hydrogen bonding motif that is described for vancomycin-KAA association. Vancomycin forms a total of 5 hydrogen bonds with KAA, through the carbonyls, secondary amines, and terminal carboxylic acid moieties on KAA. The lactone derivative replaces an amide bond with an ester bond. The amine that is present in KAA is replaced with an oxygen, leading to a reversal of the electrostatic interaction. Previous work with BSA-coated porous SiO₂ interferometric sensors functionalized with Ac-KAA demonstrated the ability of this class of sensor to also inhibit false positives due to non-specific binding of a different analyte. Perelman and coworkers used solutions of erythromycin at significantly higher concentrations than the vancomycin solutions used in this study to test the sensor response of the BSA-KAA interferometers in their study. Erythromycin was observed to not bind to the surface, and a negative sensor response was noted.

3.4.9 Optical response of BSA-coated porous SiO₂ interferometer

functionalized with KAA

Dosing BSA-KAA surfaces with solutions of vancomycin led to an increase in the OT of the sample, as observed in figure 3.4. Figure 3.5 demonstrates the response of BSA-KAA sensors to solutions of various concentrations of vancomycin. All sensors displayed the same response behavior; that is, a marked increase in OT is observed upon dosing with vancomycin. Samples were dosed until a stable OT value was
Figure 3.5 Optical response of KAA functionalized BSA coated porous SiO$_2$ to various dosing concentration of vancomycin. All samples were analyzed under a constant flow of 0.25 mL/min in a custom flow cell.
achieved for approximately 35 minutes, after which the vancomycin was rinsed off of the surface with PBS. A slow decrease in the OT was observed at this point, which is characteristic of weakly binding biomolecule systems. Samples dosed in the range of 2.1 – 16.8 µM were observed to return to baseline with sufficient rinsing (not shown), after which no further changes in OT were noted, indicating the stability and reversibility of these samples in biosensing experiments. The samples dosed with higher concentrations of vancomycin exhibited very slow decrease in OT after roughly 7200 s, and these samples were not characterized for reversibility.

A plot of average OT shift vs. concentration, a Langmuir plot, is shown in figure 3.6. Previous work on porous SiO$_2$ interferometers involving the quantitative determination of binding strength between protein A-IgG demonstrated the ability to use a Langmuir plot to measure fundamental binding constants using changes in OT$^{10,12}$. A rigorous explanation can be found in Schwartz and coworkers study, and a basic overview is contained in the experimental section of this work. A calculated fit to this data yields a value for $K_d$, the dissociation. In this study, $K_d$ was calculated to be $17.9 \pm 3.8 \mu$M, consistent the previously measured range of 1-100 µM$^{21,26}$. This measurement of this binding constant is especially remarkable due to the small size of vancomycin (~1485 g/mol) compared to typical biomolecules sensed on porous Si-based interferometers, which are typically 1-2 orders of magnitude larger$^{8,10,12}$. Furthermore, the weaker binding of vancomycin to Ac-KAA makes this biosensor platform even more powerful in elucidating fundamental binding constants of smaller or weaker binding analyte-target probe systems. This methodology provides a very simple means to attaching target probes and inhibiting NSB. Porous Si-based interferometers
Figure 3.6 Change in optical thickness of a KAA functionalized BSA coated porous SiO₂ interferometer vs. concentration of vancomycin. The quantity ΔOT is obtained from the steady-state optical thickness change of the interferometer after dosing with vancomycin. Each data point represents the average of 5 trials. The calculated $K_d$ value is $17.9 \pm 3.8 \, \mu$M. ($K_a = 5.6 \times 10^4$)
are significantly less expensive than competing platforms, such as SPR while still capable of measuring smaller molecules. This method can be applied to any target probe containing an available primary amine for coupling. BSA also contains 60 lysine residues, 41 asparagine residues, 26 arginine residues and 21 glutamine residues\(^{43}\), all which terminate in a primary amine, potentially allowing for the coupling of compounds containing reactive carboxylic acids as well. The sulfur-containing cysteine (35) and methionine (5) residues\(^{43}\) could perhaps be used to attach gold nanoparticles or other chemistries. Furthermore, it may potentially improve upon porous Si-based measurements that have been made in the past due to its unique architecture.

### 3.5 Conclusions

BSA was non-specifically adsorbed to the surface of a porous SiO\(_2\) interferometer and utilized for the attachment of acetylated-L-Lysine-D-Alanine-D-Alanine fragments for the specific detection of vancomycin binding events. The interferometer was observed to inhibit the non-specific binding of vancomycin unless specifically functionalized with the Ac-KAA target probe. Optical measurements indicate an excellent degree of selectivity and sensitivity of the sensor for vancomycin, as well as stability during flow cell experiments. The binding strength between vancomycin and Ac-KAA is quantified via calculations derived from a Langmuir plot of the data. This technique provides a general means for the attachment of primary-amine containing compounds and a new strategy for the biosensing of smaller molecular weight biomolecules. It is possible that
this technique could be extended to the attachment of other chemistries via the other functional groups contained on the peptides contained within BSA.

3.6 REFERENCES


CHAPTER 4

CONFINEMENT OF THERMORESPONSIVE HYDROGELS IN

NANOSTRUCTURED POROUS SILICON DIOXIDE TEMPLATES
4.1 Abstract

A thermoresponsive hydrogel, poly(N-isopropylacrylamide) (poly(NIPAM)), is synthesized in-situ within an oxidized porous Si template, and the nanocomposite material is characterized. Infiltration of hydrogel into the interconnecting nanometer-scale pores of the porous SiO$_2$ host is confirmed by scanning electron microscopy. The optical reflectivity spectrum of the nanocomposite hybrid displays Fabry-Pérot fringes characteristic of thin film interference, enabling direct, real-time observation of the volume phase transition of the confined poly(NIPAM) hydrogel. Reversible optical reflectivity changes are observed to correlate with the temperature-dependent volume phase transition of the hydrogel, providing a new means of studying nanometer-scale confinement of responsive hydrogels. The nano-confined hydrogel displays a swelling and shrinking response to changes in temperature that is significantly faster than for the bulk hydrogel. The porosity and pore size of the SiO$_2$ template, which are precisely controlled by the electrochemical synthesis parameters, strongly influence the extent and rate of changes in the reflectivity spectrum of the nanocomposite. The observed optical response is ascribed to changes in both the mechanical and the dielectric properties of the nanocomposite.

4.2 Introduction

Porous Si has emerged as an attractive material for use as a nanometer-scale template$^{1-5}$ because its porosity and the average pore diameter can be easily tuned by
adjusting the electrochemical preparation conditions. Porous Si is particularly suited for the fabrication of complex optical materials such as Fabry-Pérot films, photonic crystals, dielectric mirrors, and microcavities, and optical nanostructures consisting of composites of porous Si with various polymers have been demonstrated. Polymers have been placed in a porous Si template by in-situ polymerization, injection-molding, or solution casting. The potential of such hybrid materials in sensing and in controlled release drug delivery (with the free-standing films after removal from the crystalline Si substrate) has been demonstrated.

Current research efforts have been focused on the use of templates and matrices for the fabrication of stimuli-responsive hydrogels. Hydrogels are three-dimensional cross-linked polymer networks capable of undergoing a reversible volume change in response to different environmental stimuli, such as temperature, pH and solution composition. Such reversible volume changes make hydrogels excellent candidate materials for various applications including sensing, drug delivery and microfluidics. While the extent of swelling or shrinking of the hydrogels may be large, the kinetics of such changes is slow, limiting their practical application. Response times of hydrogels can be significantly reduced (from several hours to <1 min) by reducing their dimensions. This has been achieved by imprinting an interconnecting porous structure in hydrogels with silica colloidal crystal templates or by integrating hydrogels or hydrogel nanocomposites into microelectromechanical systems (MEMS).

Recently, DeLouise et al. demonstrated that a porous Si microcavity supported in a hydrogel matrix (commercially available wound dressing sheets) can be
used to detect the presence of small molecules such as sucrose. The hydrogel-supported microcavity is sensitive to a $10^{-4}$-$10^{-3}$ change in refractive index and can detect the binding of small molecules when they cause a sufficient change in the matrix porosity. However, the hydrogel is not incorporated into the Si nanostructure, limiting the ability of the device to report on the state of the entire system.

Poly(N-isopropylacrylamide) (poly(NIPAM)) is one of the most extensively studied materials in the field of responsive polymers and hydrogels. Poly(NIPAM) exhibits an abrupt volume phase transition upon heating above 31–34°C. The polymer undergoes a temperature-induced collapse from an extended coil conformation to a globular structure, resulting in a substantial decrease in volume. In this paper, we present the fabrication and characterization of porous SiO$_2$/hydrogel hybrids. Thermoresposive poly(NIPAM) hydrogel is synthesized in-situ within a nanostructured porous SiO$_2$ template. The resulting composite is a hybrid of the two materials, combining the unique optical properties of porous Si and the thermal responsiveness of the hydrogel. This hybrid enables the use of porous Si as an optical transducer in monitoring the state of the hydrogel during heating and cooling cycles. Furthermore, the hybrid demonstrates promise in applications ranging from drug delivery, actuation, and biosensing.

4.3 Experimental

4.3.1 Materials

Aqueous HF (48%) and ethanol (99.9%) were supplied by Fisher Scientific and AAper, respectively. Porous Si samples were prepared from single crystalline highly-
doped p-type Si (0.0009 $\Omega$-cm resistivity, $\langle100\rangle$ oriented, B-doped, from Siltronix Corp.) N-isopropylacrylamide (NIPAM) and 1,4-dioxane were obtained from Aldrich. N,N’-methylenebis(acrylamide) (BIS) was obtained from Fluka. Reagent grade benzoyl peroxide (97%, Sigma Aldrich Chemicals) was purified by recrystallization from ethanol. All other reagents were analytical grade and were used as received.

### 4.3.2 Etching procedure

Porous Si samples were prepared by anodization of a highly doped p-type Si wafers in ethanolic HF solution (3:1 v/v 48% aqueous HF:ethanol) using a two-electrode configuration with a platinum mesh counter-electrode. CAUTION: hydrofluoric acid is toxic and highly irritating to the skin; prompt medical attention should be obtained if the liquid contacts the skin or if the vapors are inhaled. Si wafers with an exposed area of 1.33 cm$^2$ were contacted on the back side with a strip of aluminum foil and mounted in a Teflon etching cell. Galvanostatic anodization was performed in the dark. The different etching conditions used for preparing the porous Si layers are summarized in Table 1. After etching, the samples were rinsed thoroughly with ethanol and hexane and then dried under a stream of nitrogen.

### 4.3.3 Thermal oxidation

Freshly etched porous Si samples were thermally oxidized by heat treatment in a tube furnace (Fisher Blue M). The samples were heated at 800 °C for 1 h in ambient air, and then allowed to cool to room temperature.
4.3.4 Preparation of poly(NIPAM) and porous SiO$_2$/poly(NIPAM) hybrids

Poly(NIPAM) hydrogels were synthesized by free-radical polymerization of NIPAM monomer using BIS as the cross-linking agent. NIPAM and BIS were dissolved in 1,4 dioxane at a total concentration of 0.9 mol/L, and the NIPAM:BIS concentration ratio was kept constant at 110. The pre-gel solutions were deoxygenated by bubbling with nitrogen gas for 30 min. The reaction was initiated with benzoyl peroxide and carried out at 70 °C for 24 hr. The resulting gels were soaked in Milli-Q water (18.2 MΩ), rinsed thoroughly over a period of five days and allowed to reach the equilibrium swelling state. Porous SiO$_2$/poly(NIPAM) hybrids were prepared by casting the pre-gel solution described above onto the porous SiO$_2$ sample. The sample was then covered with a glass slide to minimize the amount of free polymer above the porous template layer and allowed to polymerize at 70 °C for 24 hr. The resulting hybrids were carefully soaked in Milli-Q water, rinsed thoroughly over a period of five days and allowed to reach an equilibrium swelling state.

4.3.5 Scanning electron microscopy

Scanning electron microscope (SEM) images were obtained using a Hitachi S-4800 UHR field emission – SEM, at an accelerating voltage of 1 keV or a FEI Quanta 600 environmental SEM at an accelerating voltage of 20 keV. Porous SiO$_2$/hydrogel samples (swollen in water) were prepared for observation by freeze fracturing the hybrid in liquid nitrogen followed by freeze drying.
4.3.6 Gravimetric determination of porosity

Three porous SiO$_2$ samples were weighed on a laboratory microbalance to obtain an initial mass ($m_1$). The oxidized porous layer was then dissolved in an ethanolic HF solution (3:1 v/v 48% aqueous HF:ethanol), and the sample was weighed again ($m_2$). The porosity ($P$) was calculated using the following equation:

$$P = \frac{V_{\text{total}} - V_{\text{SiO}_2}}{V_{\text{total}}} \quad (4.1)$$

$V_{\text{total}}$, which is the total volume of the porous SiO$_2$ layer, was determined by:

$$V_{\text{total}} = St \quad (4.2)$$

where $S$ is the wafer area exposed to HF solution during the electrochemical etching and $t$ is thickness of the porous SiO$_2$ layer, determined by cross-sectional SEM measurement.

$V_{\text{SiO}_2}$, which is the volume of the SiO$_2$ fraction in the porous SiO$_2$ layer, is calculated from eq. 4.3:

$$V_{\text{SiO}_2} = \frac{m_1 - m_2}{d} \quad (4.3)$$

where $d$ is the density of bulk SiO$_2$, taken as 2.6 g/cm$^3$.

4.3.7 Determination of surface area and pore dimensions using the BET method

Nitrogen adsorption-desorption isotherms of porous SiO$_2$ templates were recorded at 77 K using a Micromeritics ASAP 2010 volumetric apparatus. Prior to the adsorption experiment, the samples were outgassed in-situ at 323 K until a static
vacuum of $5 \times 10^{-5}$ Torr was reached. Nitrogen doses were admitted and the adsorbed amount was registered as a function of the equilibrium pressure. The surface area of the sample was measured by the BET method, which yields the amount of adsorbate corresponding to a molecular monolayer. The specific surface area of the porous template and the porous volume are expressed per unit area of porous SiO$_2$ sample.

The pore dimensions were determined by using the BdB method from the nitrogen adsorption curve. The adsorption curve, albeit less often used than the desorption curve for pore size determination, presents the significant advantage of being independent of pore mouth constrictions. In the determination of the pore size distribution, the thickness of the adsorbate layer prior to adsorption was assumed to correspond to the thickness of the adsorbate layer on a non-porous fumed silica Aerosil 200. The mesopore volume was measured as the adsorbed volume at the top of the capillary condensation step of the isotherm.

### 4.3.8 Measurement of interferometric reflectance spectra

Interferometric reflectance spectra of porous SiO$_2$/hydrogel hybrids were collected using an Ocean Optics CCD S-2000 spectrometer fitted with a microscope objective lens coupled to a bifurcated fiber optic cable. A tungsten light source was focused onto the center of the porous SiO$_2$/hydrogel sample surface with a spot size approx. 1-2 mm$^2$. Reflectivity data were recorded in the wavelength range 400-1000 nm, with a spectral acquisition time of 100 ms. Both illumination of the surface and detection of the reflected light were performed along an axis coincident with the surface normal. Thermal cycling of the porous SiO$_2$/hydrogel hybrids was carried out using a
laboratory hotplate. The samples were immersed in water and the temperature of the wafer was measured using a thermocouple attached to the wafer surface. The light beam was focused on the surface of the sample and interference spectra were recorded. The samples were monitored at room temperature for an hour and then the temperature was raised to 47°C. Temperature was held at 47°C for 10-20 min and then the samples were allowed to cool to room temperature. Typically 3-5 consecutive heating/cooling cycles were performed.

4.3.9 Determination of porosity and film thickness by spectroscopic liquid infiltration method (SLIM)

Template porosity and thickness were quantified by measurement of the reflectivity spectrum as a function of liquid infiltration. The optical thickness (OT) of the sample is determined from the interferometric reflectance spectrum of the porous film in air and immersed in the solvents hexane, ethanol, acetone, and the pre-gel solution, having refractive indices of 1.372, 1.359, 1.357 and 1.426, respectively. The differences between the spectra are attributed to the change in optical thickness as the medium in the pores changes, with the assumption that all void spaces are filled equally. The data are then fit to a two-component Bruggeman effective medium approximation 38, 39. A fit to the Bruggeman equation yields a unique solution for both the porosity and the thickness of the sample. Refractive indices for the different solvents and solutions were measured using a Milton Roy Refractometer. The refractive index of the SiO₂ portion of the film is assumed to be 1.455.
4.3.10 Determination of optical thickness

The wavelength axis of the spectrum from the Ocean Optics spectrometer was calibrated using a least-squares fit of five spectral lines observed from a neon lamp, at 585.3, 614.3, 640.2, 703.2, and 811.5 nm. The data spacing of the spectrometer is approximately 0.4 nm. The x-axis was inverted and a linear interpolation was applied such that the data were spaced evenly in units of nm\(^{-1}\). A Hanning window was applied to the spectrum, it was redimensioned to 4096 data points and zero padded to the power of two. A discrete Fourier transform using a multidimensional fast prime factor decomposition algorithm from the Wavemetrics, Inc (www.wavemetrics.com) IGOR program library (FFT) was applied. The Fourier transform of the spectrum yields a peak whose position on the x-axis corresponds to the value of 2nL in eq. 4.5.

4.4 Results and Discussion

4.4.1 Preparation of porous SiO\(_2\)/hydrogel hybrids

The synthesis scheme followed to produce porous SiO\(_2\)/hydrogel hybrids is outlined in Figure 4.1. The porous Si template is prepared from a highly doped p-type single-crystal Si wafer polished on the (100) face using an anodic electrochemical etch. By varying the conditions of anodization, e.g., etching time and current density, the thickness and porosity of porous Si layers can be controlled with a high degree of precision\(^{40, 41}\). In this study, four types of porous Si samples with differing porosities and pore dimensions, but with approximately equal thicknesses were prepared. The etching conditions used to prepare each layer are summarized in Table 4.1. The
Figure 4.1 Synthesis of porous SiO₂/hydrogel hybrids. A porous Si layer is prepared by anodic electrochemical etch of a single-crystal Si wafer. The freshly-etched sample is then thermally oxidized at 800°C. A pre-gel solution is cast onto the porous SiO₂ sample. The sample is then covered with a glass slide, purged with nitrogen and allowed to polymerize at 70°C for 24 hr. The resulting hybrids are carefully soaked in Milli-Q water, rinsed thoroughly over a period of five days and allowed to reach equilibrium swelling state at room temperature.
Table 4.1 Thickness and porosity of thermally oxidized porous Si layers$^a$

<table>
<thead>
<tr>
<th>Etching Conditions</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current Density</td>
<td>Etch Time</td>
</tr>
<tr>
<td>[mA/cm²]</td>
<td>[s]</td>
</tr>
<tr>
<td>77</td>
<td>150</td>
</tr>
<tr>
<td>192</td>
<td>60</td>
</tr>
<tr>
<td>385</td>
<td>30</td>
</tr>
<tr>
<td>577</td>
<td>20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spectral Measurement$^b$</th>
<th>Gravimetry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness [nm]</td>
<td>Open Porosity [%]</td>
</tr>
<tr>
<td>8880 ± 120</td>
<td>46 ± 5</td>
</tr>
<tr>
<td>7700 ± 190</td>
<td>61 ± 7</td>
</tr>
<tr>
<td>7520 ± 230</td>
<td>69 ± 6</td>
</tr>
<tr>
<td>7050 ± 260</td>
<td>80 ± 7</td>
</tr>
</tbody>
</table>

$^a$Thickness and porosities determined by SEM, by spectral reflectivity measurements and by gravimetry. All samples have been thermally oxidized in air at 800°C. $^b$For the spectral measurement, calculation of the of the porosity and thickness is based on the application of the Bruggeman approximation to the values of optical thickness obtained from FFT of the reflectivity spectra of samples immersed in various liquids, as described in the experimental section.
resulting freshly etched porous Si sample is then thermally oxidized at 800°C to create a hydrophilic porous SiO₂ matrix. The oxidation process also helps to protect the porous structure from degradation in aqueous media ²⁷, ³⁹, ⁴².

4.4.2 Characterization of porosity and thickness of porous SiO₂ templates

The thickness and porosity of the porous SiO₂ layers are determined by SEM (thickness only), optical measurements and gravimetry. The average values are summarized in Table 4.1. Cross-sectional SEM images reveal that the etching conditions used to prepare the different layers produces films ranging from 6750 to 8800 nm in thickness. An image of a typical layer after thermal oxidation is shown in Figure 4.2. The porous SiO₂ layer is approximately 6800 nm thick (Figure 4.2a) with interconnecting cylindrical pores ranging in diameter from 60 nm to 100 nm (Figure 4.2b). The morphology of the three other types of porous films is similar (not shown); however, the pore dimensions are dependent on the current density used in the etch. The average pore diameters as observed by SEM for the different films are summarized in Table 4.1. Pore diameter generally increases with increasing current density. These results are in agreement with previous studies, in which an approximately exponential dependence of the pore diameter on current density was found for highly doped p-type samples ⁴, ³⁹, ⁴³.
Figure 4.2 Cross-sectional scanning electron micrographs (secondary electron images) of a thermally oxidized porous Si layer. (a) Interface between the porous SiO$_2$ layer (etched for 30 s at 385 mA/cm$^2$) and the single crystal Si substrate. (b) Higher magnification image of the porous SiO$_2$ layer, revealing the pore morphology.
4.4.3 Spectroscopic liquid infiltration method (SLIM) for determination of thickness and porosity

Sample porosity and thickness can be quantified by measurement of the reflectivity spectrum as a function of liquid infiltration. The optical thickness (OT) of the sample is determined from the interferometric reflectance spectrum for each filling liquid. The data are then fit to a two-component Bruggeman effective medium approximation. The refractive index of the SiO$_2$ layer is assumed to be 1.455, and the values of the refractive indices of the different liquids are obtained from the literature. The value of the open porosity (pore volume accessible to the probe molecule) $^{37}$ and the sample thickness are determined from the fit. This model has been shown to predict the porosity and thickness of porous Si and oxidized porous Si in reasonable agreement with gravimetric determinations $^{38,39,44}$. The average calculated values for the porosity and thickness of the different layers are presented in Table 4.1. The estimated porosity of the films increases with increasing the current density, with values in the range 46-80%. The calculated thickness of the different layers obtained from the Bruggeman calculations agree with the SEM measurements.

4.4.4 Comparison of gravimetric measurement with SLIM for porosity determination

Porosity can also be determined by gravimetric measurement, performed by weighing the oxidized sample before and after removal of the porous layer. The porosities determined by gravimetry are in the range of 66-86% (Table 4.1). These
values are larger than those computed using the spectroscopic liquid infiltration method (SLIM). The deviation is more significant for samples etched using lower current densities. For example, the calculated porosity based on gravimetric measurements yields a value of $66 \pm 3\%$ for layers etched at a current density of $77$ mA/cm$^2$ for 150 s, while a value of $46 \pm 5\%$ is obtained from SLIM. The observed deviation can be attributed to the existence of voids that are not accessible to the infiltrating liquids. Thus, the SLIM method measures the open porosity (pore volume accessible to the probe molecule), while the gravimetric method measures both the open and the closed porosity $^{45}$. Liquid may be excluded from the closed pores for two possible reasons: the pores may be physically closed due to sintering and pore fusion that occurs during the thermal oxidation step $^{46,47}$, or the surface tension of the liquid may be too high to adequately wet or infiltrate the smallest pores. Since the hydrogel used in the present study is only synthesized where solvent (pre gel solution) infiltrates, the SLIM porosity values provide a more realistic measure of the accessible volume within the nanostructure.

**4.4.5 BET measurements of porous SiO$_2$ templates**

Table 4.2 summarizes physical characteristics of the porous SiO$_2$ templates as determined by measurement of nitrogen adsorption isotherms and application of the BET (Brunnauer-Emmett-Teller) and BdB (Broekhof-de Boer) methods.$^{35,36,48}$ The average diameters of the pores measured with this technique are significantly lower than the visual estimates obtained from the SEM images (see Table 4.1), but the trend of increasing pore size with increasing etch current density is still observed. This
Table 4.2 Physical characteristics of porous SiO$_2$ layers measured by nitrogen adsorption measurements.

<table>
<thead>
<tr>
<th>Etching Conditions</th>
<th>BET</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Current Density</td>
<td>Etch Time</td>
<td>Average Pore Diameter</td>
<td>Porous Volume$^a$</td>
<td>Specific Surface$^b$</td>
</tr>
<tr>
<td>[mA/cm$^2$]</td>
<td>[s]</td>
<td>[nm]</td>
<td>[cm$^3$ STP/cm$^2$]</td>
<td>[m$^2$ STP/cm$^2$]</td>
</tr>
<tr>
<td>77</td>
<td>150</td>
<td>12.5</td>
<td>0.235</td>
<td>0.160</td>
</tr>
<tr>
<td>192</td>
<td>60</td>
<td>17.6</td>
<td>0.236</td>
<td>0.110</td>
</tr>
<tr>
<td>385</td>
<td>30</td>
<td>31.0</td>
<td>0.275</td>
<td>0.079</td>
</tr>
<tr>
<td>577</td>
<td>20</td>
<td>-</td>
<td>0.292</td>
<td>0.050</td>
</tr>
</tbody>
</table>

$^a$The porous volume is expressed per unit area of porous SiO$_2$ sample. $^b$The specific surface is expressed per unit area of porous SiO$_2$ sample.
discrepancy is probably related to the shape of the pores, as the methods to evaluate pore size from the condensation pressure assume an exactly cylindrical pore section. Moreover, \( \text{N}_2 \) adsorption methods are especially sensitive to small pore structures, in particular micropores (radii below 1 nm)\(^{46} \) that are not observable in the SEM micrographs. The micropores are emphasized in the BET measurement, which is relatively insensitive to macropores. Indeed, for the porous SiO\(_2\) samples with the largest pore dimensions, i.e., etched at 577 mA/cm\(^2\), the changes in relative pressure are too small to allow reliable determination of the average pore size using the BET approach. The BET method also provides a convenient means to measure the porous volume and specific surface area of the porous SiO\(_2\) templates. With increasing etch current density a decrease in the specific surface area and a minor increase in the porous volume is observed.

**4.4.6 In-situ synthesis of poly(NIPAM) in porous SiO\(_2\) templates**

The porous SiO\(_2\) nanostructure can act as a template for the in-situ polymerization of N-isopropylacrylamide (NIPAM) cross-linked with \( \text{N,N'\text{-}} \) methylenebis(acrylamide) (BIS). Pre-gel solutions, containing NIPAM, BIS and benzoyl peroxide as a free-radical initiator, all dissolved in 1,4 dioxane, are cast onto the porous SiO\(_2\) film and polymerized at 70°C for 24 hr (eq 4.4)
The obtained porous SiO$_2$/polymer gel hybrid is thoroughly rinsed with water over a period of five days and allowed to reach equilibrium swelling state at room temperature. The morphology of the porous SiO$_2$/poly(NIPAM) hybrid is depicted in Fig. 4.3. It appears that the thickness of the hydrogel layer infused in the pores is approx. 5000 nm, while the average thickness of the porous SiO$_2$ film is ~6800 nm (see Fig. 2a and Table 1). However, indirect evidence from reflectivity spectra (discussed below) indicates that water-swollen samples completely fill the porous network, and so the appearance of the image may be a result of freeze-fracturing and freeze-drying during sample preparation.
Figure 4.3 Cross-sectional scanning electron micrograph (back-scattered electron image) of porous SiO$_2$/poly(NIPAM) hybrid. The porous SiO$_2$ layer is etched for 30 s at 385 mA/cm$^2$. The poly(NIPAM) hydrogel is infused in the porous SiO$_2$ layer, partially filling the pores. The image shows just the bottom part of the hydrogel/porous SiO$_2$ composite and the interface between porous SiO$_2$ and the un-etched Si substrate.
4.4.7 Optical properties of oxidized porous SiO$_2$/poly(NIPAM) hybrids during thermal cycling

Information about the state of the poly(NIPAM) hydrogels contained in porous SiO$_2$ can be obtained by monitoring changes in the reflectivity spectrum of the hybrid. A complete overview of the experimental setup is detailed in the experimental section. Briefly, the reflectivity spectrum consists of a series of interference fringes that result from Fabry-Pérot interference at the top and bottom of the hybrid layer. The maxima of these fringes are governed by the following relationship:

$$m\lambda = 2nL$$  \hspace{1cm} (4.5)

where $m$ is an integer, $n$ is the average refractive index, $L$ is the thickness of the film and $\lambda$ is the wavelength of the incident light. The term $nL$ is referred to as the optical thickness (OT) in this work. A change in either $n$ or $L$ leads to a shift in the reflectivity spectrum and a change in OT. Accordingly, the volume phase transition of the hydrogel phase produces changes in the hybrid's refractive index and volume, which can result in a shift of OT. Thus, the optical properties of the porous SiO$_2$ template provide the basis for monitoring real-time changes in the porous SiO$_2$/hydrogel hybrid.

Figure 4.4 depicts changes in OT upon thermal cycling of a porous SiO$_2$/poly(NIPAM) hybrid. This porous SiO$_2$ template was obtained by etching Si at a current density of 577 mA/cm$^2$ for 20 seconds. The hybrid was thermally cycled between 24°C and 47°C. Three consecutive heating-cooling cycles were performed. Figure 4a shows that OT of the hybrid increases as the temperature of the system
Figure 4.4 Optical thickness changes of a porous SiO$_2$/poly(NIPAM) hybrid upon three consecutive heating/cooling cycles. The porous Si layer was etched for 20 s at 577 mA/cm$^2$ (80% porous sample). a. Optical thickness changes and system temperature vs. time. b. Optical thickness changes vs. system temperature. The temperature of the system is measured by placing a thermocouple probe on the bulk Si substrate directly adjacent to the hybrid.
approaches 30°C. Upon achieving a system temperature of 30.5-30.8°C, an abrupt, dramatic decrease of approximately 534 nm in OT is observed. This significant change in OT occurs within the temperature range that the volume phase transition for poly(NIPAM) is observed. After this dramatic decrease in OT, further heating of the system results in a slow, minor increase in OT. The system reaches a plateau in OT at 40 °C, after which no changes are observed. Cooling of the system is accompanied by a slow decrease in OT. When the temperature reaches a temperature of 31.3–31.7°C, a dramatic and rapid increase in OT is observed. Again, this temperature is in the range wherein the volume phase transition of poly(NIPAM) occurs. Finally, as the system continues to cool to room temperature, a decrease in OT occurs until the OT returns to its original value, demonstrating high reversibility of the hybrid. The dramatic changes in OT observed during heating and cooling of the hybrid are attributed to the volume phase transition of the poly(NIPAM) hydrogel, as it collapses and expands, respectively. It should be emphasized that the heating and cooling rates are different due to the characteristics of the experimental setup.

The observed OT changes upon the three consecutive heating-cooling cycles are reproducible. Figure 4b depicts the OT changes as a function of the system temperature. Plotting the OT data in this fashion provides a convenient means for determining specific temperatures at which notable transitions occur. The curves retrace each other with a measured temperature difference between consecutive heating cycles of ~0.3°C, and the difference between cooling cycles is ~0.4°C. These deviations are within the bounds of experimental error. It is noted that the hysteresis-like behavior of the system differs from typical hysteresis curves. The transition during the heating step occurs at a
lower temperature than the transition during cooling. Such peculiar behavior was previously observed in the volume phase transition of bulk poly(NIPAM) hydrogels using scanning microcalorimetry\textsuperscript{34}. At large temperature ramp rates, the transition during the cooling cycle begins at temperatures larger than the volume phase transition displayed during heating\textsuperscript{34}. In the present study, the dramatic OT shifts observed for both the heating and cooling cycles of the experiment are separated by 0.5-1.2ºC.

The system temperature was measured by placing a thermocouple on the bulk Si wafer directly adjacent to the porous SiO\textsubscript{2} hybrid. Moreover, the experimental setup is open to the surrounding environment, allowing a temperature gradient to exist between the heat source and the point of measurement. As a result, the measured temperatures probably deviate somewhat (on the order of 1-2ºC) from the temperature within the hybrid. Thus, within the experimental error, the large OT transitions observed occur in the temperature range of 31-34ºC of the poly(NIPAM) volume phase transition previously reported\textsuperscript{31, 32, 34}. However, it is likely that the phase diagram for confined and surface constrained poly(NIPAM) composites are different than bulk poly(NIPAM)\textsuperscript{31, 33}.

\subsection*{4.4.8 Effect of porosity and pore size of the porous SiO\textsubscript{2}/poly(NIPAM) hybrid on observed optical thickness changes}

Figure 4.5 depicts changes in OT for porous SiO\textsubscript{2}/poly(NIPAM) hybrids measured upon thermal cycling. The four plots correspond to samples with the four different pore sizes indicated in Tables 1 and 2. The plots are arranged in order of increasing porosity and pore size. All four hybrids display a temperature-dependent OT.
Figure 4.5 Optical thickness changes of porous SiO$_2$/poly(NIPAM) hybrids observed upon thermal cycling. Pore sizes of the template increase from a-d. The porous Si sample preparations correspond to those presented in Tables 4.1 and 4.2: a. 150 s at 77 mA/cm$^2$, b. 60 s at 192 mA/cm$^2$, c. 30 s at 385 mA/cm$^2$, d. 20 s at 577 mA/cm$^2$. Samples are immersed in Milli-Q water, and the sample temperature and optical thickness are monitored as a function of time. Heat is applied at the time points labeled (a), (c), (e) and (g), and the temperature increases at a rate of ~1°C/min. until a temperature of 40°C is attained. Heat is turned off and the sample allowed to cool at time points marked (b), (d), (f) and (h).
However, there are differences in the magnitude of the various OT shifts observed during thermal cycling.

Figure 4.5a shows optical thickness changes for a hybrid in which the porous SiO$_2$ template is characterized by a porosity of 46% and an average pore diameter of 30 nm (see Table 4.1). During heating, a small but rapid increase in OT of approximately 0.12% is observed at ~30.2°C. At 30.7°C, the OT of the hybrid decreases. When the system is allowed to cool the hybrid undergoes a reversal in which the OT increases until the system temperature reaches 31.5°C at which point OT decreases, approaching its original OT value. Figure 4.5d depicts the same hybrid system as in 4.5a, except the porous SiO$_2$ template has larger porosity (80%) and larger average pore size (100 nm). Both systems exhibit an initial increase in OT upon heating. However, the sample with larger pores displays a distinct decrease in OT as the system temperature approaches the temperature of the volume phase transition. All samples with a pore size >40 nm and a porosity >50% (Fig. 4b-4d) show this decrease in OT at the temperature attributed to the volume phase transition of the poly(NIPAM) hydrogel, with the decrease most pronounced for the samples with larger pores and higher porosities.

Figures 4.5b and 4.5c show the OT response of hybrids containing porous templates with porosities of approximately 61% and 70% and average pore diameters of 55 nm and 80 nm, respectively. As the porosity of the template increases, the magnitude of the decrease in OT is more pronounced, with a measured decrease in OT of 0.8%, 3%, and 4% for porosities of 61%, 70%, and 80%, respectively. There are notable differences in the OT response curves over the range of template porosities studied.
This is attributed to the inhomogeneous nature of the poly(NIPAM) polymer in the hybrids. Moreover, coexistence between swollen and collapsed gel phases has been demonstrated for hydrogel systems that are under stress or tension due to anisotropic swelling in a rigid substrate \(^{33,49}\).

Figure 4.6a summarizes the effects of the template’s pore morphology on the observed decrease in OT. The value of $\Delta OT$ depicted on the vertical coordinate refers to the maximum difference in OT values observed below and above the temperature corresponding to the volume phase transition for poly(NIPAM).

A substantial linear decrease in OT is observed as the pore volume to surface area ratio of the porous SiO\(_2\) template increases (Figure 4.6a), indicating a strong relationship between the optical response of the hybrid to changes in temperature and the pore morphology of the template. The volume to surface area ratio of the porous SiO\(_2\) template increases as the etch current density increases (see Table 4.2). Hence, the optical response of the hybrid can be easily tuned at the template preparation stage. Previous work suggests that interactions between poly(NIPAM) hydrogel and a surface hinders the isotropic phase transition in the near-surface region \(^{31}\). Poly(NIPAM) hydrogels contained in the templates with lower porosity and smaller pore sizes should exhibit a higher degree of surface constraint and volume confinement. Thus it is expected that the samples made with the smaller pore templates will display a more limited change in OT and a slower rate of change during heating-cooling cycles.

Figure 4.6b depicts rate of change of $\Delta OT$ (d$\Delta OT$/dt) vs. the template's ratio of porous volume to specific surface area. The $\Delta OT$ data used to derive d$\Delta OT$/dt in this plot is the same as is used for Figure 4.6a. The value of d$\Delta OT$/dt is calculated as the
Figure 4.6 Effect of the volume:surface area ratio of the porous SiO$_2$ template (as determined by measurement of the nitrogen adsorption isotherms and application of BET model, see Table 2) on (a) the value of the change in OT, $\Delta OT$ and (b) the rate of change in $\Delta OT$ for the different porous SiO$_2$/poly(NIPAM) hybrids as the samples are heated through the volume phase transition. The value $\Delta OT$ refers to the maximum difference in OT measured in the 30-32 °C temperature range corresponding to the poly(NIPAM) hydrogel volume phase transition. The rate of change in OT ($d\Delta OT/dt$, plot b) is calculated as the maximal slope of the $\Delta OT$ versus time curve in the same temperature regime.
maximal slope of the ΔOT versus time curve (measured while heating the sample in the
temperature range 30-32°C). The rate of change in ΔOT dramatically increases with
greater volume to surface area ratio (note the logarithmic scale in Figure 4.6b).

Presumably the poly(NIPAM) hydrogel in the small pore templates undergoes a slower
volume phase transition due to its interaction with the pore surfaces (see Figure 4.3).
The volume phase transition of the hydrogel is hindered by this interaction, thereby
limiting the optical response to heating-cooling cycles. As the pore size increases, the
amount of “free” hydrogel that is capable of undergoing a volume phase transition
increases. Accordingly, this is observed in the optical measurements as a large increase
in the magnitude and rate of change in ΔOT. It should be noted that the rate of change
of the signal observed with the larger pore (> 40 nm) templates is very rapid compared
with the published rates attributed to the volume phase transition in bulk hydrogels.
This result is consistent with previous studies in which nanometer-scale hydrogel
domains are observed to undergo a similarly rapid volume phase transition\textsuperscript{9,26}.

4.4.9 Interpretation of optical thickness changes observed on thermal cycling
of porous SiO\textsubscript{2}/poly(NIPAM) hybrids

Figure 4.7 presents a graphical overview of the proposed mechanism responsible
for the OT changes observed from the hybrids, focusing on the samples with porosities
>50%. The schematic illustrates changes occurring in the hybrid morphology in a
single pore, and the central plot represents the hysteresis loop for thermal cycling of an
80% porous sample. It should be emphasized that despite abundant literature on
Figure 4.7 A Schematic illustrating the structural changes proposed to occur in the porous SiO$_2$/poly(NIPAM) hybrid in a heating-cooling cycle. The OT versus temperature plot in the center of the figure is taken from a single heating-cooling cycle observed for a hybrid containing a porous SiO$_2$ template that was etched for 20 s at 577 mA/cm$^2$, displaying 80% porosity (see also, Figure 4.4b).
poly(NIPAM), very little is understood about the mechanism of its volume phase transition. Deviations from this model will be discussed as well.

The first stage of heating is accompanied by an increase in OT that occurs between 30°C and 30.8°C (Figure 4.7a). This small increase in OT can result from an increase in either the refractive index of the hybrid, n, or an increase in its thickness, L. Since poly(NIPAM) exhibits a temperature-induced collapse from an extended coil to a globular structure, an increase in the physical thickness of the film is not expected to occur. Moreover, it is well established that an increase in refractive index is observed when poly(NIPAM) hydrogels collapse. A two stage mechanism has been suggested for the collapse of poly(NIPAM) hydrogels. The first stage of this mechanism involves rapid microphase segregation of the hydrogel into two distinct phases, resulting in polymer clusters separated by water-rich regions. The formation of these polymer clusters results in an increase in refractive index of the hydrogel, which would lead to the observed increase in the OT of the hybrid.

Figure 4.7b illustrates the physical change that accompanies the rapid, dramatic decrease in OT that occurs for the higher porosity hybrids at 30.8°C. It is suggested that the transition temperature of poly(NIPAM) is affected by surface interactions with the porous SiO₂ template. As previously discussed, the interconnecting pore structure of the template provides a means for the entire hybrid to respond as a three-dimensional network. As the surface constrained poly(NIPAM) polymer collapses, it is suggested that its interaction with the pore surface causes compression of the porous template. This compression occurs most dramatically along the direction perpendicular (z-
direction) to the porous Si/bulk Si interface, resulting in a decrease in the physical thickness of the film (L). The decrease in L leads to the observed shift in OT (see eq. 4.5). The preferential compression of the hybrid along the z-direction is attributed to the highly anisotropic mechanical properties of nanostructured porous Si.

As the hybrid is heated above 31.2°C, a minor increase in OT is observed, ending at approximately 40°C (Figure 4.7c). The hybrid is proposed to undergo a minor relaxation due to reorganization of the hydrogel microstructure. It should be kept in mind that the hydrogel remains highly inhomogeneous during heating, leading to the coexistence of distinct phases, including surface constrained domains, “free” domains, aqueous domains, etc. Bulk poly(NIPAM) hydrogels undergo extremely slow reorganization of the interface between polymer-rich and water-rich phases.

Potentially, these changes in OT may also arise from the expulsion of water from the hybrid. However, due to the dramatic reduction of the diffusion coefficient of water in hydrogel from $\sim 10^{-7}$ cm$^2$/s in the swollen state to $10^{-17}$ cm$^2$/s in the collapsed state, it is thought unlikely that an appreciable amount of water would be expelled from the pores in the timescale of the measurement.

Figure 4.7d illustrates the physical change that occurs upon cooling of the hybrid. At approximately 40°C a substantial decrease in OT is observed. As the system cools, the refractive index of the poly(NIPAM) hydrogel decreases, leading to a decrease in measured OT. It is also possible that the hydrogel undergoes a different reorganization process; for example it is suggested that the mechanism for hydrogel swelling is inequivalent to the mechanism of collapse. Furthermore, information
about the collapsed state of poly(NIPAM) hydrogels above the volume phase transition is incomplete.

As the system approaches 31.7°C, a large and rapid increase in OT is observed (Figure 4.7e). Essentially, this transition mirrors the process depicted in Figure 4.7b. At this temperature poly(NIPAM) starts to swell. It is proposed that the globular domains of poly(NIPAM) begin to relax, allowing for expansion of the interconnected network of the hybrid. As the system continues to cool, the hydrogel domains continue to unfold, resulting in a decrease in refractive index, as observed by a decrease in OT (Figure 4.7f). Upon cooling to the initial ambient temperature, the hybrid’s OT returns to its original value, demonstrating the reversibility of the system through heating-cooling cycles.

4.5 CONCLUSIONS

Porous Si was used as a template for the formation of nanometer-scale poly(NIPAM) hydrogels. The interconnected pore structure of the template combined with adhesion of the poly(NIPAM) hydrogel results in a unique hybrid material with distinct properties. The thin film optical interference spectrum enables direct, real-time observation of changes in the hydrogel during its thermally induced volume phase transition. Reversible optical reflectivity changes are observed to correlate with the temperature-dependent volume phase transition of the hydrogel. The optical response of the hybrid greatly depends upon the template nanostructure, which dictates the confinement conditions of the hydrogel. As the porosity and average pore diameter of
the porous Si template can be easily tuned by adjusting the electrochemical preparation conditions, this system provides a convenient platform for studying confined materials. The fundamental approach presented in this work can be expanded to other stimuli responsive hydrogels and polymers, providing a systematic means of studying confinement effects. The ability of the template nanostructure to direct the kinetics of the phase transition has implications for many drug delivery, biosensing, and microfluidics applications.

4.6 REFERENCES


CHAPTER 5

PREPARATION AND CHARACTERIZATION OF A
MULTIFUNCTIONAL POLY(N-ISOPROPYLACRYLAMIDE-CO-
ACRYLIC ACID)/POROUS SiO$_2$ NANOHYBRID
5.1 ABSTRACT

A multifunctional nanohybrid composed of a pH- and thermoresponsive hydrogel, poly(N-isopropylacrylamide-co-acrylic acid) (poly(NIPAM-co-AAc)) synthesized in-situ within the mesopores of an oxidized porous Si template, is characterized utilizing a novel optical method. Infiltration of hydrogel into the interconnecting nanometer-scale pores of the SiO₂ template is confirmed by scanning electron microscopy. The optical reflectivity spectrum of the hybrid displays Fabry-Pérot fringes characteristic of thin film interference, enabling direct, real-time observation of the swelling and volume phase transition of the confined poly(NIPAM-co-AAc) hydrogel. Changes in the pH of solution within the hybrid lead to reversible optical reflectivity changes. The optical activity is found to correlate to the percentage of AAc contained within the hydrogel, with a maximum change observed for 20% AAc samples. The swelling kinetics of the hydrogel are significantly altered due to its nanoscale confinement, resulting in very rapid response of the hydrogel phase to environmental stimulation via pH change or heating. The inclusion of AAc is found to dramatically change the thermoresponsiveness of the hybrid at pH 7, effectively eliminating the lower critical solution temperature (LCST). The observed changes in optical reflectivity values are ascribed to changes in the hybrids’ dielectric composition and morphology.
5.2 INTRODUCTION

Porous Si is established as a unique nano-scale template for the incorporation of various polymers \(^1\)\(^-\)\(^5\) and hydrogels \(^6\),\(^7\). The potential of such hybrid materials in sensing \(^2\), biosensing \(^6\) and controlled drug delivery \(^2\) has been demonstrated. Recently, we have demonstrated that the a thermoresposive hydrogel, poly(N-\(\text{isopropylacrylamide}\)) poly(NIPAM) synthesized in situ within an oxidized porous SiO\(_2\) template exhibited unique properties \(^7\). In particular, the confined hydrogel was observed to undergo rapid volume phase transition upon heating or cooling in comparison to bulk poly(NIPAM) hydrogels. We have developed a novel optical approach to study the behavior of the hybrid, enabling direct, real-time observation of the confined hydrogel. Additionally, the porosity and pore size of the template, which are precisely controlled by the electrochemical etching conditions, strongly influence the extent and rate of hydrogel phase transition.

Current research efforts are focusing on the study of environmentally responsive hydrogels. It is of particular importance that the hydrogels display rapid response to stimuli, and miniaturization of feature size is one approach towards achieving this \(^8\)\(^-\)\(^12\). Poly(N-\(\text{isopropylacrylamide-co-acrylic acid}\)) (poly(NIPAM-co-AAc)) has emerged as a promising material for use in a variety of applications, including drug delivery \(^13\),\(^14\), sensing \(^15\), microlenses \(^13\), and microfluidic devices \(^8\),\(^13\),\(^16\). Herein, we present the fabrication and characterization of poly(NIPAM-co-AAc)/porous SiO\(_2\) hybrids. The resulting hybrid combines the optical properties of the porous SiO\(_2\) template with the multifunctionality of the pH- and thermosensitive...
hydrogel. The multifunctional hybrid enables continuous, real-time monitoring of the hybrid through the use of the porous SiO$_2$ template as an optical transducer in studying the state of the hydrogel in response to pH and thermal stimuli. The effect of the acrylic acid on pH-triggered swelling and thermally-induced volume phase transition was thoroughly investigated. Furthermore, this hybrid demonstrates potential for applications in drug delivery and sensing, where both pH and temperature changes may induce an immediate response of the system.

5.3 EXPERIMENTAL

5.3.1 Materials

Aqueous HF (48%) and ethanol (99.9%) were obtained from Fisher Scientific and AAper, respectively. Porous Si samples were prepared from single crystalline, highly doped p-type Si (9.0×10$^{-4}$ Ω·cm resistivity, <100> oriented, B-doped, from Siltronix Corp.). pH 4.0 and pH 7.0 buffers were obtained from Fisher Scientific. N-isopropylacrylamide (NIPAM), Acrylic Acid (AAc) and 1,4-dioxane were obtained from Aldrich. N,N’-methylenebis(acrylamide) (BIS) was obtained from Fluka. Reagent-grade benzoyl peroxide (97%, Sigma–Aldrich Chemicals) was purified by recrystallization from ethanol. All other reagents were analytical grade and used as received.
5.3.2 Etching procedure

Porous Si samples were prepared by anodically etching highly doped Si in a solution of 3:1 v/v 49% aqueous HF:EtOH employing a two-electrode configuration with a platinum counter electrode. CAUTION: Hydrofluoric acid is highly toxic and should be handled with great care. Medical attention should be sought in case of contact with skin or inhalation. Si wafers were placed on an aluminum back contact and put in a Teflon etching cell. 1.33 cm$^2$ of the chip was exposed to etching solution. Samples were etched at a constant current density of 377 mA/cm$^2$ for 30 s in the dark. After etching, samples were rinsed with copious amounts of ethanol and dried under a stream of nitrogen.

5.3.3 Preparation of poly(NIPAM-co-AAc) and poly(NIPAM-co-AAc)/porous SiO$_2$ hybrids

Poly(NIPAM-co-AAc) hydrogels were synthesized by free-radical polymerization of NIPAM and AAc monomers using BIS as the cross-linking agent. NIPAM, AAc and BIS were dissolved in 1,4 dioxane at a total concentration of 0.9 mol/L, and the NIPAM:BIS concentration ratio was kept constant at 110. The mol percentage of AAc monomer was varied relative to the fixed NIPAM monomer content. The pre-gel solutions were deoxygenated by bubbling with nitrogen gas for 30 min. The reaction was initiated with benzoyl peroxide and carried out at 70 °C for 24 hr. The resulting poly(NIPAM-co-AAc)/porous SiO$_2$ hybrids were soaked in pH 4 buffer, rinsed thoroughly over a period of five days and allowed to reach the equilibrium swelling state.
Porous SiO$_2$/poly(NIPAM) hybrids were prepared by casting the pre-gel solution described above (without AAc) onto the porous SiO$_2$ sample. The sample was then covered with a glass slide to minimize the amount of free polymer above the porous template layer and allowed to polymerize at 70 °C for 24 hr. The resulting hybrids were carefully soaked in Milli-Q water, rinsed thoroughly over a period of five days and allowed to reach an equilibrium swelling state.

### 5.3.4 Oxidation of porous Si films

Freshly etched porous Si films were thermally oxidized in a ceramic tube furnace (Lindberg Blue M). Samples were heated to 800°C for 1 h under ambient conditions, and then allowed to cool to room temperature.

### 5.3.5 Scanning electron microscopy

Scanning electron microscopy (SEM) images were obtained using a FEI Quanta 600 environmental scanning electron microscope at an accelerating voltage of 20 keV. Porous SiO$_2$/hydrogel hybrids were prepared for SEM analysis by first placing samples directly into liquid nitrogen for 15 min, followed by freeze drying. The samples were then freeze-fractured for SEM analysis.

### 5.3.6 Gravimetric determination of porosity

Three porous SiO$_2$ samples were weighed on a laboratory microbalance to obtain their initial mass ($m_1$). Samples were then placed in a solution of 3:1 49%
aqueous HF:EtOH in order to dissolve the SiO$_2$, and the sample was reweighed ($m_2$).

The porosity was determined using the following equation:

$$P = \frac{V_{\text{total}} - V_{\text{SiO}_2}}{V_{\text{total}}} \quad (5.1)$$

Where $V_{\text{total}}$ is the total volume of material removed from the bulk Si substrate, as determined by:

$$V_{\text{total}} = At \quad (5.2)$$

Where $A$ is the etched area, and $t$ is the total thickness of the porous film as measured by cross-sectional SEM. The total volume of SiO$_2$ removed from the porous layer, $V_{\text{SiO}_2}$, was calculated by:

$$V_{\text{SiO}_2} = \frac{(m_1 - m_2)}{d} \quad (5.3)$$

where $d$ is the density of bulk SiO$_2$, taken as 2.6 g cm$^{-3}$.

### 5.3.7 Measurement of pre-gel solution and buffer refractive indices

The refractive index of the pre-gel solutions and buffers used in this study were measured using a Milton-Roy refractometer.

### 5.3.8 Measurement of interferometric reflectance spectra

Interferometric reflectance spectra of the Porous SiO$_2$/hydrogel hybrids was measured using an Ocean Optics CCD S-2000 spectrometer. A bifurcated fiber optic cable was used to attach microscope optics to the spectrometer. The other end of the fiber optic cable was attached to a tungsten light source that was focused through the optics to a spot size of ca. 1-2 mm$^2$. Spectra were collected in the wavelength range of 400-1000 nm, with a 20 ms spectral acquisition time. 100 individual spectra were
averaged together for each spectral acquisition. Illumination of the sample and detection of reflected light were both done at 0° relative to surface normal. pH cycling experiments were carried out in a custom flow cell apparatus attached to a VICI m50 pump system. pH was held constant for one hour increments, and buffers were flowed over the hybrid at a fixed flow rate of 1 mL/min. Flow was temporarily suspended between different buffer flow in order to clean the outside of the tubing and change beakers. A total of 3 pH cycles were conducted on each sample. Thermal cycling experiments on the hybrid were conducted on top of a standard laboratory hot plate. Samples were immersed in 3 mL of buffer and a k-type thermocouple was attached to the Si wafer surface to measure its temperature. Samples were monitored at room temperature for one hour, after which the temperature was raised at approximately 1 °C/min until a final temperature of 47 °C was achieved. Samples were held at this temperature until a total of one hour had passed from the beginning of the heating cycle. Samples were then allowed to cool back to room temperature. A total of 2-5 cycles were performed on each sample.

5.3.9 Measurement of template porosity and thickness by the spectroscopic liquid infiltration method (SLIM)

The thickness and porosity of the template used in this study was measured spectroscopically using the previously-described SLIM method \(^{17,18}\). Briefly, SLIM is used to determine the porosity and thickness of the porous SiO\(_2\) template by measuring changes in the optical thickness (OT) that occur as a consequence of filling the porous film with solvents of varying refractive index. In this study we used ethanol, hexane,
acetone, and the 15% pre gel solution, with refractive indices of 1.359, 1.372, 1.357, and 1.428, respectively, to measure changes in OT. The data were then fitted to a two-component Bruggeman effective approximation.

**5.3.10 Determination of optical thickness**

The wavelength axis of the spectrum from the Ocean Optics spectrometer was calibrated using a least-squares fit of five spectral lines observed from a neon lamp, at 585.3, 614.3, 640.2, 703.2, and 811.5 nm. The data spacing of the spectrometer is approximately 0.4 nm. The x-axis was inverted and a linear interpolation was applied such that the data were spaced evenly in units of nm$^{-1}$. A Hanning window was applied to the spectrum, it was redimensioned to 4096 data points and zero padded to the power of two. A discrete Fourier transform using a multidimensional fast prime factor decomposition algorithm from the Wavemetrics, inc (www.wavemetrics.com) IGOR program library (FFT) was applied. The Fourier transform of the spectrum yields a peak whose position on the x-axis corresponds to the value of 2nL in eq. 1.

**5.4 Results and Discussion**

**5.4.1 Preparation of poly(NIPAM-co-AAc)/porous SiO$_2$ hybrids**

The synthesis scheme followed to produce poly(N-isopropylacrylamide-co-acrylic acid)/porous silica [poly(NIPAM-co-AAc)/porous SiO$_2$] hybrids is outlined in Figure 5.1.
Figure 5.1 Preparation scheme of poly(NIPAM-co-AAc)/SiO$_2$ hybrids. An anodic electrochemical etch is used to prepare a porous Si layer from a single-crystal Si wafer. The freshly-etched sample is then thermally oxidized at 800°C. A pre-gel solution is cast onto the porous SiO$_2$ sample. The sample is then covered with a glass slide, purged with nitrogen and allowed to polymerize at 70°C for 24 hr. The resulting hybrids are carefully soaked in pH 4 phthalate buffer, rinsed thoroughly over a period of five days and allowed to reach equilibrium swelling state at room temperature.
5.4.2 Preparation of porous SiO$_2$ template

The porous Si template is prepared from a highly doped p-type single-crystal Si wafer polished on the (100) face using an anodic electrochemical etch. In this study, all porous Si templates are prepared by etching Si wafers at 385 mA/cm$^2$ for 30 s. The resulting freshly etched porous Si sample is then thermally oxidized at 800°C to create a hydrophilic porous SiO$_2$ matrix. The oxidation process also helps to protect the porous structure from degradation in aqueous media$^6, 17$.

5.4.3 Characterization of the porous SiO$_2$ thickness and porosity

5.4.3.1 SEM characterization of porous SiO$_2$ template

The thickness and porosity of the porous SiO$_2$ layers are determined by scanning electron microscopy (SEM; thickness only), optical measurements and gravimetry. A cross-sectional SEM image of a typical layer after thermal oxidation is shown in Figure 5.2. The porous SiO$_2$ layer is approximately 7600 nm thick (Figure 5.2a). The interconnecting pores have a nominal thickness of 60-100 nm (Figure 5.2b). It should be noted that the pores of the porous SiO$_2$ template are interconnected, providing a long-range network of pores (Fig. 5.2b).

5.4.3.2 Spectroscopic liquid infiltration method (SLIM) analysis of porous SiO$_2$ template thickness and porosity

Sample porosity and thickness can be quantified by measurement of the reflectivity spectrum as a function of liquid infiltration$^{19}$. The optical thickness (OT) of the sample is determined from the interferometric reflectance spectrum for each
Figure 5.2 Cross-sectional scanning electron micrographs (secondary electron) of a thermally oxidized porous Si layer. (a) Interface between the porous SiO$_2$ layer (etched for 30 s at 385 mA/cm$^2$) and the single crystal Si substrate. (b) Higher magnification image of the porous SiO$_2$ layer, revealing the pore morphology.
filling liquid (e.g., ethanol, hexane). The data are then fit to a two-component Bruggeman effective medium approximation. The refractive index of the SiO$_2$ layer is assumed to be 1.455, and the values of the refractive indices of the different liquids are obtained from the literature or measured with a refractometer. The value of the open porosity (pore volume accessible to the probe molecule) and the sample thickness are determined from the fit $^20$. This model has been shown to predict the porosity and thickness of porous Si and oxidized porous Si in reasonable agreement with gravimetric determinations $^7$, $^{21,22}$. In this work, the open porosity was measured to be $71 \pm 2\%$ with a thickness of $7860 \pm 150$ nm. The thickness agrees well with the SEM measurements.

### 5.4.3.3 Gravimetric measurement of porosity

Another method for determination of porous SiO$_2$ porosity involves a gravimetric approach, wherein the sample is weighed before and after removal of the oxidized layer. Deviation from the SLIM-measured porosity and gravimetric-based porosity was previously observed $^7$. The gravimetric method is excellent at determining total porosity of the template, but in certain cases it does not reflect the open porosity accessible to liquids within the SiO$_2$ films. Previous work with porous SiO$_2$ templates thoroughly describes differences between the two methods $^7$. It was suggested that the thermal oxidation of the template leads to surface chemistry changes that alter surface wettability and morphological changes that result in inaccessible voids within the porous network. The porosity of templates used in this study was determined to be $77 \pm 5\%$, a value slightly higher than that obtained by the SLIM method.
5.4.4 In-situ synthesis of poly(NIPAM-co-AAc) in porous SiO₂ templates

The porous SiO₂ nanostructure can act as a template for the in-situ polymerization of poly(NIPAM-co-AAc) cross-linked with N,N'-methylenebis(acrylamide) (BIS). Details of the composition of the pre-gel solutions are summarized in Table 5.1. Pre-gel solutions, containing NIPAM, AAc, BIS and benzoyl peroxide as a free-radical initiator, all dissolved in 1,4 dioxane and purged with N₂ gas, are cast onto the porous SiO₂ film and polymerized at 70°C for 24 hr. The obtained porous SiO₂/polymer gel hybrid is thoroughly rinsed with pH 4 pthalate buffer over a period of five days and allowed to reach equilibrium swelling state at room temperature. The morphology of the porous poly(NIPAM-co-AAc)/porous SiO₂ hybrids is depicted in Fig. 5.3. It appears that the thickness of the hydrogel layer infused in the pores is approx. 5300 nm, while the average thickness of the porous SiO₂ film is ~7600 nm (see Fig. 2a). In previous work involving polyNIPAM/porous SiO₂ hybrids it was observed by SEM that the polyNIPAM hydrogel did not completely infiltrate the film. It was suggested by Segal and coworkers that the incomplete filling of the pores was due to the freeze drying/freeze fracturing process used to prepare the hybrids for SEM analysis. It is believed that the incomplete filling of the porous network in this work arose from the same phenomenon. As noted earlier, the pores of the porous template are arranged in a random interconnected network. A result of this morphology is that the pre-gel solution can effectively infiltrate into the template.
Table 5.1 Composition of pre-gel solutions.

<table>
<thead>
<tr>
<th>Pre-gel Component</th>
<th>Structure</th>
<th>Solution and Composition**</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-isopropylacrylamide (NIPAM)</td>
<td><img src="image1" alt="structure" /></td>
<td>A* 1.47</td>
</tr>
<tr>
<td>Acrylic Acid (AAc)</td>
<td><img src="image2" alt="structure" /></td>
<td>0.085</td>
</tr>
<tr>
<td>N-N’-methylene bisacrylamide (BIS)</td>
<td><img src="image3" alt="structure" /></td>
<td>0.027</td>
</tr>
<tr>
<td>Benzoyl peroxide (BP)</td>
<td><img src="image4" alt="structure" /></td>
<td>0.028</td>
</tr>
</tbody>
</table>

*Solutions A-E are presented in increasing concentration of Acrylic Acid monomer. All other components are held at a fixed concentration.

**All concentrations presented as solution molarity.
Figure 5.3 Cross-sectional scanning electron micrograph (back-scattered electron) of poly(NIPAM-co-AAc)/porous SiO$_2$ hybrid. The porous SiO$_2$ layer is etched for 30 s at 385 mA/cm$^2$. The poly(NIPAM-co-AAc) hydrogel containing 15% AAc is infused in the porous SiO$_2$ template. The image shows just the bottom part of the hydrogel/porous SiO$_2$ hybrid and the interface between porous SiO$_2$ and the bulk Si substrate.
5.4.5 Optical analysis of poly(NIPAM-co-AAc)/porous SiO$_2$ hybrids

The status of the poly(NIPAM-co-AAc)/porous SiO$_2$ hybrids was studied by monitoring their reflectivity spectra. Details of the experimental setup are included in the Experimental section. Briefly, a reflectivity spectrum arises from Fabry-Pérot interference occurring from reflections at the top and bottom interfaces of the hybrid thin film. The maxima of this interferogram is governed by the following relationship:

$$m\lambda = 2nL$$

where $m$ is fringe order, $\lambda$ is wavelength of incident light, $n$ is refractive index (RI), and $L$ is the physical thickness of the hybrid film. The quantity “$2nL$” is referred to as the effective optical thickness (EOT) of the hybrid. Changes in either $n$ or $L$ lead to a shift in the interference maxima, resulting in a change in EOT\textsuperscript{19, 23, 24}. As such, we will demonstrate that the porous SiO$_2$ template enables real-time monitoring of the physical behavior of the poly(NIPAM-co-AAc) hydrogel under different pH and thermal conditions by observing changes in the EOT due to physical changes occurring within the hydrogel phase.

5.4.6 Changes in EOT during pH cycling experiments

5.4.6.1 pH cycling

In order to study the pH responsiveness of the hybrids, they were placed in a custom-made flow cell assembly attached to a mechanical liquid pump. The hybrid was flowed under pH 4 buffer for one hour followed by a flow of pH 7 buffer for one hour. This process was repeated for a total of three complete cycles. Figure 5.4 demonstrates
changes in the EOT for a single cycle of a 15% AAc hybrid studied during pH cycling experiments. Upon a change in pH from 4 to 7, a sharp and rapid decrease in the EOT on the order of -6.5 nm is observed. Following this initial transition, continued exposure to the higher pH buffer leads to a rapid increase of 35 nm in the EOT. After the increase, the hybrid stabilizes and exhibits no further changes in EOT. Changing the pH from 7 back to 4 leads to a complete reversal of the hybrid to its initial state. An immediate decrease of the EOT to roughly 4 nm above the initial baseline is observed. The EOT then increases and slowly returns to its initial baseline. Subsequent cycling demonstrates the high degree of reproducibility and reversibility of the hybrid.

**5.4.6.2 Conditioning of the Hybrids Under Flow Conditions**

Freshly-prepared poly(NIPAM-co-AAc)/porous SiO$_2$ hybrids were observed to undergo small baseline shifting during the initial pH cycles (generally 1-3 cycles). The initial instability of the EOT changes led to the introduction of pre-cycling step that was used to condition the sample until it achieved baseline stability and cycle reproducibility. We have found that a total of three five minute cycles followed by a constant flow of pH 4 buffer for 2 hours were the optimal conditions for achieving baseline stability. The initial instability was attributed to the hybrid being transferred from a static environment used to reach an equilibrium condition to a dynamic flow environment within the flow cell. Previous work on pH-sensitive hydrogel actuators in microfluidic devices demonstrated that the forces of aqueous flow on constrained hydrogels led to different responses of the hydrogels to pH changes$^{25}$. The change in baseline was monitored optically and was determined to be small relative to the optical
Figure 5.4 EOT changes of a 15% AAc hybrid during a single pH cycle. Samples are contained in a liquid flow cell assembly and exposed to a constant flow of 1 mL/min. pH 4 and 7 buffers are cycled while optical changes in the hybrid are constantly monitored.
changes resulting from pH triggering. As such, the effect of aqueous flow forces was minimal, however, it was desirable that the hybrids exhibit stability and complete reproducibility.

5.4.6.3 Effect of acrylic acid content on the optical response.

The content of AAc within the hydrogel component of the hybrid has a dramatic effect on the overall optical response of the hybrid during pH cycling experiments. Figure 5.5 illustrates the changes in EOT for the different hybrids utilized in this study. All the samples in this figure were prepared using the same conditions to prepare the porous SiO$_2$ template, which was prepared via anodic etching at 385 mA/cm$^2$ for 30 s, followed by thermal oxidation at 800 ºC. It is important to note that while the hybrids contained different amounts of AAc, they still share very similar behavior. Each undergoes an initial decrease in EOT followed by a rapid increase to a constant, stable EOT value after the pH is switched from 4 to 7. Differences in the optical activity arise when the pH is switched back to pH 4. In the cases of 5 and 25% AAc, the hybrid returns to baseline immediately after the pH change. For 10, 15, and 20% samples, the transition back to baseline is more gradual.

The optical response was quantified by measuring the difference in EOT between the sharp minimum in the EOT curve observed during transition from pH 4 to 7 and the subsequent higher baseline that is achieved. Figure 5.6 demonstrates the average response of the hybrids over the range of AAc incorporation that was studied. A total of three sample runs were averaged for each data point. The response of the hybrids gradually increases with increasing AAc content. A maximum EOT change of
**Figure 5.5** Effective optical thickness response of hybrids with different AAc content during pH 4-7 cycles. The samples were studied within a flow cell under a constant buffer flow of 1 mL/min at 25 °C. EOT changes were triggered by cycling the pH within the flow cell.
Figure 5.6 Changes in effective optical thickness (EOT) as a function of AAc content in poly(NIPAM-co-AAc)/porous SiO$_2$ hybrids. A total of three sample runs were averaged for each data point. The solid line is used to guide the eye.
~50 nm is observed for the 20% AAc hybrid, after which the response begins to decline.

5.4.6.4 Rapid cycling of pH

In order to further test the dynamics of the hybrid, some samples were cycled at a much higher frequency than the 2 hour cycles used to examine the overall behavior of the system. Figure 5.7 depicts the EOT changes during rapid pH cycling of a 25% AAc sample. The sample was exposed to each buffer (pHs 4 and 7) for only 5 minutes in this experiment. The response of the hybrid during this cycling indicates an excellent reproducibility and reversibility of the hybrid. The total average time between baselines is 2 min, indicating that the cycle time could potentially be decreased even more in these hybrids. The rapid response time is attributed to the nanoscale features of the hydrogel contained within the template \(^ {7} \). This acceleration of swelling transition is important for various applications, including microfluidics \(^ {8, 25} \) and sensor devices \(^ {26, 27} \).

5.4.7 Interpretation of EOT changes during pH cycling

Previous work on thermo-sensitive hydrogel/porous SiO\(_2\) hybrids showed that the optical properties of the porous SiO\(_2\) template can be used to monitor changes in optical thickness and correlate these changes to physical phenomena within the hydrogel phase of the hybrid \(^ {7} \). In this present work, we utilize these optical properties to study a hydrogel phase that is multifunctional, containing both pH- and thermo-sensitive components, AAc and NIPAM, respectively.
Figure 5.7 Changes in EOT during rapid pH cycling of a 25% poly(NIPAM-co-AAc)/Porous SiO$_2$ hybrid. The sample was studied within a flow cell under a constant buffer flow of 1 mL/min at 25 ºC. EOT changes were triggered by changes in the pH of the buffers every 5 min.
Bulk poly(NIPAM-co-AAc) hydrogels with varying compositions have been well studied in the past. It has been determined that the inclusion of the AAc moiety provides the hydrogel with sensitivity to pH. In contrast, neat polyNIPAM hydrogels do not display sensitivity to pH changes over the range studied in this present work. Furthermore, bulk hydrogels require significantly longer time to achieve equilibrium swelling during pH changes, on the order of hours to days. At pHs below the pKa of AAc, it has been suggested that the carboxylic acids are engaged in intra- and intermolecular hydrogen bonds with each other and amines on NIPAM and BIS, effectively compartmentalizing water within the hydrogel and lowering the total water content of the gels. It was found that these hydrogels exhibit a dependence of their swelling ratio on pH. The swelling ratio of anionic hydrogels, such as poly(NIPAM-co-AAc), is determined by two major forces, the electrostatic repulsion between the carboxylic acid groups of the AAc and the ionic osmotic pressure generated from the attraction of mobile counterions to charged ions within the hydrogel network. This abrupt change in osmotic pressure between the hydrogel and its surrounding environments is known as the Donnan equilibrium. As such, varying the AAc content within these hydrogels leads to dramatically different equilibrium swelling ratios.

In the poly(NIPAM-co-AAc)/porous SiO\textsubscript{2} hybrids, the feature size of the hydrogel component is dictated by the template morphology. Given a nominal pore size of 60-100 nm, it is clear that the hydrogel features are significantly smaller than those typically studied in the bulk, and, as such, exhibit much faster response times. Furthermore, the optical properties of the template are sensitive enough to sense
very small changes in refractive index of the solutions contained within the hydrogel phase of the hybrid, as well as the minute physical changes occurring during pH cycling.

The initial decrease in EOT upon transition from pH 4 buffer to pH 7 buffer, as observed in figures 4 and 5, is attributed to a change in refractive index of the hybrid film. The measured refractive indices of pH 4 and 7 buffers are 1.3355 and 1.3350, respectively. It should be noted that both buffers have the same ionic strength of 0.05 M. Since hydrogels can be especially sensitive to ionic strength, it is important that both buffers have the same concentration of ions. As the pH 7 buffer infiltrates into the porous network, replacing the pH 4 buffer, the dominant effect is to lower the overall refractive index of the hybrid. As a result of this decrease in refractive index, the EOT decreases (see eq. 5.4). Shortly after the initial decrease in EOT, a sharp increase is observed, attributed to physical changes to the hydrogel phase of the hybrid.

At some point after the introduction of pH 7 buffer into the hybrid, mixing of the new buffer (pH 7) and the remaining buffer (pH 4) occurs, and the aggregate pH of the solution contained within the hydrogel rises above the pKa of AAc, which is reported as 4.25 at 25 ºC \(^{29,31}\). At this point, the charge distribution on the pendant acid groups changes, leading to a conformational change in the hydrogel. This behavior has been observed previously in both bulk \(^{29}\) and thin films \(^9\) of poly(NIPAM-co-AAc) hydrogels. The anionic groups repel each other, causing a swelling of the hydrogel. The conformational change of the hydrogel is modeled as an increase in polymer chain-to-chain distance, or, alternatively, an unfolding of the crosslinking chains into an extended linear conformation \(^9,30\). The observed increase in EOT may be in part due to
the swelling of the hydrogel, which may lead to an increase in the physical thickness, \( L \), of the entire hybrid (see Eq. 5.4). It was suggested in previous work on poly(NIPAM)/porous SiO\(_2\) hybrids that some of the observed optical changes were due to changes in the thickness of hybrid as a result of volume phase changes of the hydrogel\(^7\). Another consequence of the change in charge within the hydrogel is an increase in its hydrophilicity\(^9\). The increase in hydrophilicity, and hence, wettability, coupled to an influx of counterions that mask the negative charge of the AAc\(^{29,31}\), leads to an increase of refractive index.

The extent of the increase in EOT upon introduction of pH 7 buffer is observed to be a function of %AAc content within the hydrogel (See Figure 5.6). Previous work on bulk poly(NIPAM-co-AAc) hydrogels indicates that the %AAc content of the gels has a dramatic effect on the equilibrium swelling ratio of the hydrogels, particularly in the range of compositions studied in this work\(^{28,29}\). As mentioned earlier, the carboxylic acids of the AAc groups hydrogen bond to each other at pHs below the pKa of carboxylic acid. As the pH rises above the pKa, the hydrogen bonds break due to a change in charge, and the hydrogels swell. It is possible that the EOT changes increase from 5 to 20\% AAc because of the initial extent of hydrogen bonding within these gels. At lower AAc concentrations, there is a lesser extent of hydrogen bonding, and water can more effectively permeate the hydrogel. As the %AAc increases, there are significantly more opportunities for the formation of hydrogen bonds below the pKa of the acid, leading to greater expulsion of water. When these higher %AAc gels are exposed to higher pH, the influx of water and counterions is potentially much greater than in lower %AAc gels, leading to a larger increase in EOT.
The 25% AAc samples exhibit a lower increase in EOT than the 20% samples. In previous work involving poly(NIPAM-co-AAc) hydrogels, a substantial decrease in equilibrium swelling ratio between 20% and 30% AAc samples was observed. The authors of this work did not suggest an explanation for their findings, as they did not observe a clear pattern in equilibrium swelling ratios in samples containing more than 20% AAc. It is possible that samples containing more than 20% AAc begin to demonstrate physical properties distinct from lower %AAc poly(NIPAM-co-AAc) hydrogels. It is likely that the initial conformation and configuration of the hydrogel within the porous SiO$_2$ template is heavily dictated by the number and proximity of hydrogen bonds of the carboxylic acids. As such, it is possible that the higher %AAc samples adopt a distinct morphology from the lower %AAc samples.

As the pH is changed from 7 back to 4, a complete reversal of the system is observed. There was a rapid decrease in the EOT, likely stemming from expulsion of water and counterions from the hydrogel as the carboxylic acid groups become protonated and reform hydrogen bonds. In the 5% AAc hybrids, this decrease ends below the original pH 4 baseline. For the 10, 15, 20% AAc hybrids, the decrease in EOT ends above the initial baseline. In all cases, the decrease is followed by a small increase in EOT. In the 5 and 25% AAc hybrids, the increase stops at the baseline, after which no changes are observed. The increase may be due to diffusion of water back into the hydrogel after the initial expulsion. In the 10, 15, and 20% AAc hybrids, the increase if followed by a gradual decrease in the EOT until it returns to the original pH 4 baseline. Due to the greater amount of AAc in these samples, it may take longer for
them to achieve equilibrium, as the diffusion of water out of the hydrogel phase (observed as a decrease in EOT) may take much longer in these samples.

Two negative controls (not shown) were studied to ensure that it was indeed the hydrogel leading to EOT changes and not a result of charge restructuring on the porous SiO$_2$ surface or some other anomalous effect. In the first control, a neat template containing no hydrogel was pH cycled. The optical response was observed to scale with the refractive index of the buffer used, with a ~20 nm decrease in EOT between pH 4 and pH 7. The system exhibited two distinct baselines (one at pH 4, one at pH 7) and no other optical activity. The EOT immediately decreased upon changing from pH 4 to pH 7. It should be noted that samples containing the copolymer hydrogel, the decrease in EOT upon a change in buffers from pH 4 to pH 7 never exceeded -12 nm. This may be due to a decrease in overall free volume due to the presence of hydrogel in the pores, or the swelling process begins rapidly enough to counteract the change in refractive index between the two buffers. In the second control, a poly(NIPAM)/porous SiO$_2$ hybrid was prepared and pH cycled. Since the hybrid contained no acid-sensitive moieties, no large differences from the first control were expected. Indeed, the sample behaves the same way as a blank template. The EOT decreases roughly 10-15 nm as the buffer is changed from pH 4 to pH 7 and returns when the buffer is changed back to pH 4.

5.4.8 Changes in the EOT of poly(NIPAM-co-AAc)/Porous SiO$_2$ hybrids during thermocycling

Poly(NIPAM) hydrogels are well-known to undergo a volume phase transition at the lower critical solution temperature of the material$^{33-36}$. The inclusion of relatively
small amounts of AAc leads to dramatic changes in the thermal response of the hybrid\textsuperscript{28-31}. Previous work on poly(NIPAM)/porous SiO\textsubscript{2} hybrids demonstrated the utility of using the optical properties of the porous SiO\textsubscript{2} template to study the volume phase transition of the hydrogel contained within the hybrid \textsuperscript{7}. Figure 5.8 illustrates EOT changes during thermocycling a 10\% AAc hybrid in pH 7 buffer. Upon heating, the hybrid is observed to undergo a decrease of in EOT, consistent with our previous findings. As the hybrid cools, the EOT returns to baseline.

\textit{5.4.9 Explanation of EOT changes in poly(NIPAM-co-AAc)/porous SiO\textsubscript{2} hybrids during thermocycling experiments}

Previous work on poly(NIPAM)/porous SiO\textsubscript{2} hybrids allowed for accurate prediction of the LCST of polyNIPAM by monitoring changes in the EOT of the hybrid \textsuperscript{7}. In Segal’s work, the optical transitions were observed to be very sharp and occur at the LCST of polyNIPAM. In this present work, the transitions are observed to be much broader.

This phenomenon was observed in bulk thermoresponsive hydrogels containing AAc as well \textsuperscript{28,30}. In fact, the inclusion of the acid moieties can, in some cases, lead to the loss of a sharp LCST for the gels at pHs above the pKa of the acid used \textsuperscript{30}. The measurement of temperature dependence of the heat capacity of these samples at a pH of 7 is observed to broaden over a range of \textasciitilde30 °C, beginning around 23 °C \textsuperscript{28}. The mechanism of broadening is proposed to be a combination of electrostatic repulsion forces and increased hydrophilicity of the sample \textsuperscript{28}. The repulsion forces work against
Figure 5.8 Thermal cycling of a 10% AAc poly(NIPAM-co-AAc)/porous SiO$_2$ hybrid at pH 7. A pronounced decrease in the EOT is observed upon heating. As the hybrid cools to room temperature, the EOT returns to its initial value, indicating thermal reversibility of the hybrid.
the collapse of the temperature sensitive units. At high enough pH, the sample is charged enough to effectively broaden the entire transition region. Furthermore, the volume phase transition in bulk polyNIPAM hydrogels is generally viewed as a result of a hydrophilic-to-hydrophobic transition within the material\textsuperscript{33-36}. Because poly(NIPAM-co-AAc) hydrogel contains charged species, this transition is dampened, as water can still effectively wet the copolymer hydrogel.

5.5 CONCLUSIONS

A poly(NIPAM-co-AAc) hydrogel was synthesized in situ within a mesoporous SiO\textsubscript{2} template. The interconnected pore structure of the template combined with the unique properties of poly(NIPAM-co-AAc) hydrogels results in a novel hybrid material with unique thermo- and pH-responsive properties. The optical properties of the porous SiO\textsubscript{2} template enable real-time observation of morphological changes in the hydrogel phase of the hybrid during both pH and thermally induced volume changes. The extent of AAc in the hydrogel phase of the hybrid is determined to have a dramatic effect on the magnitude of optical change, thus suggesting the utility of this optical methodology in studying the behavior of hydrogels confined in the nanometer-scale porous network. The inclusion of AAc in the hydrogel leads to changes in the thermal response of the hybrid, as predicted by theory and previous experimental work. The ability to tune the response of the hybrid via changes in the chemical composition of the hydrogel has implications for many drug delivery, biosensing, and microfluidic applications.
5.6 REFERENCES


