Molecular Imprinting in Templated Mesoporous Organosilica Studies in Material Composition and Morphology

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Department of Chemistry
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Abstract

This thesis presents a study of molecular imprinting in P123-templated mesoporous organosilica with varying chemical composition and morphology. Case studies in molecular imprinting in sol-gel silica materials over the past fifteen years were used to establish the context of this work and highlight the key considerations that motivated the research presented here. In the proof-of-concept of molecular imprinting inside the walls of a templated mesoporous hybrid organosilica, a clear imprinting effect was demonstrated by comparing its target binding behaviour to that of nonimprinted and blank control materials. A comparison of a set of hybrid materials using tetraethoxysilane as the crosslinker with an all-organosilica set using 1,2-bis(triethoxysilyl)ethene as the crosslinker indicated that nonspecific binding interactions between a target and the matrix can enhance target binding selectivity. In a set of four imprinted materials with the same chemical composition but varying pore structure and particle morphology, faster binding kinetics were observed for shorter mesopore channels and greater external surface area. As a whole, the work presented in this thesis establishes trends in target binding behaviour for variations in chemical composition and material morphology for this new class of materials that correspond with binding trends in imprinted organic polymers and nonspecific adsorption kinetics in sol-gel materials.
dedicated to my husband
Acknowledgments

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# Table of Contents

Acknowledgments.......................................................................................................................... iv  
Table of Contents........................................................................................................................... v  
List of Figures.................................................................................................................................... ix  
List of Tables...................................................................................................................................... xix
List of Abbreviations.......................................................................................................................... xx  
Preface ...................................................................................................................................................... xxx  
Works Cited ........................................................................................................................................ xxix  

1 Introduction and Overview ............................................................................................................... 1  

2 Controlling Morphology and Porosity for Better Molecularily Imprinted Sol-Gel Silica.............. 3  
   2.1 Introduction ................................................................................................................................ 3  
   2.2 An Overview of Molecular Imprinting ............................................................................................ 4  
      2.2.1 A Definition ............................................................................................................................... 4  
      2.2.2 A Brief History, Mostly in Silica .............................................................................................. 5  
      2.2.3 Types of Molecular Imprinting ............................................................................................... 8  
         2.2.3.1 Noncovalent Imprinting ...................................................................................................... 9  
         2.2.3.2 Covalent Imprinting .......................................................................................................... 9  
         2.2.3.3 Semicovalent Imprinting ................................................................................................. 10  
         2.2.3.4 Imprinting Using Coordination Chemistry ....................................................................... 10  
         2.2.3.5 Combining Methods and Optimization ........................................................................... 11  
         2.2.3.6 Interactions in Molecular Imprinting .................................................................................. 11  
      2.2.4 Evaluating Imprinted Polymers ............................................................................................... 12  
         2.2.4.1 Batch Rebinding ................................................................................................................ 13  
         2.2.4.2 Chromatographic Separations ........................................................................................... 14  
         2.2.4.3 Final Note on the Performance of Molecularily Imprinted Polymers ........................... 14  
      2.2.5 Modern Molecular Imprinting in Sol-Gel Silica .................................................................... 15  
         2.2.5.1 The Sol-Gel Method ......................................................................................................... 15  
            2.2.5.1.1 Why Imprint in Sol-Gel Silica? ..................................................................................... 17  
         2.2.5.2 Sol-Gel Organosilica Materials .......................................................................................... 18  
      2.2.6 Controlling Morphology in Molecularly Imprinted Organosilica ........................................... 19  
      2.3 Case Studies in Micron-Scale Morphology Control ................................................................. 19  
      2.3.1 Imprinting in Bulk Organosilica ............................................................................................. 20  
      2.3.2 Imprinted Silica/Organosilica Spheres .................................................................................... 21  
      2.3.3 Imprinting in Thin Films ....................................................................................................... 23  
      2.3.4 Comparing Monoliths to Powders to Thin Films .................................................................... 27  
      2.4 Case Studies in Nanoscale Morphology Control: Templated Pores .......................................... 30  
      2.4.1 Templated Pores with Controlled Shape and Size .................................................................. 30  
         2.4.1.1 Basic Templating Approaches ............................................................................................ 31  
         2.4.1.2 Interactions in Mesopore Templating .............................................................................. 33  
      2.4.2 Surface Imprint Grafting on Periodic Mesoporous Silica ....................................................... 34  
      2.4.3 Synthesizing Mesoporous (Organo)silica with Surface Imprints ........................................... 37
2.4.4 Imprinting Inside the Walls of a Mesoporous Material ........................................ 40
2.5 Outlook for Imprinting in Silica .............................................................................. 44
Works Cited .................................................................................................................. 46

3 Characterization Techniques ...................................................................................... 53

3.1 Characterization of Porous Solids by Gas Sorption .................................................. 54
3.1.1 Classification of Pores .......................................................................................... 55
3.1.2 Adsorption at the Gas-Solid Interface ................................................................ 56
3.1.3 Sorption Isotherms ............................................................................................. 58
   3.1.3.1 Adsorption Potentials in Pores of Different Sizes ........................................ 58
   3.1.3.2 Classifying Sorption Isotherms ........................................................................ 59
   3.1.3.3 Adsorption in Mesopores ............................................................................... 60
   3.1.3.4 Isotherm Hysteresis ....................................................................................... 62
      3.1.3.4.1 Types of Hysteresis .................................................................................. 62
      3.1.3.4.2 Origin of Hysteresis ................................................................................ 64
3.1.4 Collecting an Experimental Isotherm .................................................................. 66
3.1.5 Analyzing Experimental Isotherms ................................................................. 69
   3.1.5.1 Determining Specific Surface Area by BET Theory .................................... 70
   3.1.5.2 Determining Pore Size Distribution .............................................................. 72
      3.1.5.2.1 The Barrett, Joyner, and Halenda Method ............................................. 75
      3.1.5.2.2 Non-Local Density Functional Theory .................................................... 74
      3.1.5.2.3 Reasons to Use NLDFT and the Adsorption Branch ............................... 76
   3.1.5.3 Additional Empirical Assessment: the $\alpha$ Method ...................................... 78
3.1.6 Final Thoughts on Gas Sorption ......................................................................... 80
3.2 Solid State Nuclear Magnetic Resonance .............................................................. 81
3.2.1 Solid-State versus Solution Phase ........................................................................ 82
   3.2.1.1 Heteronuclear Dipolar Coupling .................................................................... 83
   3.2.1.2 Homonuclear Dipolar Coupling ...................................................................... 83
   3.2.1.3 Chemical Shift Anisotropy ........................................................................... 84
   3.2.1.4 The Magic Angle ......................................................................................... 85
3.2.2 Experimental Method ......................................................................................... 85
3.2.3 Interpreting $^{13}$C and $^{29}$Si SSNMR Spectra ...................................................... 87
3.3 Small Angle X-Ray Scattering .............................................................................. 89
3.4 Other Characterization Methods ............................................................................. 91
3.5 Conclusions ............................................................................................................. 91
Works Cited .................................................................................................................. 92

4 Molecularly Imprinted Mesoporous Organosilica ...................................................... 95
4.1 Introduction .............................................................................................................. 95
   4.1.1 Material Synthesis ........................................................................................... 97
4.2 Material Characterization ....................................................................................... 99
   4.2.1 Chemical Composition of the Materials ............................................................ 100
   4.2.2 Quantification of the Imprint Sites .................................................................... 105
   4.2.3 The Effect of the Organic Groups on the Pore Structure .................................. 104
   4.2.4 Determining the Location of the Imprint Sites ............................................... 105
4.3 Target Binding Tests ............................................................................................. 107
   4.3.1 Confirming Imprinting by Solid-Phase Extraction Tests .................................... 107
4.3.2 Confirming Size-Selectivity by Static Binding Tests ............................................. 110
4.4 Conclusions ............................................................................................................. 111
4.5 Experimental Methods .......................................................................................... 112
  4.5.1 Materials ........................................................................................................... 112
  4.5.2 Imprint Precursor Synthesis ........................................................................... 113
  4.5.3 Mesoporous Material Synthesis ....................................................................... 113
  4.5.4 Imprint Removal ............................................................................................... 113
  4.5.5 Solid-Phase Extraction and Static Adsorption Experiments ......................... 114
  4.5.6 Instrumentation ................................................................................................. 114
Works Cited .................................................................................................................. 117

5 The Effect of Crosslinker on Binding Behaviour in Molecularly Imprinted Mesoporous
Organosilica ................................................................................................................... 119
  5.1 Introduction .......................................................................................................... 119
    5.1.1 Material Synthesis ......................................................................................... 121
  5.2 Characterization Results ....................................................................................... 123
    5.2.1 Chemical Characterization ............................................................................ 124
    5.2.2 Porosity Characterization ............................................................................. 125
  5.3 Binding Studies ..................................................................................................... 128
    5.3.1 Solid-Phase Extraction in Aqueous Media .................................................... 129
    5.3.2 Static Binding in Nonpolar Media .................................................................. 130
    5.3.3 Quantifying the Imprinting Effect .................................................................. 132
    5.3.4 Do Porosity and Surface Area Influence Binding Patterns? ......................... 134
  5.4 Conclusions ......................................................................................................... 134
  5.5 Experimental Methods ....................................................................................... 135
    5.5.1 Materials ....................................................................................................... 135
    5.5.2 Mesoporous Material Synthesis .................................................................... 135
    5.5.3 Imprint Removal ............................................................................................ 136
    5.5.4 Solid-Phase Extraction and Static Adsorption Experiments ....................... 136
    5.5.5 Instrumentation ............................................................................................. 137
Works Cited .................................................................................................................. 139

6 The Effect of Pore Structure on the Kinetics of Target Binding in Molecularly Imprinted
Mesoporous Organosilica .............................................................................................. 141
  6.1 Introduction .......................................................................................................... 141
    6.1.1 Material Synthesis ......................................................................................... 143
  6.2 Characterization Results ....................................................................................... 145
    6.2.1 Electron Microscopy ....................................................................................... 144
    6.2.2 Nitrogen Sorption and Porosity Parameters .................................................. 147
  6.3 Binding Studies ..................................................................................................... 151
    6.3.1 Kinetics of Target Binding ............................................................................. 151
    6.3.2 Binding Rate Constants ............................................................................... 153
    6.3.3 Ruling Out Binding Due to Porosity Differences ......................................... 155
  6.4 Conclusions ......................................................................................................... 156
  6.5 Experimental Methods ....................................................................................... 156
    6.5.1 Materials ....................................................................................................... 156
    6.5.2 Mesoporous Material Synthesis .................................................................... 156
6.5.3 Imprint Removal

6.5.4 Kinetics of Target Binding Experiments

6.5.5 Instrumentation

Works Cited

7 Summary and Future Directions

7.1 Summary and Optimization

7.2 Future Directions, Beyond Optimization

7.2.1 Tuning Chemical Composition

7.2.2 Tuning Morphology

7.2.3 And Almost Anything Else

Works Cited

Appendix A Supporting Information – Chapter 4

Appendix B Supporting Information – Chapter 5

Appendix C Supporting Information – Chapter 6

Appendix D Supporting Information – Chapter 7
List of Figures

Figure 2-1. General procedure for molecular imprinting. An imprint molecule interacts with appropriate functional monomers to form a complex. Polymerization of the functional groups Y on the functional monomer with a compatible crosslinker generates the molecularly imprinted polymer. The imprint is removed to liberate a cavity with residual functional groups on the surface that can interact with and bind an appropriate target molecule.

Figure 2-2. Plot of the number of publications per year in the field of molecular imprinting from 1931 to 2012.

Figure 2-3. Structure of alkyl orange dye used to prepare imprinted silica gel sorbents from aqueous sodium silicate in the presence of glacial acetic acid. R = methyl, ethyl, n-propyl, or n-butyl.

Figure 2-4. Schematic representation of the five main types of molecular imprinting: i) noncovalent, ii) electrostatic/ionic, iii) covalent, iv) semicovalent, and v) metal centre coordination. An imprint molecule is combined with an appropriately chosen functional monomer, through noncovalent, covalent, or ligand (L) to metal (M) interactions with complementary functional groups on the imprint. A complex of the imprint and functional monomer (IC) is formed, in which the functional monomer is bound to the imprint molecule I) by hydrogen bonding or van der Waals interactions, II) by electrostatic or ionic interactions (the charges on the imprint and functional monomer may vary), III) through a covalent bond, IV) through a covalent bond with a spacer (orange), or V) by ligand–metal or metal–ligand coordination. The functional monomer contains a functional group, Y, which is able to form covalent bonds with an appropriate crosslinker. After polymerization of the complex with a crosslinker to form the solid polymer matrix (in grey), the imprint–functional monomer interactions are intact. The imprint is removed through washing, cleavage of chemical bonds, or ligand exchange, and leaves behind an imprint cavity with functional groups on the walls. Subsequent uptake of a target molecule is achieved by noncovalent interactions (in types i, ii and iv), the formation of a covalent bond (in type iii), or by ligand exchange (in type v) with target molecules that fit into the cavity and possess the correct structure. The matrix may also participate in target recognition and binding through non-specific surface interactions that results from surface features created around the imprint molecule during crosslinking. Adapted from Ref. 9.

Figure 2-5. General mechanisms of hydrolysis and condensation of alkoxysilane precursors to form silica in a) acid catalyzed conditions and b) base catalyzed conditions. Condensation can produce either water or alcohol as a byproduct.

Figure 2-6. Structures of selected commercially available molecular precursors for silica. a) functional monomers: 1 methyltriethoxysilane (MTES); 2 3-aminopropyltriethoxysilane (APTES); 3 3-isocyanatopropytriethoxysilane (ICPTES); 4 N-(aminoethyl)-3-aminopropyltriethoxysilane (AATES); b) crosslinkers: 5 tetramethoxysilane (TMOS); 6 tetraethoxysilane (TEOS); 7 1,2-bis(triethoxysilyl)ethane (BTEA); 8 1,2-bis(triethoxysilyl)ethene (BTEE); 9 bis(triethoxysilyl)methane (BTEM); 10 1,4-bis(triethoxysilyl)benzene (BTEB). Ethoxy groups are shown for all structures except TMOS, but many are also available with methoxy groups, which are more reactive.
Figure 2-7. a) Imprinting and imprint removal scheme for two-point imprinted material: 1 material synthesis from an IC linked by a carbamate bond (magenta) using TEOS as a crosslinker, n-decyl alcohol, hydroxypropyl cellulose, and sorbitan monooleate as additives, and aqueous NHON as a catalyst; 2 imprint removal by thermal treatment in DMSO with i) H2O to produce Imp-A with a terminal amine or ii) ethylene glycol to produce Imp-B with a terminal alcohol (blue). Target interaction with estrone (green) likely goes by hydrogen bonding. Control silica particles were prepared with APTES instead of the IC and in the absence of estrone. b) Scanning electron micrograph of spheres produced, showing schematic representation of structure of the particles and the location of the imprint sites (turquoise). c) Amount of bound estrone by 3 Imp-B, 4 Imp-A, and 5 the control; amount of bound testosterone propionate by 6 Imp-B, 7 Imp-A, and 8 the control. Reproduced with permission from ref. 51. Copyright 2002 American Chemical Society.

Figure 2-8. a) Imprinting and imprint removal scheme for estrone imprinted core-shell spheres: 1 material synthesis from an IC linked by a carbamate bond (magenta) using TEOS as a crosslinker, n-decyl alcohol, hydroxypropyl cellulose, and sorbitan monooleate as additives, and aqueous NHON as a catalyst; 2 imprint removal by thermal treatment in DMSO with i) H2O to produce Imp-A with a terminal amine or ii) ethylene glycol to produce Imp-B with a terminal alcohol (blue). Target interaction with estrone (green) likely goes by hydrogen bonding. Control silica particles were prepared with APTES instead of the IC and in the absence of estrone. b) Scanning electron micrograph of spheres produced, showing schematic representation of structure of the particles and the location of the imprint sites (turquoise). c) Amount of bound estrone by 3 Imp-B, 4 Imp-A, and 5 the control; amount of bound testosterone propionate by 6 Imp-B, 7 Imp-A, and 8 the control. Reproduced with permission from ref. 51. Copyright 2002 American Chemical Society.

Figure 2-9. a) Sol-gel precursors and imprint molecule for noncovalently imprinted thin film sensor: 1 TMOS; 2 methyltrimethoxysilane (MTMOS); 5 phenyltrimethoxysilane (PTMOS); 4 dopamine (DA). b) Percent voltammetric response in imprinted thin film sensor of various targets: 4 dopamine; 5 catechol; 6 norepinephrine; 7 epinephrine; 8 catechol violet; 9 (dihydroxyphenyl)alanine; 10 ascorbic acid. Responses were normalized to the response of that target at a bare glassy carbon electrode. c) Imprint molecule propranolol (11), functional monomers methacrylic acid (MAA, 12, P-1) and trimethylolpropane trimethacrylate (TRIM, 13, P-2), and crosslinker ethylene glycoldimethacrylate (EGDMA, 14) for the preparation of an imprinted organic thin film. A MIO film was prepared with 1, 2, and 3 as crosslinker and combined functional monomers, respectively, using 11 as the imprint. d) Steady-state binding of 11 in acrylic (P-1 and P-2) and MIO thin films: imprinted (black) and nonimprinted controls (grey). Kinetic binding of 11 in e) P-1 imprinted (solid circles) and nonimprinted (open circles) and f) MIO imprinted (solid circles) and nonimprinted (open circles). Figures d, e, and f reproduced with permission from ref. 55. Copyright 2001 American Chemical Society.

Figure 2-10. a) Imprinting, imprint removal, and site-selective tagging scheme for a site-selective tagged and template xerogel (SSTTX): 1 material synthesis from an IC linked by a carbamate bond (magenta) using TMOS as a crosslinker, water, and HCl as a catalyst; 2 imprint removal by carbamate cleavage using LiAlH4 in THF under argon; 3 target uptake of 9-anthrol (green) or its tautomer 9-anthrone, followed by site-selective tagging of the second aminopropyl group by the fluorescent probe 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD, blue) in THF. After tagging, 9-anthrol is washed out to produce a sensor. b) Steady-state emission spectra from imprinted material in a before (——) and after (---) LiAlH4 treatment, showing >90% imprint removal. c) Photograph of monoliths of control...
materials (A–D) and the SSTTX (E, as prepared in a) excited at 488 nm and filtered for NBD fluorescence only. Controls varied from E as follows: A no imprint; B noncovalent imprint analogue; C one-point imprint analogue; D no imprint removal step. d) NBD response profiles for the SSTTX and controls A–D exposed to varying [9-anthrol]. Controls do not give a significant response, while the SSTTX does. Reproduced with permission from ref. 56. Copyright 2006 American Chemical Society.

Figure 2-11. Schematic representation of the synthetic approach to surfactant-templated mesoporous materials using self-assembly between a template (a typical triblock copolymer shown here) and molecular precursors of different types (blue: tetraalkoxysilane, orange: alkyltrialkoxyisilane, yellow: silsesquioxane precursor). The surfactant forms micelles in solution, the shape of which (spherical or rod-like) is determined by concentration. At low template concentrations, cooperative self-assembly (1a, 1b) between micelles and sol-gel precursors occurs to form the liquid crystalline phase. At high template concentrations, a liquid crystalline micelle phase is formed independently, and sol-gel precursors assemble around the micelles when added to solution (3). Sol-gel processing through catalyzed hydrolysis and polycondensation followed by removal of the template (4) yields ordered mesoporous materials of varying chemical structure (5). Periodic mesoporous silica (PMS) is pure sol-gel silica. Hybrid organosilica prepared from cocondensation of a mix of precursor types (either blue + yellow or orange, as shown, or yellow + different yellow or orange, not shown) will have the R' group localized on pore surfaces if it is terminal (orange precursor) or distributed throughout the pore walls if it is bridging between two Si atoms (yellow precursor). It can also be prepared by grafting an organosilica precursor onto the pore surface of PMS (6). A terminal R' group will dangle into the pore, while a bridging R' group will lie on the surface or dangle into the pore, depending on the grafting method used. Periodic mesoporous organosilica (PMO) in this case is pure organosilica where every silicon atom is bound to a bridging R' group........................................... 32

Figure 2-12. Interactions between the sol-gel species and the template in acidic, basic, and neutral media. Electrostatic interactions are a) S^+T (basic), b) S^-X^+ (acidic), c) S^-M^+T (basic), d) S^-T (acidic), and hydrogen bonding interactions are e) S^-T / N^-T (neutral) and f) S^-(XI)^+ (acidic). Reproduced with permission from ref. 69. Copyright 2006 Wiley VCH. Additional ionic interactions are possible if a neutral template is protonated in acidic media and a mediator anion is present: g) (N^-H^+)(X^-T^-). Penetration of the hydrophilic block (magenta) of a nonionic triblock copolymer template into the sol-gel phase (grey) depending on synthetic conditions occurs to varying degrees from g) not at all to h) partially to i) completely, which will affect the templated pore diameter...... 35

Figure 2-13. Schematic representation of imprint coating on MCM-41-type PMS with a pore diameter of 2.5 nm. Grafting of an IC formed between Cu^{2+} and two equivalents of AATMS (i) or pure AATMS (ii) yields a hybrid organosilica material in which the amine moieties are paired and can strongly bind Cu^{2+} (I), or are randomly distributed on the surface and not necessarily paired (II). Adapted from 90............................................................................................................... 35

Figure 2-14. a) Structure of rectangular and triangular imprint molecules. b) Schematic representation of imprint coating on SBA-15-type PMS with a pore diameter of 2.5 nm: grafting of the IC or APTMS, followed by residual silanol capping using either octadecyltrimethoxysilane (OTS) or trimethoxypropylsilane (TMPS). Triangular imprinting yields triangular cavities, rectangular imprinting yields linear cavities, and random APTMS grafting yields point cavities. c) Specific
adsorption $S$ of triangular molecules on triangular (1), linear (2) and point (3) cavities. d) Specific adsorption $S$ of rectangular molecules on linear (1), triangular (2) and point (3) cavities. Reproduced with permission from ref. 92. Copyright 2000 Wiley VCH.

Figure 2-15. Schematic representation of template-directed surface molecular imprinting of a PMO for selective adsorption of 2,4,6-trinitrotoluene (TNT, 1), using Brij 76 (2) and dinitrobenzene-capped Brij 76 (3) as the template and IC, respectively. Template micelles with 12.5% 3 form in solution, and cooperatively self-assemble with two crosslinkers (1,4- bis(trimethoxysilyl)benzene (BTMEB, 4) and bis(trimethoxysilylmethane (BTMA, 5) to form an optimized surface-imprinted PMO. Removal of the mixed surfactant template leaves surface imprint cavities. Adapted from ref. 93.

Figure 2-16. a) Structure of quaternized aminosilane C$_{18}$-TMS and schematic of micelle-pore wall structure, where C$_{18}$-TMS in dimers templates the pores and interacts with the chiral amino acid L-proline (green) to produce chiral surface imprint sites. b) Solid-state induced circular dichroism of calcined mesoporous samples imprinted with i) L-proline, ii) DL-proline, and iii) D-proline. Reproduced with permission from 97. Copyright 2011 American Chemical Society. c) Adsorption of L- and D-proline on calcined mesoporous materials imprinted with L-proline, D-proline, and DL-proline, showing selectivity for the imprinted enantiomer. Reproduced with permission from ref. 98. Copyright 2012 American Chemical Society.

Figure 2-17. a) Schematic representation of imprinted PMO material before and after imprint removal, showing the carbamate bond used in sacrificial spacer imprinting (magenta) and the fit of the imprint molecule (green) inside the imprint cavity. b) Molecular structures of targets used static binding tests: diethylstilbestrol (DES), bisphenol-A (BPA), 4,4'-biphenol (BP), and hydroquinone (HQ). c) Static binding tests for four targets using the imprinted PMO and a nonimprinted control PMO. d) Kinetic binding profile for DES of the imprinted PMO and the nonimprinted control. e) Transmission electron micrographs of imprinted PMO showing the spherical shape and small particle size produced. From ref. 99. Reproduced by permission from the Royal Society of Chemistry. f) Fluorescence quenching of CdSe quantum dots embedded in BPA-imprinted PMO and nonimprinted control as a way of detecting the binding of four targets in solution. From ref. 100. Reproduced by permission from the Royal Society of Chemistry.

Figure 2-18. Molecular imprinting of bisphenol A in mesoporous SBA-15-type organosilica. a) Schematic representation of the functional groups present in MIMO with embedded imprint cavities after imprint removal (I), non-imprinted mesoporous organosilica with 5-aminopropyl groups on the pore surface (II), and control SBA-15 with surface silanols (III) and some residual ethoxy groups (not shown). b) Structures of target molecules used: phenol (Ph), resorcinol (R), 4,4'-biphenol (BP), bisphenol A (BPA, imprint molecule), and bromothymol blue (BTB). c) Stacked solid-phase extraction plots of a mixed aqueous solution of Ph, R, BP, and BPA (each at a concentration of $1 \times 10^{-4}$ M). d) Fraction of BTB bound to I, II, and III in static binding, found by the absorbance at 615 nm of the solution relative to the stock solution of BTB. Reproduced with permission from 101. Copyright 2011 American Chemical Society.

Figure 5-1. Schematic representation of the types of pores that may be present in a porous solid. Closed pores may be spherical (a), though this shape is rarely of interest as these pores are not
accessible and only affect the overall density of the porous solid; open pores are channels with at least two openings; blind pores have only one opening; open and blind pores may be cylindrical (b), branched (c), interconnected channels (d), ink bottle-shaped (e), or funnel-shaped (f).

Figure 3-2. Schematic representation of the adsorption potential for macropores (a), where $U_s(z)$ is the gas-solid interaction potential, $z$ is the distance between a molecule and the surface, and $-\varepsilon_s$ is the minimum interaction energy between the molecule and the surface at distance $z_m$; mesopores (b); and micropores (c). Macropores are so wide (their opposite walls are so far away) that they can be considered flat surfaces. In micropores, the adsorption potentials at the two walls overlap, and adsorption is governed almost entirely by interactions between the fluid and the walls. In mesopores, the fluid-wall interactions does not dominate near the core, and fluid-fluid interactions are also significant. Adsorption in each of the three classes of pores can be considered a distinct phenomenon. Adapted from ref. 3.

Figure 3-3. IUPAC classification of sorption isotherms. Type I: sorption is limited to, at most, a few molecular layers, as seen in chemisorption and in microporous materials. Type II: non-porous or macroporous sorbent with unrestricted monolayer–multilayer adsorption. The “knee” at B represents the stage where monolayer coverage is complete and multilayer coverage begins. Type III: convex to the relative pressure; attractive adsorbate-adsorbent interactions are weak and adsorbate-adsorbate interactions dominate; very uncommon isotherm shape. Type IV: typical of mesoporous materials; the most characteristic feature is the hysteresis loop that occurs as a result of capillary condensation in mesopores. Type V: also shows pore condensation and hysteresis, but adsorbate-adsorbent interactions are very weak (related to type III). Type VI: special case that represents stepwise multilayer adsorption on a uniform, non-porous surface.


Figure 3-5. IUPAC classification of the commonly encountered hysteresis loops. H1: porous materials with well-defined cylinder-like pore channels or agglomerates of uniform spheres. H2: disordered pores, large pore size distribution, ill-defined pore shape. H3: non-rigid aggregates of plate-like particles that produce slit-shaped pores. H4: narrow slit pores and micropores. Adapted from ref. 8.

Figure 3-6. a) Real isotherm of argon at 87 K on MCM-41 compared with NLDFT isotherm calculated for a single ideal pore of diameter 4.8 nm (corresponding to approximate pore size of real sample). The experimental adsorption branch corresponds with spinodal condensation, while desorption is an equilibrium transition. Reproduced from ref. 11, copyright 2001, with permission from Elsevier. b) NLDFT isotherm at 77 K in a spherical cavity of diameter 15.5 nm, where $p_a$ is the liquid-like spinodal pressure, $p_e$ is the equilibrium pressure, and $p_{sv}$ is the vapour-like spinodal pressure corresponding to the transitions shown in a). The capillary condensation pressure $p_c$ corresponds to $p_{sv}$, while the desorption pressure $p_a$ range covers three regions of evaporation with borders at $p_e$ and the cavitation pressure $p_{cav}$. Reproduced with permission from ref. 13. Copyright 2002 American Chemical Society.
Figure 3-7. Schematic diagram of the manifold of the Quantachrome Instruments Autosorb 1-C and photograph of the instrument. The sample cell is located at A, the degassing stations at B, the Dewar containing the appropriate coolant (liquid nitrogen) at C, adsorptive valves at D, and vacuum valves at E. Manifold scheme adapted from ref. 3.  

Figure 3-8. Typical type IV sorption isotherm showing H1 hysteresis for an ordered mesoporous sample. Adsorption points are shown in solid circles and desorption points in hollow circles. Capillary condensation and hysteresis are observed in the 0.60 < P/P₀ < 0.70 range, and a small amount of low pressure hysteresis is also observed on the desorption branch for P/P₀ < 0.60.  

Figure 3-9. Schematic representation of the surface of an adsorbent exposed to an adsorptive gas as seen by Langmuir and BET theories. In Langmuir theory, a complete monolayer of adsorbed gas forms at sufficient relative pressure. By contrast, BET theory does not assume monolayer coverage, and instead postulates the existence of stacks of adsorbed molecules of varying height (thickness), depending on inhomogeneities in surface sites. The top molecule in each stack is in dynamic equilibrium with the gas phase, and all surface sites are not necessarily filled.  

Figure 3-10. a) Experimental type IV sorption isotherm, with selected data points for BET analysis shown in magenta. b) BET plot for selected data points, showing linear regression results. This sample has a BET specific surface area of 683 m²/g.  

Figure 3-11. a) BJH cumulative pore volume plot from the desorption branch of the experimental isotherm shown in Figure 3-10a. b) First derivative of cumulative pore volume plot, which gives the BJH pore size distribution and a mode pore diameter of 5.45 nm.  

Figure 3-12. a) Adsorption branch of experimental isotherm (blue) and NLDFT fitted curve (magenta), showing excellent fitting for the spinodal condensation transition. b) cumulative pore volume generated from NLDFT fit (green, primary axis) and first derivative (magenta, secondary axis) showing the NLDFT pore size distribution, which has a mode value of 6.79 nm.  

Figure 3-13. Isotherms and corresponding BJH pore size distributions from the desorption branch for three different mesoporous samples. Sample A shows H1 hysteresis and narrow pore size distribution at ~5.5 nm with a slight rise at 5.7 nm. Sample B shows H1 hysteresis that closes at low relative pressure (around P/P₀ = 0.45), and a pore size distribution with two peaks, one at ~6 nm and one at 3.7 nm. Sample C shows asymmetric hysteresis with a sharp drop closing the loop around P/P₀ = 0.45, and a pore size distribution with one small broad peak at ~5 nm and one large sharp peak at 3.7 nm.  

Figure 3-14. Pore size distributions for sample A in Figure 3-15: a) calculated by BJH theory (green) and NLDFT (magenta) from the desorption branch of the isotherm for b) calculated by NLDFT from the adsorption branch (green) and the desorption branch (magenta).  

Figure 3-15. a) Raw adsorption isotherm for a mesoporous sample that also contains micropores. b) α₃ plot from experimental isotherm, showing linear regression of selected points in the first linear region of the plot. The intercept (in units of mol·g⁻¹) of the first linear portion is used to calculate the micropore volume, and the slope can be used to calculate the specific surface area of the primary
mesopores. The intercept of the second linear portion is used to find the mesopore volume, and the slope to find the external specific surface area.

Figure 3.16. a) Schematic representation of the energy levels associated with a nucleus with magnetic spin \(1/2\) aligning itself parallel (\(m=1/2\)) and anti-parallel (\(m=-1/2\)) to an external magnetic field \(B_0\). The difference in energy of the spins is \(\Delta E\). b) A nucleus of spin \(\mu\) placed in an external magnetic field \(B_0\) will precess around the field with frequency \(\omega\). This is known as the Larmor precession rate. c) Two nuclei of spins I and S placed in a magnetic field along the z-axis, where \(\Theta\) is the angle between the internuclear vector and \(B_0\).

Figure 3.17. Schematic representation of the different chemical shifts that result from orienting the electron cloud of a carbonyl bond along each axis when an external field is applied along the z-axis. Reproduced with permission from ref. 26. Copyright 2002 John Wiley and Sons.

Figure 3.18. a) The magic angle shown between the z-axis and the space diagonal of a cube. b) Schematic drawing of the interior of a MAS apparatus for SSNMR, showing the drive and bearing air inlets and orientation of the sample holder. Reproduced with permission from ref. 26. Copyright 2002 John Wiley and Sons.

Figure 3.19. a) Schematic diagram of the resonance frequencies of \(^1\)H and \(^{13}\)C before and after the application of appropriately selected RF pulses. In the lab frame, the frequencies are mismatched. In the doubly rotating frame where RF pulses are applied to both spins (causing both of them to rotate), the frequencies are matched and polarization transfer can occur. b) Schematic pulse sequence of a typical CP MAS experiment.

Figure 3.20. Experimental HPDEC \(^{29}\)Si SSNMR spectrum showing deconvolution of overlapping peaks using Dmfit, and structures of different silicon species present in an organosilica sample.

Figure 3.21. a) Schematic diagram of Bragg diffraction in a lattice. The path length difference for reflected and transmitted light is given by the line segment ABC. b) Diffraction planes (solid lines) in a 2D hexagonal porous structure. Parallel lines show the (100) diffraction path. The centre-to-centre distance \(a\) is equal to the sum of the diameter of the pore \(\Theta\) and the wall thickness \(t\). c) Side length to height relationship for an equilateral triangle.

Figure 3.22. a) Relaxation transitions of electrons after ejection of an electron from the K-shell of a copper atom. b) Schematic diagram of experimental setup for collecting a SAXS spectrum. c) Experimental SAXS spectrum showing the first diffraction peak. d) Diffraction intensity plotted as a function of \(2\Theta\), with the first peak positioned at 0.914°.

Figure 4.1. Synthesis of BPAP by a direct coupling reaction between stoichiometric amounts of phenol on BPA and isocyanate on ICPTES.

Figure 4.2. Synthesis of molecularly imprinted mesoporous organosilica (MIMO), imprint removal to yield M and interaction of a target molecule with the imprint site: a) the mixture of TEOS and BPAP cooperatively self-assembles around and between the hexagonal close-packed core-shell micelles of P123 in acidic aqueous media; b) stirring at room temperature for 24 hours followed by quiescent
curing at 80°C for 24 hours; c) P123 template removal by Soxhlet extraction with ethanol for 20 hours; d) thermal cleavage of the imprint by heating in wet DMSO for 5 hours; e) sequestration of an appropriately sized target bisphenol molecule by hydrogen bonding between phenols and amines.  

Figure 4-3. Fourier transform infrared spectra of MIMO (solid trace), M (dot-dashed trace), NIMO (dashed trace) and N (dotted trace). All spectra show no trace of the isocyanate peak at 2270 cm⁻¹.  

Figure 4-4. a) ¹³C SSNMR spectra and b) ²⁹Si SSNMR spectra of all samples before and after thermal treatment.  

Figure 4-5. ²⁹Si HPDEC spectra of a) M and b) N (grey traces) and fitting (black traces) showing individual Gaussian peaks and Si species assignments. Insets: integration of the individual Gaussian peaks for each plot and the corresponding Si species.  

Figure 4-6. TEM images of all samples before and after thermal treatment. All images are shown to the same scale. Scale bar in upper left image = 100 nm.  

Figure 4-7. Small-angle X-ray scattering plots for all samples before (dotted traces) and after (solid traces) thermal treatment, showing the (100) peak. All plots are to the same scale. Inset: enlargement of second-order (110) and (200) diffraction peaks for P, which shows evidence of long-range 2D hexagonal ordering.  

Figure 4-8. a) Compounds in target binding tests with corresponding solubilities in water: Series 1) bisphenol F (BPF), bisphenol A (BPA), hexafluorobisphenol A (BPAF); Series 2) phenol (Ph), resorcinol (R), 4,4'-biphenol (BP), and bisphenol A (BPA). Asterisks in Series 1 indicate the bridging carbon at which different substitution gives rise to different hydrophobicity in this series. b) Solid-phase extraction method employing load, rinse and elute steps: a) a syringe packed with powder is loaded with 1.0 mL of stock solution; b) the load flows through by gravity into a vial; 1.0 mL of rinse water is added immediately following the load, which is collected quantitatively and analyzed; c) the rinse flows through by gravity into a second vial; 6.0 mL of ACN is added immediately following the rinse, which is collected quantitatively and analyzed; d) a total of 6.0 mL of ACN is collected in a third vial as the eluent and evaporated to dryness; the residue is redissolved in 1.0 mL ACN and analyzed.  

Figure 4-9. a) stacked solid-phase extraction plots for Series 1: the concentration of BPF, BPA and BPAF relative to an aqueous stock solution of BPF, BPA and BPAF, each at a concentration of $1 \times 10^{-4}$ M; b) stacked solid-phase extraction plots for Series 2: the concentration of Ph, R, BP and BPA relative to an aqueous stock solution of Ph, R, BP and BPA, each at a concentration of $1 \times 10^{-4}$ M. Numbers at the top of each column indicate the total percent of each target recovered. 

Figure 4-10. a) structure of bromothymol blue (BTB) as compared to BPA, which has the same backbone linking the phenol moieties; b) Recovered and alkalized aqueous solutions of BTB after static adsorption for 48 hours: stock (1), P (2), M (3) and N (4); c) relative absorbance of solutions 1 to 4 at 615 nm, with relative absorbances indicated at the top of each column.  

Figure 5-1. a) Structures of target molecules used to test binding behaviour of imprinted materials. b) Synthetic strategy for semicovalent imprinting of bisphenol A in Pluronic P123-templated MIMO
materials using one of three crosslinkers: tetraethoxysilane (TEOS), 1,2-bis(triethoxysilyl)ethane (BTEE), or 1,4-bis(triethoxysilyl)benzene (BTEB).

Figure 5-2. Solid-state NMR spectra of T and E series: a) T series $^{13}$C CP MAS, b) T series $^{29}$Si CP MAS, c) E series $^{13}$C CP MAS, d) E series $^{29}$Si CP MAS. $^{13}$C spectra show spinning side bands (indicated by *) for E series. Peaks between 68 and 74 ppm are attributed to a small amount of residual template. Sharp peaks at 39 ppm are from residual DMSO.

Figure 5-3. Nitrogen sorption isotherms for a) T series and b) E series. All are Type IV isotherms with a distinct pore condensation step in the mesopore relative pressure range, and show either H1 hysteresis ($M_T$, $M_E$) or H1/H2 mixed hysteresis ($N_T$, $P_T$, $N_E$, $P_E$).

Figure 5-4. Pore size distributions found by NLDFT using the adsorption branch and a spinodal condensation transition: a) T series, b) E series. All samples show a narrow pore size distribution with a clearly dominant mesopore size. Smaller mesopores and micropores are evident in most cases, while larger mesopores are either entirely absent or present in minimal amounts. $N_T$ and $N_E$ show the largest relative contribution of pores smaller than the dominant mesopore.

Figure 5-5. a) Basic SPE method for aqueous binding studies. Each added volume was used to push the previous volume through the cartridge. b) Basic SB method for nonpolar binding studies. All HPLC samples were taken and filtered after 3 hours.

Figure 5-6. Stacked normalized solid phase extraction plots for a) T series and b) E series show the relative amount of each target present (compared to the stock solution) in the corresponding fraction after passing through the SPE cartridge. N=3.

Figure 5-7. Fraction of each target bound (removed from solution) for a) T series and b) E series. BP and BPA (blue) are plotted on the primary vertical axis, and biphen and tBuBP (orange) are plotted on the secondary vertical axis (right side). Numbers above the orange bars indicate the fraction bound $\times 10^{-3}$. N=3.

Figure 6-1. Scanning electron micrographs of representative samples of a) B, b) T, c) R, and d) PL.

Figure 6-2. Scanning (first two in each row) and transmission (last one in each row) electron microscope images of a) B, b) T, c) R, and d) PL, taken from the same particles shown in Figure 6-1, showing surface texture and matching scanning/transmission images of the same spot of each sample. All images are shown to the same scale. Scale bar at top left is 200 nm.

Figure 6-3. Transmission electron micrographs of T, R, and PL, showing the difference in pore structure. Arrows point to dead-end pores in T. All images are shown to the same scale. Scale bar at bottom left is 100 nm.

Figure 6-4. Nitrogen sorption isotherms for a) B, b) T, c) R, and d) PL. Hysteresis about the capillary condensation step for T, R, and PL is primarily H1.

Figure 6-5. NLDFT pore size distributions for a) B, b) T, c) R, and d) PL, using the adsorption branch of the isotherm and a spinodal condensation transition. The mode pore diameter for T, R, and PL is 6.79 nm.
Figure 6-6. $\alpha$, plots for a) B, b) T, c) R, and d) PL, all of which show a zero intercept for the first linear portion.

Figure 6-7. Binding plots of BPA only in solution as a function of time for a) B, b) T, c) R, and d) PL. N = 3.

Figure 6-8. Competitive binding plots as a function of time for a) B, b) T, c) R, and d) PL, with Ph and BPA plotted on the primary axis and BP plotted on the secondary axis. N = 3.

Figure 6-9. Experimental ($A_{1000}$, blue) and calculated ($A_e$, magenta) equilibrium amounts bound for all four materials for a) BPA only and b) competitive binding trials.
List of Tables

Table 2-1. Summary of binding energies of noncovalent interactions. ................................................. 11
Table 3-1. Relevant SSNMR parameters for $^1$H, $^{13}$C, and $^{29}$Si nuclei. ........................................ 86
Table 4-1. Physicochemical properties of the prepared mesoporous materials determined from nitrogen adsorption and X-ray diffraction................................................................. 106
Table 5-1. Sample codes for library of six materials after thermal treatment to remove the imprint, indicating the crosslinker used. Series are named according to the one-letter identifier for the organic bridging group in the crosslinker: E – BTEE, ethane; T – TEOS, no organic bridging group......... 123
Table 5-2. Summary of physicochemical properties of the six materials............................................. 127
Table 5-3. a) Imprinting factors for M vs. N, M vs. P, and N vs. P for Ph, BP, and BPA from the load fraction in aqueous SPE trials, and for tBuBP from SB in cyclohexane. b) Selectivity factors for all six samples for BP vs. Ph and BPA vs. Ph in aqueous SPE, and for tBuBP vs. biphen in SB in cyclohexane. ......................................................................................................................... 133
Table 6-1. Synthetic conditions for the synthesis of P123-templated MIMO materials of different pore morphology, and MIO prepared with no template. ................................................................. 143
Table 6-2. Summary of physicochemical properties of the four materials............................................. 150
Table 6-3. Pseudo-second order kinetic fitting results for BPA only and competitive binding trials. 155
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP</td>
<td>5-aminopropyl</td>
</tr>
<tr>
<td>APTES</td>
<td>3-aminopropyltriethoxysilane</td>
</tr>
<tr>
<td>BET</td>
<td>Brunauer, Emmett, and Teller</td>
</tr>
<tr>
<td>BJH</td>
<td>Barrett, Joyner, and Halenda</td>
</tr>
<tr>
<td>BP</td>
<td>4,4’-biphenol</td>
</tr>
<tr>
<td>BPA</td>
<td>bisphenol A</td>
</tr>
<tr>
<td>BPAF</td>
<td>hexafluorobisphenol A</td>
</tr>
<tr>
<td>BPF</td>
<td>bisphenol F</td>
</tr>
<tr>
<td>BTB</td>
<td>bromothymol blue</td>
</tr>
<tr>
<td>BTEB</td>
<td>1,4-bis(triethoxysilyl)benzene</td>
</tr>
<tr>
<td>BTEE</td>
<td>1,2-bis(triethoxysilyl)ethene</td>
</tr>
<tr>
<td>CP MAS</td>
<td>Cross-polarized magic angle spinning</td>
</tr>
<tr>
<td>CTAB</td>
<td>Cetyl trimethylammonium bromide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>HPDEC</td>
<td>High-powered proton decoupling</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>IC</td>
<td>Imprint–functional monomer complex</td>
</tr>
<tr>
<td>ICPTES</td>
<td>5-isocyanatopropyltriethoxysilane</td>
</tr>
<tr>
<td>IF</td>
<td>Imprinting factor</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemists</td>
</tr>
<tr>
<td>MCM-41</td>
<td>CTAB-templated periodic mesoporous silica</td>
</tr>
<tr>
<td>MIMO</td>
<td>Molecularly imprinted mesoporous organosilica</td>
</tr>
<tr>
<td>MIO</td>
<td>Molecularly imprinted organosilica</td>
</tr>
<tr>
<td>MIP</td>
<td>Molecularly imprinted polymer</td>
</tr>
<tr>
<td>NIMO</td>
<td>Nonimprinted mesoporous organosilica</td>
</tr>
<tr>
<td>NIP</td>
<td>Nonimprinted polymer</td>
</tr>
<tr>
<td>NLDFT</td>
<td>Non Local Density Functional Theory</td>
</tr>
<tr>
<td>P125</td>
<td>Pluronic surfactant ((\text{PEO})<em>{20}(\text{PPO})</em>{70}(\text{PEO})_{20})</td>
</tr>
<tr>
<td>PEO</td>
<td>Poly(ethylene oxide)</td>
</tr>
<tr>
<td>Ph</td>
<td>Phenol</td>
</tr>
<tr>
<td>PMO</td>
<td>Periodic mesoporous organosilica</td>
</tr>
<tr>
<td>PMS</td>
<td>Periodic mesoporous silica</td>
</tr>
<tr>
<td>PPO</td>
<td>Poly(propylene oxide)</td>
</tr>
<tr>
<td>PSD</td>
<td>Pore size distribution</td>
</tr>
<tr>
<td>R</td>
<td>resorcinol</td>
</tr>
<tr>
<td>SBA-15</td>
<td>P125-templated periodic mesoporous silica</td>
</tr>
<tr>
<td>SPE</td>
<td>Solid-phase extraction</td>
</tr>
<tr>
<td>SSA</td>
<td>Specific surface area</td>
</tr>
<tr>
<td>SSNMR</td>
<td>Solid-state nuclear magnetic resonance</td>
</tr>
<tr>
<td>T</td>
<td>target</td>
</tr>
<tr>
<td>TEOS</td>
<td>tetraethoxysilane</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TMOS</td>
<td>tetramethoxysilane</td>
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As I have written my thesis, I’ve often looked back over my earliest results, half-baked as they were, and wondered if there was any way I could give them their due. Not for nothing, a Ph.D. is a long haul. There were many times I felt I was achieving less than I should, like I was somehow failing to measure up in a world of enormous expectation, painstaking attention to detail, and immeasurable knowledge. I heard the way senior members of the group talked about their work and science in general, and I thought, “that will never be me.” But here I am, six long years later, putting the final touches on what I can honestly say has been the hardest thing I have ever done. As I think back, I remember the first steps I took, and recognize how important they really were for me, both scientifically and personally.

I’m pretty sure a grand total of six people will read my thesis, and five of them are on my examination committee. But on the off chance that people do find this work among the masses of amazing science (because science really is amazing), and do take the time to read it for more than the one sentence they need for a citation (that sounds more jaded than I mean), I wanted to write something about the human side of doing science. We deliberately remove ourselves from our work in order to establish that the results we obtain exist not because we’re people and we’ve interpreted them in a certain way, but because science says so. I’m not convinced I agree with the practice of dehumanizing scientific findings, and so I’m writing this to communicate at least some of what I believe is a great strength of scientific discovery: the people who do the work (in this case, me).

So this chapter shows some of the work that will never make it to publication because it simply doesn’t warrant it, in order to give voice to the other discoveries I’m made along the way. The little bits, things that couldn’t possibly measure up against all of the great science that gets published every day, were instrumental in producing the results you will read in the coming chapters; but more than this, they gave me the life lessons and moments of personal growth that shaped my experience as a doctoral candidate, and have led me to where I am today. These things are as important to me as the science, and my thesis would be incomplete if I chose not to write about them.
Starting Out

I began my degree with big hopes, lots of ideas, and absolutely no clue what I was getting myself into. Having made the mistake of thinking I could totally handle things on my own (wrong), I cannot stress enough the importance of seeking guidance from peers, talking through ideas with more experienced scientists, and considering their advice. That’s not to say it’s not important to make choices for yourself, and use the critical thinking skills that got you to graduate school in the first place. The point is you need to find a balance between self-confidence and humility, use good judgment, and trust your instincts. Geoff was omnipresent, but it was Danny Puzzo and Wes Whitnall who took me under their wings in the lab and actually got me going.

I spent my first four months working under Danny’s guidance on a project that went nowhere: the learning curve was steep for me, we got scooped, and it was clear the other researchers had already given the idea more than a year of solid, experience-based research. So in December of 2007 I decided to take a new direction. I know now that this is far less unusual than it felt, and my sense of panic at having “lost” months of good research time was unfounded. In reality, I think it helped me gain a better understanding of what it means to do research:

sometimes, things just don’t work.

It’s nothing to get upset about.

Getting my hands dirty in the lab for the first time was like getting into the lake on the May long weekend when I was a kid. I knew that it wouldn’t feel as cold after I’d been in for a few seconds, but I still went through the painful process of dipping one foot at a time in the water, moving slowly, and chickening out at least four times. That’s the wrong approach. The right approach? Think morning dip before breakfast at camp:

you’re not really awake yet and you definitely don’t want to get wet, but if you don’t go now, they’ll throw you in with your clothes on at lunch.

I knew I didn’t have a choice, so I did it. And once I actually cracked open my first lab book (yeah, you know that sound) to make my first lab notes, I felt a million times better.
I realize that not everyone has this problem. Some people are water babies, and can’t wait to jump in, no matter how early in the season it is. People do polar bear dip in Lake Ontario on New Year’s Day. Shudder. Same goes for scientists. Different strokes.

First Results

I wanted to do molecular imprinting in PMOs, but I was pretty sure that there was no way it was as simple as mixing a bunch of the right stuff together and making a molecularly imprinted templated mesoporous organosilica. Wes was incredibly helpful, because he knew all about incorporating crazy big molecules into PMOs, and the importance of confirming that they were actually where your idealistic cartoon drawing showed them to be. At the same time, I needed an imprinting strategy that would survive sol-gel synthesis conditions and produce a well-enough-defined imprint cavity. Basically, I needed a semicovalent imprinting method with a small molecule imprint that was easy to work with and had lots of structural analogues.

So I picked the dihydroxy benzenes. Boring little molecules, really, but the phenols are good reactive groups and my limited knowledge of organic chemistry told me it was a good choice. The literature on molecular imprinting in silica, as illustrated in Chapter 2, is pretty biased toward phenol-containing imprint molecules, and I figured I wouldn’t mess with a good thing. The organic molecular imprinting community does the same thing with propranolol-imprinted polymers, as I learned from the many jokes about it in introductions to talks at the 2010 International Conference on Molecular Imprinting.¹ While it is repetitive, it’s also a really good way to ensure a reasonable level of comparison between different publications. When you’re dealing with a subject as touchy as molecular imprinting, there’s actually a lot to be said for consistency.

¹ Fantastic conference, fantastic community. Get involved with them if at all possible!
I initially tried two methods to make my imprint–functional monomer complex (IC),\(^1,^2\) using two different catalysts (one of them, dibutyltin dilaurate (DBDU), is really toxic) and far too much solvent in column chromatography runs to purify the products (Figure i). I should have done my homework. When I found the method I've used since then, which is just a straight coupling reaction with near-quantitative yield and no purification other than removal of the solvent,\(^3\) the success had a very clear message:

science can be very complicated, but that doesn't mean it should.

Likewise, if you can avoid toxic substances, why wouldn't you? It is easy to forget how far-reaching the environmental impact of chemical research actually is. I am so proud of the members of my group who mobilized our department with a green chemistry initiative, and Laura Hoch, in particular, for her incredibly brave change of research direction for her own Ph.D. to pursue green synthesis (go, girl).

Material synthesis took time. I explored different molar loading of the IC, and confirmed a trend that is very common in PMOs incorporating various amounts of large, flexible precursors: while 5% IC (low loading) resulted in very good mesopore ordering, 20% IC (high loading) yielded very disordered materials (Figure ii). I settled on 10% loading, to achieve a balance between order, loading, and overall structural integrity. I could certainly have explored a variety of loading amounts, but you have to draw the line somewhere.
Cleavage of the carbamate bond and liberation of the imprint cavities also took some work. Seeing the parallel to the tBoc amine protecting group, Wes and I tried strong acid, but to no avail. Following a literature procedure, we tried ioditrimethylsilane, but the sample dissolved. Huh? Moving on. Reduction with LiAlH₄, another candidate from the literature, struck me as an unnecessarily hazardous option. Fortunately, I was able to find yet another simple route: thermal cleavage in a high boiling solvent with a bit of water. Perfect. Provided you take the necessary precautions, there’s nothing wrong with using dangerous chemicals in the lab, and there’s no reason to be afraid of them if you treat them with respect. But honestly, why take risks like that if you don’t have to? I don’t exactly share my husband’s insatiable need for adrenaline…
Figure iii. Raw chromatograms of solutions passed through solid phase extraction cartridges containing MIMOs imprinted with resorcinol (blue, meta) and hydroquinone (magenta, para). Though the meta sample shows poor selectivity (none of the targets is retained), the para sample shows a significantly reduced hydroquinone peak at 6.5 minutes, indicating that species is retained, while structural analogues with different phenol arrangements are not.

The first two ICs that moved forward to testing were made with resorcinol (1,3-benzenediol, “meta”) and hydroquinone (1,4-benzenediol, “para”). Unfortunately, when faced with the task of HPLC method development and long hours of analytical chemistry (something I thoroughly disliked in undergrad), and finding myself suddenly on my own as Wes had left the group to pursue his next steps, I took an ill-advised detour into opals in an effort to avoid yet more (albeit different) chromatography. I thought that I could make molecularly imprinted silica opal structures, and use spectroscopy to detect targets. Yeah, that went nowhere. Eventually I realized I was just practicing avoidance, and decided to face up to a harsh reality of doctoral research:

sometimes you have to just suck it up and do the boring stuff.

It was a grind, but unlike undergraduate experiments that are already tested and have no surprises, real research carries with it that exciting moment when you see the first confirmation that the work you’ve spent the last year doing actually made sense. It was a small achievement, but the first chromatogram showing target binding (Figure iii), only days before I had to present my research findings to date in a departmental seminar, gave me more satisfaction than I could have anticipated.

Achieving this result took time, which was hard for me because I’m an impatient person. So impatient, in fact, that instead of fully developing the results of this early imprinting with very simple molecules and writing a paper right away, I launched myself into imprinting with a “more
interesting” molecule. Bisphenol A (BPA) was prevalent in the news at the time, and I decided that if I was going to put the effort into producing a complete proof-of-concept, I should do it with a target that could lead to real applications. And so I moved forward, and produced the work you will read in Chapter 4.

**The Not Science Side of Things**

At this point I think it’s appropriate to express the gratitude I feel for the amount of freedom Geoff allowed me in my graduate work. There were many times when I thought I was wrong to pursue a Ph.D., and should maybe cut my losses, write up a Master’s thesis (or maybe just quit altogether), and move on. I struggled with motivation. I struggled to make ends meet in an incredibly expensive city, and was frequently tempted by the lifestyles of my friends, who had real jobs and were reaping the benefits of grown-up salaries. I dealt with family illness that affected my ability to work. I began and somehow sustained a trans-Atlantic relationship. And through all of it, Geoff was patient and supportive. I knew I would bitterly regret not finishing this project, having started it, and I’m grateful to have had the opportunity to see it through.

My experience as a graduate student has taught me many lessons, and allowed me to discover a lot of things about myself. But two things stand out for me above all else, and encompass my experience as a doctoral candidate:

1. Life happens whether you want it to or not. You decide whether or not to let it get in the way.
2. If you don’t take ownership of what you’re doing, no matter what it is, you will be unhappy.

I don’t know where I would be today if I hadn’t gone to graduate school. I don’t know if I would be the same person I am, or if I’d be completely different. What I do know is that I’m glad I made the choices I did. One of the reasons I went to graduate school, other than the fact that I wanted to do research (yay science!), was that I didn’t want to be a grown-up yet. I liked being a student and wanted to stay that way a little longer. I didn’t count on how much growing up you have to do as a Ph.D. candidate if you want to maintain balance in your life.

At the symposium held in honour of Geoff’s 65th birthday, Ludo, a recently minted Ph.D. from the group, gave a talk entitled something like, “Things I Learned in Geoff’s Group.” I thought it was a little silly and cutesy at the time, but now that I find myself reflecting on exactly that topic, I think
he was onto something. I've scanned through a lot of theses in the past six months, trying to decide on
the voice I wanted to use in my own writing. While I came to the conclusion that it was important to
remove myself personally from the results and take a traditional scientific approach, I was
uncomfortable with the idea of producing a thesis that didn’t actually show who I am.

My Point

So here's my point. We learn a lot as graduate students. This is what makes graduate school such an
incredible place: it fosters and reinforces a sense of lifelong learning, curiosity, and a need to explore
just a little bit further. But well beyond scientific discovery, we pursue personal discovery. We learn
whether we have the chops to make it through something this challenging. We learn what it means
to enjoy the great satisfaction of achieving an ambitious goal. We learn to look at the world around us
and ask questions. Regardless of whether or not we choose to continue in research, I don’t believe any
one of us stops asking questions. No matter what career path we follow after earning our Ph.D., we
carry with us all that we have learned and apply it to whatever we do. And this is ultimately why I
needed to write this chapter, knowing that my life may lead me very far away from research in the
pursuit of other things. Having devoted thousands of words to experimental work, I needed to claim a
little bit of space for me. My Ph.D. is about chemistry, but the experience has gone well beyond
science.

J.E.L.

July 2013
Works Cited


1

Introduction and Overview

This thesis presents the development of molecular imprinting in the walls of templated mesoporous organosilica. As it sits at the intersection of two worlds (molecular imprinting and templated mesoporous materials), this project covers a lot of ground in synthesis, characterization, and material testing. The results presented are modest by the standards of the state-of-the-art in any of the related specialist fields, but significant in their own right because they represent early steps in the development of a new class of hierarchically templated functional hybrid materials.

Chapter 2 presents a tutorial overview of the concepts of molecular imprinting, sol-gel chemistry, and templated mesoporous materials. It also contains a critical review of case studies in molecular imprinting in sol-gel silica over the past fifteen years, with a focus on the way that control over morphology in general, and porosity in particular, has affected material performance.

Chapter 3 provides a discussion of the key characterization methods used in this thesis, with a focus on gas sorption for characterizing the porosity of materials. Solid-state nuclear magnetic resonance and small-angle X-ray scattering are also discussed as they pertain to the characterization of periodic mesoporous organosilica materials.

Chapter 4 presents the proof-of-concept report of molecular imprinting in Pluronic P123-templated periodic mesoporous organosilica, reproduced from its 2011 publication in ACS Nano, and slightly updated to reflect the rigorous discussion of gas sorption data treatment presented in Chapter 3.

Chapter 5 presents a study of the connection between inherent nonspecific interactions between an imprint molecule and an organosilica material, and that material’s performance in target binding when imprinted with that particular molecule.

Chapter 6 presents a study of the effect that particle and pore morphology have on the kinetics of binding in molecularly imprinted mesoporous organosilica, using four materials with the same chemical composition but very different size, shape, and pore structure.
Chapter 7 summarizes the key findings of this thesis, and discusses some future directions that this area of study might take.

This thesis is a scientific work, but it is also a very personal thing. It is my sincere hope that whoever reads this work is able to recognize this fact. Our world is breathtaking, and science lets us see it in ways that are changing every day. With deep gratitude for the opportunity to walk this path, and humility in the shadows of those that have come before me, I offer this small contribution.
2
Controlling Morphology and Porosity for Better Molecularly Imprinted Sol-Gel Silica

This chapter was submitted for publication to Chemical Society Reviews on 26 July 2013.

ABSTRACT: The wealth of molecular precursors for organic and inorganic polymers has resulted in an incredible volume of molecular imprinting literature. The vast majority of reports deal with organic polymer systems, and molecular imprinting in silica can still be considered a small niche in the field. In this chapter, we present key concepts of molecular imprinting, sol-gel processing, and the synthesis of templated mesoporous silica. We take a small fraction of the literature and use it to understand the ways in which molecular imprinting in siliceous materials of controlled morphology has achieved success in the past fifteen years. Using selected case studies rather than a comprehensive review of the entire field, our goal is to illustrate the key aspects of imprinted silica-based materials as demonstrated by judiciously controlled systems, looking first at control on the micrometre-based materials as demonstrated by judiciously controlled systems, looking first at control on the micrometre scale in bulk phase materials, and then on the nanometre scale in templated mesoporous materials.

2.1 Introduction

The field of molecular imprinting encompasses a vast array of methods, materials, and applications. The number of combinations that can produce a molecularly imprinted polymer (MIP) is essentially infinite, and the pursuit of optimized systems has resulted in thousands of publications. The starting point for new research in molecular imprinting can either be a target molecule or a desired matrix. In this chapter, the relatively small field of molecular imprinting in silica (small relative to that of organic MIPs) will be discussed using a small number of illustrative examples selected from the literature. A number of excellent reviews give exceptionally thorough accounts of imprinting in silica up to 2012. Instead of providing a comprehensive review of the field, the goal here is to examine the ways that researchers have approached molecular imprinting in silica with controlled morphologies. Next, the key concepts of molecular imprinting and sol-gel processing will establish a fundamental understanding of the field. Finally, selected cases will present the variety of approaches used recently (that is, in the last fifteen years) to produce imprinted silica materials, and discuss in each case the particular advantages and/or challenges of the given system (including the imprinting method and material morphology). In particular, we will highlight the use of templated controlled porosity as a deliberate morphological choice in imprinted silica, and discuss its impact on material performance.
Figure 2-1. General procedure for molecular imprinting. An imprint molecule interacts with appropriate functional monomers to form a complex. Polymerization of the functional groups Y on the functional monomer with a compatible crosslinker generates the molecularly imprinted polymer. The imprint is removed to liberate a cavity with residual functional groups on the surface that can interact with and bind an appropriate target molecule.

2.2 An Overview of Molecular Imprinting

2.2.1 A Definition

Molecular imprinting is defined as the assembly of a crosslinked polymer matrix around an imprint molecule that is held in place, either covalently or noncovalently, by judiciously chosen functional monomers (Figure 2-1). The removal of the imprint molecule yields an imprint cavity of a specific size and shape. The surface of the imprint cavity contains functional groups that are able to interact, either covalently or noncovalently, with complementary moieties on an appropriately sized target molecule. The target molecule may be, but is not always, the same as the imprint molecule. The matrix that surrounds the imprint molecule is made up of a crosslinked polymer, formed from crosslinking monomers. The functional monomers (there may be one or many, of one type or several) that hold the imprint molecule in place are compatible with the crosslinker but contain a distinct functional group that interacts preferentially or forms a bond with the imprint molecule. In the final MIP, the imprint cavity remains when the imprint molecule is removed, and is able to interact with a target molecule through any combination of size, shape, and functional group matching.

The goal of molecular imprinting is selectivity, either for a specific species or for a molecular fragment. Either way, the applications for MIPs are grouped into two main categories: sensing and separation. Depending on the intended application, the approach to imprinting and preparation of the MIP will change.
2.2.2 A Brief History, Mostly in Silica

The field of molecular imprinting is drawing close to its centennial. The number of publications related to molecular imprinting published annually has risen steadily in the past three decades (Figure 2-2), and a search of the concept “molecular imprint” in Scifinder returns more than 12,000 references. Review articles number in the dozens. As this field continues to grow, a look back at the earliest discoveries gives perspective and illuminates the crucial role that interdisciplinary chemistry plays in molecular imprinting. This brief history is by no means meant to be exhaustive, as an excellent review has already provided an exceptionally thorough account of the development of the field.

In 1931, Soviet chemist M.K. Polyakov reported that silica particles prepared from sodium silicate in the presence of organic additives (benzene, toluene, or xylene) demonstrated an increased uptake capacity for the associated additive over the other two structural analogues. Because the explanation for this preferential uptake was related to a templating effect from the additive used, this report is the earliest example of the concept of molecular imprinting to be found in the literature. At that time, researchers like Linus Pauling were pondering the origin of the selectivity of antibodies. Pauling’s theory was that antigens templated the formation of antibodies, and created structures complementary to themselves. In essence, he was arguing for bio-imprinting, and was the first in his field to do so. Published in 1942, experimental results of the preparation of artificial antibodies using
methyl blue dye as the antigen are the earliest known reports of the connection between the biological templating that happens in nature and a synthetic imitation of this process. Like Paul Ehrlich before him, Pauling drew on Emil Fischer's “lock-and-key” principle of enzyme action to describe the complementarity that also governed antigen–antibody interactions.

Pauling’s student, Frank Dickey, published a study some years later in which he extended Pauling’s theory of bio-imprinting and the lock-and-key concept backwards to silica, presumably with no knowledge of Polyakov’s report almost two decades earlier. Unlike Polyakov, who introduced the chosen organic additive late in the silica gel synthesis, Dickey added alkyl orange dyes to the initial mixture of sodium silicate and glacial acetic acid used to synthesize what he called “specific adsorbents” (Figure 2-3). The results of affinity tests for the different dyes pointed to an imprinting effect, particularly in the case of propyl orange. Dickey noted that the results would likely be more pronounced if the dyes differed in structure by more than just the alkyl groups on the tertiary amine. In a later paper, Dickey attempted to explain the mechanism by which a specific adsorbent was created. He suggested that two related processes might be responsible: the first one involved the dye attracting parts of the gel that were still fluid to form an attraction-favouring configuration that becomes part of the final rigid structure; the second relied on the fact that an attraction-favouring configuration that forms spontaneously would likely survive the condensation of the surrounding matrix and inhibit further reaction at that site. This explanation bears a striking resemblance to our current understanding of how molecular imprinting works.

More thorough follow-up work using the same silica and alkyl orange dye system resulted in the first use of the term “imprint” to describe the micropores in the adsorbent created by the dye molecule used. This term and Dickey’s proposed mechanism were contested shortly thereafter by Morrison and coworkers at the University of Alberta, who favoured the following explanation: residual (unextractable) dye molecules remaining in the silica gel act as attraction centres for specific adsorption, and are the main species responsible for the specific adsorption of matching dye species.
In other words, their findings led them to believe that unextractable dye molecules acted as a sort of crystallization centre (holding only loosely to the definition) onto which other molecules would stick, and to reject the idea that silica gels acted as specific adsorbents as a result of lock-and-key-type interactions between targets and physical cavities in the matrix. Their theory was based in part on their observation that the materials they made showed constant leaching of the imprint, indicating that a significant amount of the imprint molecule remained in the gel after the extraction step.

Perhaps one of the most significant arguments that Morrison and coworkers could make against the mechanism Dickey had first proposed was that silica as a matrix is extremely complicated, and prone to such problematic behaviour as reactivity at incompletely condensed moieties and therefore shrinkage upon drying. They postulated that any cavities left behind by an imprint molecule must certainly close as the gel dries, and that only unextractable molecules remaining in the silica gel could preserve the cavities they created. Research into this debate did not succeed in disproving the idea of attraction centres, and although it pointed most strongly to a true imprinting mechanism, the issue remained unresolved.

Research in molecular imprinting shifted away from silica in the 1970s, after the first two reports of molecular imprinting in organic polymers appeared in 1972: Wulff used an imprint molecule that was covalently bound to the polymer matrix; Klotz added methyl orange dye to a polymerization mixture in a noncovalent manner similar to earlier imprinting in silica. The wide range of organic polymer precursors and compatible functional monomers available, coupled with the limited number of silica precursors available at the time, is the likely reason that molecular imprinting in silica declined so sharply. Additionally, early researchers in molecular imprinting were organic and biological chemists, so it is not surprising that they chose to work with organic polymers rather than with inorganic substances like silica. Indeed, the dominant context for molecular imprinting in the 1970s and 1980s was focused on biomimetic concepts like enzyme mimics, biological receptors, and artificial antibodies.

With the transition to organic polymers for molecular imprinting came a wider variety of methods to create imprint sites, as more easily manipulated molecular precursors and solvent systems offered a
Figure 2-4. Schematic representation of the five main types of molecular imprinting: i) noncovalent, ii) electrostatic/ionic, iii) covalent, iv) semicovalent, and v) metal centre coordination. An imprint molecule is combined with an appropriately chosen functional monomer, through noncovalent, covalent, or ligand (L) to metal (M) interactions with complementary functional groups on the imprint. A complex of the imprint and functional monomer (IC) is formed, in which the functional monomer is bound to the imprint molecule (I) by hydrogen bonding or van der Waals interactions, (the charges on the imprint and functional monomer may vary), through a covalent bond, through a covalent bond with a spacer (orange), or by ligand–metal or metal–ligand coordination. The functional monomer contains a functional group, Y, which is able to form covalent bonds with an appropriate crosslinker. After polymerization of the complex with a crosslinker to form the solid polymer matrix (in grey), the imprint–functional monomer interactions are intact. The imprint is removed through washing, cleavage of chemical bonds, or ligand exchange, and leaves behind an imprint cavity with functional groups on the walls. Subsequent uptake of a target molecule is achieved by noncovalent interactions (in types i, ii and iv), the formation of a covalent bond (in type iii), or by ligand exchange (in type v) with target molecules that fit into the cavity and possess the correct structure. The matrix may also participate in target recognition and binding through non-specific surface interactions that results from surface features created around the imprint molecule during crosslinking. Adapted from Ref. 9.

full range of covalent and noncovalent interactions. These methods have been studied extensively over the last four decades, and although the range of specific methods is as broad as the selection of molecular precursors available, they can be grouped into categories based on the dominant interaction(s) between the imprint molecule and the functional monomer.

2.2.3 Types of Molecular Imprinting

Molecular imprinting is achieved by a combination of covalent and noncovalent interactions between a chosen imprint molecule and complementary functional monomers, the exact constellation of which distinguishes the different types of molecular imprinting from each other (Figure 2-4). In the first
step, an imprint–functional monomer complex (IC) is assembled via the appropriate interactions, depending on the method of imprinting chosen. A compatible crosslinking monomer is then used to form the solid MIP matrix. Depending on the imprinting method, the complex formation occurs either before crosslinker addition or in situ during polymerization of the matrix. Removal of the imprint molecule through disruption of the functional monomer–imprint interactions liberates an imprint cavity of a defined size and shape. This cavity has the residual organic functional groups of the functional monomer(s) bound in a precise configuration to the inner surface, which can then preferentially interact with a target molecule of an appropriate size, shape, and chemical structure.

2.2.3.1 Noncovalent Imprinting

Noncovalent molecular imprinting can proceed by ionic or nonionic interactions. Most commonly, the dominant interaction is hydrogen bonding, which occurs for example between methacrylic acid groups and primary amines in nonpolar solvents.26 Such weak interactions require the use of an excess of functional monomer because the equilibrium of the system does not favour the formation of the IC. Ionic interactions such as the formation of ion pairs are dominant in polar solvents and are strong enough to allow for the formation of stoichiometric ICs.27 This reduces the occurrence of non-specific binding sites in the final MIP.

Noncovalent imprinting is certainly a simple method, particularly when ICs self-assemble in the pre-polymerization mixture. However, noncovalent imprinting is sensitive to even slight disruption of the interactions holding the complex together (for example, the presence of water, which can disrupt hydrogen bonding), and is therefore not very robust. Additionally, if excess functional monomer is present, the existence of nonspecific binding sites where excess functional monomers were incorporated into the matrix can affect the selectivity of the MIP. While it is possible to cap these excess groups in a post-polymerization step,28 this treatment must be done with the utmost caution and control, as it can easily disrupt the desired interactions and damage imprint cavities.

2.2.3.2 Covalent Imprinting

In order to achieve highly specific imprinting and target binding that is robust, the use of reversible covalent bonds is an obvious choice. This is one of the classical methods of molecular imprinting,24 and often uses such readily reversible condensation reactions as those that form boronate esters,29
ketals/acetals,\textsuperscript{30} and Schiff's base.\textsuperscript{31} Covalent imprinting, being stoichiometric, ensures that functional monomer residues exist only in the imprint cavities; this can greatly reduce nonspecific interactions. However, the need for a distinct synthesis step to generate the initial IC, as well as the bond cleavage required to remove the imprint and bond formation required to bind a target molecule increase the complexity of this method, rendering it slower and less versatile.

\subsection*{2.2.3.3 Semicovalent Imprinting}

An optimized combination of the durability of covalent imprinting and the rapid target uptake of noncovalent imprinting, semicovalent imprinting most commonly makes use of a small sacrificial spacer fragment, such as carbon dioxide.\textsuperscript{32} This spacer adds just enough length to the covalent bond holding the IC together to facilitate the transition from covalent bonds in the imprinting step to noncovalent interactions in the target uptake step. Despite the additional step required to synthesize the initial, covalently attached IC, the final target uptake step proceeds by rapid noncovalent binding. This method combines the precision of covalent imprinting in the creation of the imprint cavity with the speed of target uptake characteristic of noncovalent imprinting.

\subsection*{2.2.3.4 Imprinting Using Coordination Chemistry}

Metal ions can participate in imprinting in one of two ways: either they form part of a complex that is covalently bound to an imprint cavity and participate in target recognition through metal–ligand bonding interactions (where the target is a ligand for the metal ion in question, Figure 2-4v), or they can act as the actual imprint when metal ion uptake is the goal. When a metal ion is the centre of a complex that is bound to the walls of an imprint cavity, it can undergo ligand exchange to bind an appropriate target molecule. The range of choices for the metal and its ligands is vast, making this method easy to tailor to specific needs, providing at least one of the most strongly bound ligands contains a polymerizable moiety that is compatible with an appropriate crosslinker. Another key criterion is that the ligand exchange between the placeholder ligand (L, Figure 2-4) and the imprint molecule (and later the target) occurs under conditions compatible with the system in question.\textsuperscript{35} Alternatively, a metal ion may act as an ionic imprint species to create an imprint cavity that can interact with an appropriate target metal ion. The level of selectivity that is possible in this kind of imprinting is governed less by the size of the cavity created (in other words, it is less likely that the
size-selectivity that exists in the imprinting of relatively complex organic molecules exists here) and more by the (partial) charge that exists in the cavity and the strength of the interactions between the target metal ion and the ligands in the cavity.34

2.2.3.5 Combining Methods and Optimization

Although each of the previously discussed methods of molecular imprinting may be used on its own, combinations of the different types are also useful. For example, a combination of ionic and hydrogen bond interactions can enhance the overall effectiveness of a noncovalent imprinting system.35 The choice of combination depends on the imprint and target, the matrix, the synthesis conditions, and the intended application of the MIP.

The types of molecular imprinting are categorized according to the functional monomer–imprint interaction present. However, the choice of crosslinker, polymerization method, porogenic additive, and solvent(s) will also influence the performance of the MIP. Exploiting non-specific binding interactions between target molecules and the crosslink matrix, for example, can serve to enhance the target retention. Conversely, these interactions can have detrimental consequences on a system’s selectivity. MIP optimization needs to take into account a wide variety of factors that extend far beyond the type of imprinting selected.

2.2.3.6 Interactions in Molecular Imprinting

So far, we have looked at the specific interactions that occur between complementary functional groups on imprint molecules and functional monomers. Depending on the imprinting strategy selected, the binding energies of noncovalent interactions in the system may vary (Table 2-1).36 If a
covalent imprinting strategy is used, the binding energies will be significantly higher, but if a semicovalent approach is used, the noncovalent interactions that also participate in the formation of imprint sites will not be negligible during target binding events. When considering an imprinting strategy and selecting an imprint molecule, it is useful to consider the following:

1. There must be at least one binding interaction possible.
   a. The choice of imprint and functional monomer should ensure complementarity.
   b. Additional interactions may also exist.
2. Stronger binding interaction(s) are better.
   a. Covalent imprinting affords a narrower distribution of binding sites, but results in slow or energetically costly target binding events.
   b. There should be a balance between the strength of IC-forming and target binding interactions before and after MIP polymerization.
3. More selective binding sites are produced from interactions with specific directionality.
   a. Species that can bond in more than one direction (such as primary amines participating in hydrogen bonding) afford lower selectivity.
4. Better binding and selectivity arise when more interactions between the imprint and the polymer are present.
   a. Nonspecific interactions between the crosslinker and the imprint can enhance binding and selectivity.
   b. Rigid species reduce the conformational freedom within an imprint site and improve selectivity.
5. In a bi- or multifunctional imprint, selectivity by interactions with multiple functional groups is best when the intramolecular separation of the groups is maximized.
   a. Greater spacing between functional groups results in reduced interference.

It is clear that the choice of imprinting method and molecular precursors requires some thought, and that a strategic approach with the intended application(s) kept in mind is best.

2.2.4 Evaluating Imprinted Polymers

The evaluation of a MIP generally involves the use of a range of target molecules: the imprint molecule itself, any number of structurally similar species, and other molecules that are significantly different from the imprint in size, structure, and/or shape. In a static binding test, the most common method of evaluation, a known mass of MIP is added to a solution of known target concentration. The system is allowed to come to equilibrium, and the change in concentration of target in the solution is found. This allows the amount of bound target to be determined by difference.

It is customary for initial target binding experiments in imprinted polymers to be conducted in the same solvent used in the synthesis of the polymer. This is mainly due to the variety of interactions
that take part in the imprinting process, and the sensitivity of many of them to environmental factors like the polarity, acidity/basicity, and ion content of the solvent, which either enhance binding or compete with it. Because organic polymers are, for the most part, synthesized using organic solvents, this can severely limit their application for biological and environmental samples, which are commonly or sometimes necessarily prepared in aqueous media. If a different solvent is used, it is not guaranteed that the binding interactions will be the same as in the imprinting.

2.2.4.1 Batch Rebinding

In batch rebinding, precise masses of MIP are suspended in solutions containing precisely known concentration(s) of target molecule(s). Targets other than the imprint molecule include structural analogues, larger targets to prove size exclusion in the imprint cavities, and similar targets that lack the required functional groups for specific binding. Batch rebinding experiments are typically fitted with classical adsorption models. The binding event for a target T in solution exposed to a sample of MIP of known mass and allowed to reach equilibrium is described by

\[
\text{MIP} + \text{T} = \text{MIP:T}
\]

and the partition coefficient \( K_p \) is

\[
K_p = \frac{[\text{MIP:T}]}{[\text{T}]}
\]

which is found by measuring the difference in target concentration in solution after the binding event. This is an equilibrium constant, and can be used to calculate the free energy of binding:

\[
\Delta G = -RT \ln K_p
\]

From the difference of the free energies of binding of two targets, we find that to evaluate the selectivity of the MIP for target \( T_1 \) over \( T_2 \) with a different structure, the ratio of their partition coefficients gives the selectivity factor, \( \alpha \):

\[
\alpha = \frac{K_{p1}}{K_{p2}}
\]

To determine if a true imprint feature has been created, the imprinting factor IF is found by comparing the MIP and its corresponding NIP for a given target:
\[ IF = \frac{K_{\text{MIP}}}{K_{\text{NIP}}} \]  

A value greater than one confirms an imprinting effect, and the greater the value of IF, the more pronounced the imprinting effect.

The time required to reach equilibrium can be determined by a kinetic binding study. This follows the same method as batch rebinding, except that aliquots of the suspension are taken at regular time intervals, separated from the MIP immediately, and analyzed to determine the residual target concentration. When the target concentration stops decreasing, the system is considered to be at equilibrium.

### 2.2.4.2 Chromatographic Separations

Molecularly imprinted polymers can also be used as chromatographic stationary phases, in which case their performance is evaluated by their ability to resolve mixtures of targets. A MIP powder with a sufficiently narrow particle size distribution may be packed into a standard HPLC column. Alternatively, solid-phase extraction (SPE) is a common method used to test imprinted polymers intended for preparative or separation applications. The general method involves three steps: loading the cartridge with a mixture of species in solution, rinsing the cartridge with a solvent to remove weakly bound species, and finally eluting the cartridge with a strong solvent to collect strongly bound species. Provided the SPE process is performed under equilibrium conditions, the relationships presented in the discussion of batch rebinding may also be used to evaluate the MIP.

An important note to keep in mind is that MIPs contain a distribution of binding sites, which are of varying quality in the binding event. Because there is a distribution, and because the interactions that are present in the binding event are sensitive to experimental conditions (solvent, pH, ionic strength, temperature, competing species), it is important to ensure that comparisons are made primarily for values obtained under the same experimental conditions. If experimental conditions are different, it is essential to note this when making comparisons.

### 2.2.4.3 Final Note on the Performance of Molecularly Imprinted Polymers

The early work in molecular imprinting was inspired by a desire to mimic the recognition processes that occur in biological systems, particularly enzyme–substrate and antibody–antigen binding events.
Compared to biological systems, however, MIPs are inferior. Unlike enzymes and antibodies, which contain perfectly formed and selective homogeneous binding sites, MIPs almost invariably contain a heterogeneous distribution of strong and weak binding sites. The strength of binding in antibody–antigen (Ab–Ag) systems is quantified in terms of the dissociation constant, $K_d$:

$$K_d = \frac{[\text{Ab}][\text{Ag}]}{[\text{AbAg}]} \quad (2-6)$$

which, in a heterogeneous system like a suspension of solid MIP in a solvent, is simply the reciprocal of the partition coefficient in Equation 2-2. A lower value of $K_d$ corresponds to better overall binding. In biological systems, the values of $K_d$ are generally on the order of between $10^{-9}$ and $10^{-15}$ M.$^{58}$ By contrast, MIP materials have $K_d$ values on the order of $10^{-3}$ to $10^{-6}$ M.$^9$ In general, while MIP materials have been shown to perform very well for chromatographic separation applications and as sensors when sensitive reporter species are used to signal a binding event, MIPs are still inferior to nature in such biological applications as biosensing and biocatalysis.

### 2.2.5 Modern Molecular Imprinting in Sol-Gel Silica

As stated in Section 2.2.2, a likely reason for the decline in the use of silica for molecular imprinting was the limited number of silica precursors available in the 1970s compared to the wealth of molecular precursors available for organic polymers. However, the rise of sol-gel methods in the 1980s created a new avenue by which to achieve molecular imprinting in silica.$^{39}$ In particular, the work of Schmidt in the mid-1980s on “developing composites on an atomic scale” that could combine the chemical properties of both organic and inorganic materials was an important step for modern work in hybrid organic–inorganic materials.$^{40,41}$

#### 2.2.5.1 The Sol-Gel Method

Sol-gel methods use metal alkoxide molecular precursors to produce a metal oxide according to the overall reaction

$$n\text{Si(OR)}_4 + 2n\text{H}_2\text{O} \rightarrow n\text{SiO}_2 + 4n\text{ROH} \quad (2-7)$$

where R is an alkyl group and the metal in this case is Si.$^{42}$ The precursors first form a colloidal solution (a sol), then an integrated network (or gel) of amorphous material. For silica, tetraalkoxysilanes (such as tetramethoxysilane, TMOS, or tetraethoxysilane, TEOS) hydrolyze and polycondense to form highly crosslinked silica materials.
The reactions proceed either by acid catalysis at pH < 2 or by base catalysis at pH > 2 (Figure 2-5). The crossover at pH = 2 marks the isoelectric point of silica, where the electric mobility of silica is zero and reaction rates are extremely low. Sol-gel processing is done at low temperatures (generally between 0 °C and 100 °C) and in mild chemical conditions (pH and water content are easily tunable).

Although the balanced reaction (Equation 2-7) suggests that two equivalents of water are sufficient to produce one equivalent of completely condensed SiO₂, this is rarely the case. Depending on the porosity of the final material, a significant amount of incompletely condensed SiOH and sometimes SiOR species will be present. The precise amount can be determined by techniques like solid-state ²⁹Si NMR, and the true empirical formula for sol-gel silica is written as [SiO₄(OH)₃(OR)ₓ]ₙ, where 2x + y + z = 4. The degree of condensation can be controlled to a certain extent by the synthesis method used and post-synthetic treatments to drive off water and alcohol and increase condensation, but this is not necessarily required. The water and alcohol produced in the sol-gel reactions serve as porogens in the material, and when drying is done in ambient conditions, the resultant material is called a xerogel. If drying is done in supercritical conditions, an aerogel is produced. Aerogels have extremely low densities, high surface areas, and large porosities, and they have excellent thermal and
electrical insulation properties. However, their extremely large surface area also makes them less robust than xerogels, which have smaller porosities and lower surface areas.

The rate of reaction for hydrolysis depends on pH, water content of the synthesis solution, and the nature of the alkyl group. Generally, larger and/or bulkier alkyl groups slow the rate of hydrolysis. Likewise, steric effects from larger alkyl groups on neighbouring species hinder condensation.

2.2.5.1.1 Why Imprint in Sol-Gel Silica?

The reasons to use sol-gel silica for molecular imprinting are numerous. Its reactivity is low in all but extreme conditions, which include exposure to very strong acid, very strong base, oxidizers, and toxic fluoride species. This makes it a robust matrix for a wide variety of applications and chemical environments. The rigid, highly crosslinked structure of xerogel silica allows the creation of delicate imprint sites with the potential for a high degree of shape selectivity compared to more flexible organic polymers. This template fidelity is likely a major contribution factor to the success of early silica imprinting work. At the sol stage, tremendous control over the shape of the silica is possible, which will be discussed throughout the case studies presented in Sections 2.3 and 2.4. Silica exhibits minimal swelling in the presence of solvents and shows excellent thermal stability. These attributes, too, allow it to maintain the shape and size of imprint cavities. Silica is very stable against oxidation and ageing, which are problematic in many organic polymers. Silica is also remarkably compatible with aqueous and biological systems, and is able to successfully encapsulate enzymes and antibodies without damaging their activity.

However, there are also challenges associated with silica’s rigid and highly crosslinked structure. Although sol-gel silica is often highly porous, the porosity is disordered and diffusion pathways are not direct. Because the distance between crosslinks in silica is much less than in organic polymers, silica does not swell easily (in fact, swelling in silica is almost negligible in most cases). Overall, this means that it is crucial to exert control over the diffusion distances and pathways in a silica MIP. This can be done by any combination of incorporating various kinds of porosity, controlling the shape of particles, and shrinking dimensions.
2.2.5.2 Sol-Gel Organosilica Materials

Atomic-scale composite materials, or hybrid materials, are easily produced by the sol-gel method simply by the addition of molecular precursors that are able to undergo the same hydrolysis and condensation reactions as the metal alkoxide. Organosilica describes any hybrid siliceous material in which silicon atoms (some or all) are covalently bound to at least one carbon atom; this can include both materials that have been modified to contain organic groups and that were synthesized with organic groups. It is common for materials composed of mostly silica (with only a small fraction of Si atoms bound to C atoms) to simply be referred to as silica. However, for the sake of clarity, silica will only be used here to refer to materials that contain no Si–C bonds whatsoever.

Looking at the molecular precursors that are commercially available, it is easy to see the connection between sol-gel methods and molecular imprinting. The R’ fragment of alkylalkoxysilane groups of the form \((\text{RO})_n\text{SiR'}_i\) interacts covalently or noncovalently with an imprint molecule, and the alkoxy silane fragment behaves like the crosslinking group Y (Figure 2-4). This allows the easy covalent incorporation of functional monomers into an inorganic silica matrix (Figure 2-6a). Crosslinking tetraalkoxysilane monomers of the form \(\text{Si(OR)}_4\), or silsesquioxane precursors of the form \((\text{RO})_5\text{SiR'Si(OR)}_5\) condense with the functional monomers to form the matrix (Figure 2-6b). In contrast to early approaches to molecular imprinting, which simply relied on the formation of cavities

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i. In most cases, \(m = 3\).
in the silica around imprint molecules, sol-gel processing allows organic residues to be covalently bound by Si–C bonds to imprint cavities in the same way they are by C–C bonds in organic MIPs, and participate in the same kinds of target interactions as in all-organic systems. The choice of functional monomer will depend on the imprint molecule, while the choice of crosslinker will depend on the desired properties of the matrix.

2.2.6 Controlling Morphology in Molecularly Imprinted Organosilica

In the following two sections, representative selections from the literature over the past fifteen years will illustrate the different approaches to preparing molecularly imprinted organosilica (MIO) of different shapes. The work presented here is only a fraction of the literature; many other excellent reports exist that are only omitted here because of a lack of space. The examples are arranged in terms of the morphological control exerted over the matrix, and are divided into two categories according to the smallest length scale at which morphological control is exerted: micrometres and nanometres. The approaches range from simple post-synthetic procedures (grinding and sieving bulk monoliths of gel, spin coating thin films) to highly specific synthetic methods (highly ordered porous materials prepared by surfactant templating).

2.3 Case Studies in Micron-Scale Morphology Control

Sol-gel silica is that prepared with minimal additional water or solvent (only enough to effect hydrolysis or prevent phase separation at critical stages) and allowed to gelate and harden as a xerogel monolith takes on shape of its container. Any chemical species (solvent, additives, etc.) that are present in solution can become trapped in the matrix, creating pores. Depending on the degree of drying and condensation and the dimensions of the monolith, diffusion can be highly problematic. Ink-bottle-shaped pores, which have very narrow necks that hamper access to the pore interiors, are common in bulk sol-gel silica. The customary solution is simply to grind the monolith and sieve the resultant particles to achieve a moderately narrow size distribution in the range of tens of microns.
2.3.1 Imprinting in Bulk Organosilica

The Davis group reported the preparation of bulk microporous MIO that displayed a measurable increase in microporosity upon removal of the imprint molecule. Using a semicovalent imprinting method, the authors prepared imprinted materials using one-, two-, and three-point imprint molecules linked to APTES by a carbamate bond (Figure 2-7a). As-synthesized monoliths were ground to a powder of particles smaller than 10 μm before further steps were performed. The powder was treated with chlorotrimethylsilane and hexamethyldisilazane, which reacted with residual silanols in the rest of the matrix to cap them with trimethylsilyl (TMS) groups. This silanol capping was done in order to avoid non-specific binding interactions between a target molecule and the imprint cavity/surrounding silica matrix. Cleavage of the carbamate bond to remove the imprint was achieved using trimethylsilyliodide. Gas adsorption before and after imprint removal showed an increase in overall volume adsorbed (Figure 2-7b), which corresponded to the introduction of additional microporosity (pore diameters smaller than 20 Å) through the post-synthetic treatment.
steps (Figure 2-7c). The authors attributed this added porosity to the imprint cavities vacated after imprint removal. As can be seen in the pore size distribution, all of the porosity is in the micropore range, which corresponds to very narrow diffusion pathways for solvent and target molecules to travel. The two-point imprinted material was used as a base catalyst for the Knoevenagel condensation reaction between malononitrile and isophthalaldehyde, which was restricted to a one-to-one ratio in the imprinted material because of the size restriction inside the imprint cavities. A control amorphous silica with surface aminopropyl groups was able to condense a second malononitrile molecule to the second aldehyde, which the authors used to confirm the size-selectivity of the imprint cavities.

In this report, no direct discussion was given of the need for small particles. It is reasonable to assume that previous literature in which imprinted bulk monoliths were ground to a powder informed the decision. Although no direct experimental evidence of improved diffusion as a result of the grinding was presented, the catalytic turnover rate of 74 per amine site per hour, as compared to 367 turnovers per hour for surface amine groups in the control material indicates that moderately good diffusion rates were achieved in the particles. However, the fact that the catalysis in the MIO proceeded at only one-fifth the rate of the control clearly shows the influence of access on the performance of the material. This report was one of the early successes in the revitalization of molecular imprinting in silica, and continues to appear as a reference in more recent work. The carbamate bond used in this example is a popular linker for MIO materials because it is easy to form and cleave and is stable for most of the chemical environments used in sol-gel processing. The sacrificial spacer, CO₂, widens the overall imprint site slightly, and facilitates noncovalent interactions like hydrogen bonding.

2.3.2 Imprinted Silica/Organosilica Spheres

Although it is a simple procedure, mechanical grinding to produce a powder from a monolith has the distinct disadvantage of poor material economy: the effort to obtain at least a moderate particle size distribution can result in the loss of up to 80% of the total material. This loss is unacceptable for any

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ii. The amine protecting group N-tert-butoxycarbonyl (tBOC) used in organic synthesis is a carbamate bond with primary and secondary amines.
sort of industrial or commercial application. One alternative to mechanical grinding is to vary the synthesis conditions and produce particles directly. The Stöber method from 1968 is a well-established protocol for producing silica spheres in solution from the ammonia-catalyzed reaction of tetraalkyloxysilanes in alcohol.48 After more than 40 years of development, variations of this method abound, and include the use of different solvents, catalysts, and additives. Depending on the intended application, the degree of polydispersity in size is easily tuned. Excellent size control from tens of nanometres up to several microns has been demonstrated through synthetic and templating approaches.49,50 The typical process involves creating small monodisperse seed spheres, and then growing them either continuously or in a stepwise fashion.

The Chang group reported the preparation of spherical MIO particles imprinted for estrone, a naturally occurring estrogen.51 An estrone IC, formed via a carbamate linker, was incorporated into spherical particles of controlled size (between 1.5 and 5 μm in diameter) using TEOS as a crosslinker (Figure 2-8a). Two final MIO materials were produced by using a different nucleophile in the
carbamate cleavage: $\text{H}_2\text{O (OH)}$ produced Imp-A with a terminal amine group, while ethylene glycol ($\text{HOCH}_2\text{CH}_2\text{O}$) produced Imp-B with a terminal alcohol. Anticipating the difficulty of removing molecules from the centre of micron-sized particles of highly crosslinked organosilica, the authors controlled the placement of the imprint sites by adding the IC partway through the synthesis of the particles, thus creating core-shell $\text{SiO}_2@\text{MIO}$ spherical particles (Figure 2-8b). Confirmation of the position of the imprints was obtained by changes in FTIR spectra corresponding to loss of imprint functional groups after mechanically grinding away the outer shell. No mention is made of the estimated thickness of the shell (or the depth at which imprint sites would be buried). Static binding tests were performed in chloroform for two targets (estrone and testosterone propionate), and interestingly Imp-B showed the best binding behaviour overall for estrone (Figure 2-8c). The authors did not speculate as to the reason the alcohol-terminated imprint cavity showed better binding performance than the one with the amine, but did point out the possibility of introducing a variety of different functional groups (perhaps with reference to groups not compatible with the synthesis of MIOs by the sol-gel method) in a post-synthetic step. This example shows a deliberate attempt to circumvent the diffusion problem that may be encountered in highly crosslinked sol-gel silica. The ease with which additional layers of (organo)silica can be added to existing material through particle regrowth and grafting is a very positive attribute of silica.

2.3.3 Imprinting in Thin Films

One of the great advantages of sol-gel processing is the ease with which sols can be used to produce thin films of high quality. Most commonly achieved through spin-coating, films of controlled thickness can be prepared on a variety of substrates by adjusting the spinning speed during the coating process and controlling the viscosity of the sol by varying the aging time before coating (viscosity increases over time). The obvious advantage of thin films is the fact that they are thin; shorter diffusion lengths than in bulk sol-gel silica are produced inherently, which means the binding kinetics for imprint sites should be higher. Also, films can be coated on a variety of substrates commonly used in sensor assemblies, making it easy to integrate custom sol-gel materials to build new sensors. However, challenges do arise from the inherently lower porosity of spin-coated sol-gel thin films, which can impede diffusion as compared to bulk sol-gel materials where larger pore volumes are present.32
Early in the development of modern molecular imprinting in silica, the Collinson group reported noncovalent imprinting of dopamine (DA, 4) in a hybrid sol-gel thin film formed on a glassy carbon electrode substrate from a mixture of three sol-gel precursors: TMOS (1) as a crosslinker, methyltrimethoxysilane (MTMOS, 2) to increase hydrophobicity and film stability, and phenyltrimethoxysilane (PTMOS, 3) as a functional monomer (Figure 2-9a). DA was physically entrapped inside the 450 nm-thick film, presumably in close proximity to 3 as a result of an IC formed by favourable hydrophobic and aromatic interactions. Extraction of the DA imprints was achieved in the final film by soaking it in a phosphate buffer solution for 24 hours and confirmed by UV-Vis spectroscopy. A non-imprinted control film was prepared in the same manner but in the absence of DA. To test the film’s sensing capabilities, it was exposed to buffered aqueous solutions (pH 7) of various targets and the normalized voltammetric response of the film was recorded.

Figure 2-9. a) Sol-gel precursors and imprint molecule for noncovalently imprinted thin film sensor: 1 TMOS; 2 methyltrimethoxysilane (MTMOS); 3 phenyltrimethoxysilane (PTMOS); 4 dopamine (DA). b) Percent voltammetric response in imprinted thin film sensor of various targets: 4 dopamine; 5 catechol; 6 norepinephrine; 7 epinephrine; 8 catechol violet; 9 (dihydroxyphenyl)alanine; 10 ascorbic acid. Responses were normalized to the response of that target at a bare glassy carbon electrode. c) Imprint molecule propranolol (11), functional monomers methacrylic acid (MAA, 12, P-1) and trimethylolpropane trimethacrylate (TRIM, 13, P-2), and crosslinker ethylene glycol dimethacrylate (EGDMA, 14) for the preparation of an imprinted organic thin film. A MIO film was prepared with 1, 2, and 3 as crosslinker and combined functional monomers, respectively, using 11 as the imprint. d) Steady-state binding of 11 in acrylic (P-1 and P-2) and MIO thin films: imprinted (black) and nonimprinted controls (grey). Kinetic binding of 11 in e) P-1 imprinted (solid circles) and nonimprinted (open circles) and f) MIO imprinted (solid circles) and nonimprinted (open circles). Figures d, e, and f reproduced with permission from ref. 55. Copyright 2001 American Chemical Society.
(Figure 2-9b). Similar responses were observed for DA and 5, which is expected for a target with the same key functionality (in this case, the catechol ring) but a smaller size (no 2-aminoethane group). Selectivity was confirmed by normalized responses for structurally similar molecules of varying chemical functionality at the 4 position on the aromatic ring (6, 7), of larger size (8), or of zwitterionic (9) or acidic nature (10) in aqueous buffer solution. Structures 6 and 7 showed about half the response of DA, while 8, 9, and 10 showed no measurable response. The organosilica sensor film is negatively charged in pH 7 buffer, which can explain the exclusion of negatively charged species that cannot participate in the same interactions as the imprint molecule. Exclusion or poor recognition of larger or bulkier targets than the imprint is often used as evidence for imprint sites that are size-selective.

The response time of the film was evaluated by the time required for stabilization of the peak current; this was achieved in five minutes or less. Based on the lack of voltammetric response observed for all targets in the non-imprinted control film, the authors suggested that the films were likely fairly dense, and that porosity was mostly generated in the imprinted film as a result of DA removal (which would have vacated imprint cavities). However, they noted that it was not possible to directly characterize the porous nature of the film because of the very small amount of organosilica material present on the substrate. Although they did compare atomic force microscope images of their films to previously reported work, this point highlights a major challenge when controlling the morphology of thin films. It is entirely possible that greater porosity of the films could contribute to faster response times and better target uptake, but without conclusive evidence of porosity it is impossible to confirm. Gas sorption is a powerful method of characterizing porous materials, but because it requires that the sample’s mass be precisely known and cannot distinguish between adsorption on different surfaces, it cannot work with very thin film samples on substrates. Ellipsometric porosimetry with water vapour has recently been used to characterize the porosity (pore volume and average pore diameter) of thin organosilica films, but this technique may not have been readily available at the time this report was published. Unfortunately, in the absence of direct evidence of the difference in porosity of the imprinted and non-imprinted films in this report, it is impossible to confirm that porosity is the major reason for the difference in voltammetric response. The difficulty of controlling and then reliably
interrogating the porous structure of sol-gel thin films is a significant challenge. However, this early report was one of the first of its kind, and showed excellent evidence for the potential of using sol-gel thin films for sensing.

Marx and coworkers compared the binding properties in both organic and aqueous media of a thin film MIO to an imprinted acrylic thin film for propranolol (11), a beta-blocking drug that is an immensely popular archetypal imprint in organic polymers (Figure 2-9c). Thin MIO films (700 nm thick) were simple to produce, but a new polymerization system was required to create thin films of the imprinted acrylic polymer (1 μm thick). As with the previous example, PTMOS (2) and MTMOS (3) were used as functional monomers and TMOS (1) was used as the crosslinker in the MIO film (Figure 2-9a). Standard spin coating produced a high quality transparent film. The acrylic polymer film was synthesized via modified radical polymerization using methacrylic acid (MAA, 12, P-1) or trimethylolpropane trimethacrylate (TRIM, 13, P-2) as the functional monomer and ethylene glycoldimethacrylate (EGDMA, 14) as the crosslinker. Although this polymer combination is the most popular system for molecular imprinting, preparing a high quality thin film of the acrylic polymer is not a trivial achievement. High degrees (80–100%) of crosslinking can be achieved, but this results in opacity, brittleness, and cracking. The solution was to use a functional monomer/crosslinker ratio that reduces the degree of crosslinking (in this case, to 34%) and establish a precisely controlled polymerization environment inside a closed polymerization cell with controlled pressure and atmosphere.

Aside from the complication in producing a high quality organic polymer thin film, the MIO displayed another advantage over the organic system in target binding studies. As a result of the large amount of functional monomer in P-1 and P-2, the organic films showed both high capacity for 11 and high nonspecific binding (shown by high target uptake in the control non-imprinted films, Figure 2-9d). This binding behaviour was similar to literature reports of the same system using bulk imprinted acrylic polymer. By contrast, the MIO film showed lower total capacity but much lower

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iii. Ellipsometric porosimetry (EP) is performed with water vapour. The treatment of raw data to determine porosity features involves the contact angle of water on the given material. Contact angles are generally measured on a bulk surface, and it is debatable whether this contact angle is valid on the nanometre scale. Also, when films are hydrophobic, this causes challenges for water adsorption in very small pores.
nonspecific binding (relative and absolute) in the nonimprinted control. The kinetic binding behaviour of the films also showed the superiority of the MIO film: it reached saturation in less than 1 hour and showed minimal nonspecific binding, while P-1 showed a slower uptake profile, only reached saturation after ~10 hours, and showed nonspecific binding that increased with time. Organic solvents used as porogens in organic polymer syntheses are more volatile than the polar protic solvents (water, alcohol) used in sol-gel processing. As a result, despite their low crosslink density, the acrylic thin films likely had lower overall porosity (most of the solvent evaporated during drying) than the MIO film, which resulted in poorer target binding kinetics.

Even rigid and highly crosslinked organic polymers will contain a significant amount of porosity if appropriate porogenic solvents are used. However, unless compatible nonvolatile solvents are selected, the degree of porosity generated can be lower if the desired morphology is a thin film. By contrast, the ease with which porosity can be tuned in sol-gel thin films (despite their lower porosity relative to bulk monolithic sol-gels) gives them a distinct advantage in imprinted thin film applications.

2.3.4 Comparing Monoliths to Powders to Thin Films

One benefit to imprinting in thin films is that it facilitates the production of sensing devices, in which short diffusion distances presumably allow for rapid response times. However, complete characterization of an imprinting strategy in organosilica is difficult to achieve if only a thin film is produced. Challenges in physicochemical characterization for thin films arise because of the very small amount of MIO produced in a single film (vide supra); this is an unnecessary evil, given the ease with which gram-scale amounts of silica-based sol-gel materials can be produced at low cost. Therefore, the preparation of various morphologies of the same MIO is useful for obtaining a more complete understanding of the characteristics of the system. Naturally, the different processing conditions can introduce variations in the materials, and these must be considered when drawing conclusions, but given the flexibility of the sol-gel method and the ease of changing one variable at a time, it is possible to account for these inconsistencies.
Figure 2-10. a) Imprinting, imprint removal, and site-selective tagging scheme for a site-selective tagged and template xerogel (SSTTX): 1 material synthesis from an IC linked by a carbamate bond (magenta) using TMOS as a crosslinker, water, and HCl as a catalyst; 2 imprint removal by carbamate cleavage using LiAlH₄ in THF under argon; 3 target uptake of 9-anthrol (green) or its tautomer 9-anthrone, followed by site-selective tagging of the second aminopropyl group by the fluorescent probe 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD, blue) in THF. After tagging, 9-anthrol is washed out to produce a sensor. b) Steady-state emission spectra from imprinted material in a before (—) and after (--) LiAlH₄ treatment, showing >90% imprint removal. c) Photograph of monoliths of control materials (A–D) and the SSTTX (E, as prepared in a) excited at 488 nm and filtered for NBD fluorescence only. Controls varied from E as follows: A no imprint; B noncovalent imprint analogue; C one-point imprint analogue; D no imprint removal step. d) NBD response profiles for the SSTTX and controls A–D exposed to varying [9-anthrol]. Controls do not give a significant response, while the SSTTX does. Reproduced with permission from ref. 56. Copyright 2006 American Chemical Society.

An imprinting approach using multiple morphologies to achieve comprehensive characterization appears in the work of the Bright group, who used 9,10-anthracenediol as an imprint molecule in bulk organosilica and thin films with TMOS as a crosslinker (Figure 2-10a).⁵⁶ The bulk monolithic materials and thin films were labeled with a polarity-sensitive probe molecule, 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD), which was used to sense the presence of the target by its fluorescence response. After labeling, the so-called site selectively template and tagged xerogel (SSTTX) to detect 9-anthrol, which is structurally similar to the imprint molecule. To achieve a better understanding of the performance of the MIOs, four control materials were prepared to eliminate four possible artifacts, namely fluorescence from nonspecific matrix binding (A), fluorescence from imprint site binding by encapsulation only (B), fluorescence in the absence of the target molecule (C), and fluorescence from NBD not covalently bound in the imprint site or other undesired fluorescence (D). All five materials (controls A–D and the SSTTX, E) were prepared as monoliths in duplicate and thin films. One monolith of each material was ground to a fine powder (irregular particles 20–500 μm in
diameter), and one was left whole. The maximum diffusion length for imprint sites buried most deeply in the material varies from 2 μm for films to as much as 250 μm for particles (assuming a maximum diameter of 500 μm) and 0.5 cm for monoliths (which were prepared in 1.0-cm cuvettes). Longer reaction times were accordingly allowed for longer diffusion distances. The imprint removal step by treatment with LiAlH₄ in THF was performed for various times depending on the material morphology: 10–20 minutes for thin films, 24 hours for powders, and at least 48 hours for monoliths. Likewise, washing to remove cleaved imprints, loading of 9-anthrol, and labeling with NBD were carried out for longer times in samples with longer diffusion lengths. Confirmation of successful imprint cleavage and removal was confirmed through the steady-state fluorescence from the anthracene moiety; long reaction and washing times resulted in the removal of more than 90% of the imprint from the monolithic imprinted material (Figure 2-10b).

Monolithic samples were used to confirm that the complete SSTTX system was responsible for a positive response to the target by comparing fluorescence signals. Control materials A–D showed no analytical response, whereas the E showed significant fluorescent response when excited at 488 nm (Figure 2-10c). Also of note in this result is the uniformity of the intensity of fluorescence throughout monolith E (not easily seen in the figure). This suggests a uniform distribution of the imprint sites throughout the monolith, which is a highly desirable result if the monolith is to be ground to a powder for separation applications. Obtaining confirmation that the binding sites are homogeneously distributed throughout a MIP is not trivial. Of note here is the time allowed for the target to penetrate the monolith: it was exposed to a solution of 9-anthrone for fifteen minutes. Conversely, the 2 μm thick film sample exhibited 90% of full scale fluorescent response after 45 seconds of exposure to the target in solution. This is a relatively long response time, which is to be expected for a dense organosilica film. Increasing porosity has been shown to have a significant impact on diffusion of gases in sol-gel thin films⁵⁷ and the corresponding response time of thin film sol-gel gas sensors.⁵⁸ Accordingly, even in thin films with single micron diffusion lengths, the porosity of the material will affect response times for sensing small molecules that would require even greater pore sizes for easy diffusion than diatomic gas molecules.

The main reason to use different morphologies to characterize the same MIO system is that different shapes give different information. Film thicknesses are remarkably easy to vary, which would allow
for the construction a calibration curve of the response time of a sensor as a function of diffusion length, provided porosity is controlled. Monoliths with the fluorescent response in the example above are useful for interrogating the distribution imprint sites. Powders are easily packed into columns for multiple cycles of response testing, repeated separation runs, or easy determination of the response as a function of target concentration (Figure 2-10d). Altogether, the different pieces of information that the various MIO morphologies give contribute to a complete understanding of many aspects of the imprinting system.

### 2.4 Case Studies in Nanoscale Morphology Control: Templated Pores

Ideally, MIO materials would be prepared with minimized diffusion through very small dimensions in at least one direction. This is possible to a limited extent by grinding monoliths to very fine powders (three-dimensional decrease in diffusion lengths) or spinning ultra-thin films (one-dimensional decrease), but even these approaches are unlikely to achieve nanometre diffusion lengths. Thin films that are tens of nanometres thick are easy to fabricate, but the resultant decrease in the volume of MIO material translates to fewer imprint cavities, which means that the signal to noise ratio may suffer for sensing applications. Optimization of any one parameter often results in compromises in another.

As previously discussed, thin films are inherently denser than monoliths, and the decrease in diffusion length achieved is likely to be counterbalanced by an accompanying decrease in porosity. Thus, the best solution to the problem of improving diffusion to achieve fast imprint–target interactions in rigid polymers like organosilica is porosity. Studies have clearly shown that porosity affects diffusion, though it is hardly a logical stretch to understand this point. It is only natural, then, to want to introduce more porosity into molecularly imprinted materials. However, random porosity generated by solvents in bulk organosilica is less than ideal, as the diffusion pathways are not guaranteed to be optimal and the connectivity of pores cannot be controlled.

### 2.4.1 Templated Pores with Controlled Shape and Size

The IUPAC classifies pores into three size categories based on their smallest diameter: micropores have diameters smaller than 2 nm, macropores have diameters greater than 50 nm, and mesopores
have diameters between 2 and 50 nm. Pores of irregular shape behave according to the smallest diameter they possess. Long channels that are open at one or both ends are classified according to the diameter and not the length of the channel. Typical solvent pores in bulk sol-gel silica range are a mix of micropores and mesopores with broad pore size and shape distributions.

In 1992, a report from Kresge and coworkers gave rise to an entirely new class of sol-gel materials: ordered mesoporous silica. The addition of a quaternary ammonium surfactant to a sol-gel molecular sieve synthesis produced a highly porous material with an unprecedented structure: the pores were long channels of precisely uniform diameter in the meso range arranged in a two-dimensional hexagonal (honeycomb) lattice. The pore structure of the so-called MCM-41 periodic mesoporous silica (PMS) was templated by the surfactant, which formed a 2D hexagonal liquid crystalline phase in solution.

Intense research activity with different surfactants, sol-gel precursors, and synthetic conditions over the past two decades has led to a massive wealth of ordered mesoporous materials of highly controlled pore size, shape, and arrangement, interesting chemical functionality, and various higher order morphologies tailored to specific applications. Two notable achievements in the first decade were the first successful use of a nonionic block copolymer surfactant template (triblock copolymer Pluronic P123) to produce so-called SBA-15 PMS, which allowed for the synthesis of materials with larger pore diameters (and easier pore size tuning over a wide size range), and the simultaneous first reports of periodic mesoporous organosilica (PMO) materials from three all-organosilica (RO)SiR'Si(OR)3-type silsesquioxane molecular precursors: BTE, BTEA, and 1,2-bis-(trimethoxysilyl)ethane (BTMA). In the past decade, ordered mesoporous organosilica has been the subject of many excellent reviews.

2.4.1.1 Basic Templating Approaches

The basic approach to preparing PMS/PMO involves the organization of molecular sol-gel (organo)silica precursors around a micelle structure of a surfactant or polymer in solution, acid- or base-catalyzed hydrolysis and polycondensation of the alkoxyasilane groups, and removal of the micelle structure to liberate a final ordered porous material (Figure 2-11). In contrast to molecular
imprinting where the desired pores are created by an *imprint* molecule, the desired porosity in PMS/PMO is produced by the surfactant or other amphiphile, which is a supramolecular *template*. As with bulk sol-gel organosilicas, the variations possible in synthetic approach, chemical composition, and post-synthetic treatment steps allow for a wide variety of mesoporous materials that can be tailored to specific applications.
Figure 2-12. Interactions between the sol-gel species and the template in acidic, basic, and neutral media. Electrostatic interactions are a) \( S^+I^- \) (basic), b) \( S^+X^-I^+ \) (acidic), c) \( S^-M^+I^- \) (basic), d) \( S^-I^+ \) (acidic), and hydrogen bonding interactions are e) \( N^0H^+/(X^-I^+)^0 \) (neutral) and f) \( S^0(XI)^0 \) (acidic). Reproduced with permission from ref. 69. Copyright 2006 Wiley VCH. Additional ionic interactions are possible if a neutral template is protonated in acidic media and a mediator anion is present: g) \( (N^0H^+)(X^-I^+)^0 \). Penetration of the hydrophilic block (magenta) of a nonionic triblock copolymer template into the sol-gel phase (grey) depending on synthetic conditions occurs to varying degrees from g) not at all to h) partially to i) completely, which will affect the templated pore diameter.

Depending on the synthetic conditions and choice of starting materials, pore diameters in these materials can range from ~3 nm to tens of nanometres. Wall thicknesses are typically between 5 and 8 nm, and particle sizes are easily tuned. PMO materials can be prepared in such controlled morphologies as spheres,\(^{81}\) hollow spheres,\(^{82}\) “nanorice”,\(^{83}\) coiled rods,\(^{84}\) thin films,\(^{85}\) and freestanding membranes.\(^{86}\) The materials typically have high surface area, reaching more than 1000 m\(^2\)/g in some cases, and allow tunable microporosity in the walls through various processing conditions. The potential for better MIO materials through mesopore templating is quite clear: when open pores are of uniform shape and size, access to porosity is improved,\(^{87}\) and better imprinted materials are possible.

### 2.4.1.2 Interactions in Mesopore Templating

In order to successfully use a micelle system to template pores in a sol-gel material, there must be at least one type of attractive interaction between the micelle system and the sol-gel precursor(s) at the early stages of hydrolysis and condensation. Therefore, depending on the template species used, the optimal experimental conditions will involve different interactions (Figure 2-12). The template (S, surfactant, or N, nonionic copolymer) can have inherent attractive interactions (a, d, e). Alternatively, mediator ions (M\(^+\), metal cations, or X\(^-\), typically halide anions) enable cooperative self-assembly (b,
c, f, g). While surfactant templates form well-defined micelles, the hydrophilic blocks of nonionic copolymer templates can also participate in attractive interactions with the sol-gel species. This can result in the partial or complete penetration of the hydrophilic block into the walls of the templated pores, which can introduce additional microporosity and affect the pore diameter as a result (h, i, j). This penetration can be more pronounced if silsesquioxane precursors with bridging organic groups are used.

Molecular imprinting in PMS/PMO materials faces a particular limitation that is not present in most bulk MIOs: unlike bulk materials (monoliths, thin films) where entrapment of the imprint or IC is certain, the volume of solvent used to synthesize most template mesoporous materials is large enough that the location of the imprint or IC is not certain unless specific control over it is exerted. Noncovalent imprinting of organic compounds is especially challenging, as hydrophobic interactions can overcome IC interactions, and result in the imprint molecule being sequestered within the micelles rather than bound inside the condensing sol-gel phase. Alternatively, a covalently bound IC may interact favourably with a portion of the template, resulting in a material in which the imprint sites are located on the surface of the pores rather than buried in the walls. While this case is not necessarily problematic (as the following section, which deals with imprinting that is deliberately located on pore surfaces, will show), it means that care needs to be taken when making statements about where exactly the imprint sites are located.

### 2.4.2 Surface Imprint Grafting on Periodic Mesoporous Silica

One approach to overcoming the diffusion problem that we have not yet discussed is controlling the depth at which imprint sites are located. With the right imprinting strategy, the sites can in fact be located on the surface of a substrate. Grafting alkylalkoxysilanes onto PMS surfaces (as opposed to planar silica substrates) has the distinct advantage of incredibly high available surface area: provided the species to be grafted is size-matched to the pores of the support mesoporous material, hundreds of square metres per gram of surface area are available. This allows for much higher loading of imprints (or any other grafted species) than on planar substrates, while maintaining the fast kinetics of access to surface sites.
Dai and coworkers reported imprint coating in MCM-41-type PMS powder with an average pore diameter of 2.5 nm and a specific surface area exceeding 1000 m²/g. This was achieved by grafting an IC of Cu²⁺ and AATMS (trimethoxysilane analogue of 5, Figure 2-6a) onto the surface of the mesopores (I, Figure 2-13). A non-imprinted control material was synthesized by grafting pure AATMS with no Cu²⁺ onto another sample of the same PMS (II). Additionally, commercially available amorphous silica gel with an average pore diameter of 6.0 nm and a specific surface area of 600 m²/g was coated with the same IC (III). Because amines become protonated below pH = 3 and lose their ability to complex metal cations, removal of the imprint ions was achieved simply by soaking the imprinted material in slightly acidic aqueous solution for 20 minutes and then neutralizing it to pH = 7 before drying. If the uniform shape of pores had no impact the imprinting effect observed, then similar behaviour should have been observed for I and III in uptake and selectivity experiments. On the contrary, I showed an imprinting factor IF for Cu²⁺ of 5.7 over II, while III showed a maximum IF of 1.54. Because the cylindrical pores in the PMS used in this work were of the ideal size for the specific IC used, the authors attributed the better performance of I to two major characteristics: pore curvature and size that matched the stereochemical requirements for imprinting the Cu²⁺ ion, and a uniform pore size distribution that limited the coordination environment to the desired configuration.
Neither of these characteristics is present in amorphous sol-gel silica, and this report clearly demonstrates the value of mesopores for surface metal ion imprinting. The same group later used cocondensation of the same IC with TEOS was used to yield a so-called “hierarchically imprinted sorbent material” 91. This phrase highlights the conceptual connection between molecular imprinting and templated mesoporous materials: surfactant templating could arguably be seen as a form of molecular imprinting, but at the supramolecular level. When combined, the methods yield hierarchically porous materials.

Grafted surface imprinting has also been reported in PMS using small molecule imprints. Triangular and rectangular imprint molecules attached by a carbamate bond to APTMS (trimethoxysilane analogue of 3, Figure 2-6a) was grafted onto the pore surfaces of SBA-15-type PMS (Figure 2-14a, b). 92 Size-selective imprint cavities in this case were created by capping residual surface silanol groups on the pore walls with octadecytrimethoxysilane (OTS). The resultant imprinted
2,4,6-trinitrotoluene (TNT, 1) using Brij 76 (2) and dinitrobenzene-capped Brij 76 (3) as the template and IC, respectively. Template micelles with 12.5% 3 form in solution, and cooperatively self-assemble with two crosslinkers (1,4-bis(trimethoxysilylylethyl)benzene (BTMEB, 4) and bis(trimethoxysilylmethane (BTMA, 5)) to form an optimized surface-imprinted PMO. Removal of the mixed surfactant template leaves surface imprint cavities. Adapted from ref. 93.

materials showed shape selectivity for the imprint molecules even though the monolayer of octadecyl groups is not rigid or even well defined (Figure 2-14c, d). Size and shape selectivity of small organic molecules is clearly possible through grafted surface imprinting on PMS materials.

### 2.4.3 Synthesizing Mesoporous (Organo)silica with Surface Imprints

Post-synthetic grafting methods are certainly easy to use and modify, but this approach does suffer from one flaw in particular: grafted species can block pores or decrease their diameters significantly: in the previous example, the pore diameter went from 4.5 nm in the original PMS to 2.4 nm after OTS coating. This pore diameter decrease can have major implications for accessibility, which could defeat the purpose of using a mesoporous material altogether. The obvious alternative is building mesoporous (organo)silica with imprinted surface sites formed *in situ*. This strategy arguably makes better use of the mesoporous nature of PMS/PMO because it does not introduce any additional species into the pore channels.

Taking a creative approach to synthesizing an IC, Johnson and coworkers reported template-directed surface molecular imprinting for the selective adsorption of 2,4,6-trinitrotoluene (TNT) (Figure 2-15). The mixed ethane- and diethylbenzene-bridged PMO was templated using an oligomeric alkyl poly(ethylene oxide), Brij-76 (1), as both the template and the functional monomer.

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iv. The term “bridged” here is used with the parent name of the organic fragment that joins two Si(OR)$_3$ groups in a silsesquioxane organosilica precursor molecule. It is common to call PMO synthesized from BTEA “ethane PMO” or “ethane-bridged PMO”.
The terminal alcohol (green) was reacted with 3,5-dinitrobenzoyl chloride to form an IC (2) containing a terminal 3,5-dinitrobenzoate group (fuchsia). Two crosslinkers were used: 1,4-bis(trimethoxysilylethyl)benzene (BTMEB, 3) for its ability to interact noncovalently with the imprint molecule, and BTMA (4, trimethoxysilane analogue of 9, Figure 2-6a) for good structural integrity. Using a variety of template/IC and BTMEB/BTMA ratios, a series of molecularly imprinted mesoporous organosilica (MIMO) materials were synthesized that displayed varying physicochemical and binding properties. When 2 comprised 12.5% of the total amount of template and when 3 comprised 30% of the crosslinker, the best compromise of structure, capacity, and selectivity was achieved in the MIMO. When the amount of 2 was increased in the template mixture, greater capacity but lower selectivity was observed. An increase in the amount of BTMEB resulted in both greater uptake capacity of TNT and better selectivity for it. However, when BTMEB comprised more than 40% of the total crosslinker, a transition from mesopores to micropores and a decrease in the overall pore order and uniformity were observed, likely due to the fact that this high loading of a non-rigid precursor weakens the structural integrity of the MIMO and its ability to hold the templated shape. In binding tests of TNT and structural analogues, binding equilibrium in static adsorption on the optimized MIMO was achieved in under 5 minutes. By comparison, MIMOs with lower pore size and shape uniformity took longer to reach adsorption equilibrium (up to 50 minutes). This provides excellent evidence for the advantage of ordered mesopores over poorly ordered mesopores or micropores for rapid target binding, even when a surface imprinting method is used to allow for the easiest access possible to imprint sites. Additionally, this example highlights the balance that exists in optimizing different parameters in a MIP system: superior performance is not necessarily achieved with maximum loading of imprint sites.

An attractive feature of sol-gel silica is its high crosslink density, which gives it rigidity and the ability to form very fine features. Silica is not chiral, but because of its utility in so many separation and sensing applications (either as a solid support or an active material itself), significant interest exists in

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v. The less rigid the organic linker in a silsesquioxane PMO precursor, the poorer the structural integrity of the PMO after template removal. PMOs made from precursors containing propyl or longer flexible groups collapse upon template removal. A mixed precursor approach can circumvent this problem.
imbuing it with a chiral nature. The Marx group has reported a chirally imprinted thin film of sol-gel organosilica (not containing templated mesopes) that displayed enantioselectivity through careful tailoring of the R’ groups on (RO)_3SiR’ precursors to match specific functionalities on a chosen imprint molecule. In this case, an enantioselective negative chiral image was incorporated into imprint cavities through the spatial arrangement and orientation of organic functional monomers. However, this kind of chiral imprinting relies almost entirely on the presence of organic functional groups for enantioselectivity. To determine exactly how finely featured silica can be, it is necessary to remove the organics and look at the purely inorganic matrix.

In MCM-41 synthesis, a common practice is to remove the template by calcination because the strong ionic interactions between the cationic surfactant template and the silica matrix make solvent extraction less reliable. Under typical calcination conditions (450 °C or higher, flowing air, oxygen, and/or nitrogen atmosphere), all organic fragments within a sample (template, R’ groups, residual OR groups) are burned away, and additional condensation often occurs. The Coronas group made use of this to demonstrate the creation of chiral imprint cavities in calcined imprinted MCM-41 (Figure 2-16). As with the surface imprinting reported by Johnson (vide supra), the authors used a
modified surfactant as the functional monomer. However, in this case, the quaternized aminosilane surfactant functional monomer was used exclusively as the pore template (no unmodified surfactant was used, Figure 2-16a). The positively charged quaternary ammonium group interacted with the deprotonated form of the imprint amino acid (in this case, L- or D-proline) in basic media, and created imprint cavities on the surface of the pores. A control material imprinted with DL-proline (racemic mixture) was also prepared. The benefit to using an alkoxyilane-containing functional monomer of this type is that by virtue of its ability to participate in three interactions simultaneously (with the imprint, the crosslinker, and the template), it in effect guaranteed the precise placement of imprint cavities on the surface of the templated pores. Because the template was covalently bound to the mesoporous material by Si–C bonds, it was necessary to use calcination to vacate the pores. Confirmation of the presence of chiral sites after calcination was obtained by solid-state induced circular dichroism, which measures the asymmetry induced on an achiral molecule (in this case, phenol) by a chiral environment (Figure 2-16b).

The signals for the chiral imprinted materials are of the same intensity but opposite value (indicating opposite chiral handedness), while there is no observed signal for the DL-imprinted material. In a binding test using a racemic mixture of D- and L-proline, clear enantioselectivity was observed for both chiral materials, while the DL-imprinted material shows no preferential adsorption. (Figure 2-16c). Despite observed shrinkage in the silica matrix after calcination and the absence of organic functional groups, this work successfully demonstrated the generation of extremely fine imprint sites in pure sol-gel silica. Morrison’s 1959 assertion that silica shrinkage would necessarily destroy imprint sites seems to have been conclusively proven incorrect.

2.4.4 Imprinting Inside the Walls of a Mesoporous Material

Achieving surface imprinting in PMOs is a relatively easy task when grafting is used, as there is no ambiguity as to the location of the imprint sites. Likewise, cocondensation with any (RO)3SiR’ species will create a material with R’ on the pore surface, allowing very simple surface imprinting. In situ surface imprinting by cocondensation can also be controlled by exploiting strong template-functional monomer interactions to create “dents” in the pore walls, as discussed in the previous examples.
Figure 2-17. a) Schematic representation of imprinted PMO material before and after imprint removal, showing the carbamate bond used in sacrificial spacer imprinting (magenta) and the fit of the imprint molecule (green) inside the imprint cavity. b) Molecular structures of targets used static binding tests: diethylstilbestrol (DES), bisphenol-A (BPA), 4,4'-biphenol (BP), and hydroquinone (HQ). c) Static binding tests for four targets using the imprinted PMO and a nonimprinted control PMO. d) Kinetic binding profile for DES of the imprinted PMO and the nonimprinted control. e) Transmission electron micrographs of imprinted PMO showing the spherical shape and small particle size produced. From ref. 99. Reproduced by permission from the Royal Society of Chemistry. f) Fluorescence quenching of CdSe quantum dots embedded in BPA-imprinted PMO and nonimprinted control as a way of detecting the binding of four targets in solution. From ref. 100. Reproduced by permission from the Royal Society of Chemistry.

Making the transition to imprinting inside the pore walls, however, is a less straightforward task. Producing a material with the correct chemical functionality is not the main challenge; this is as straightforward as synthesizing an appropriate IC and cocondensing it with a given crosslinker in the presence of an appropriate template. The challenge is finding evidence that the imprint sites are indeed inside the walls of the material. If the IC is of the form \((R\text{O})_3\text{SiR'}\text{Si}(	ext{OR})_3\), it is reasonable to predict that it will be integrated into the matrix of the pore walls. However, depending on the system, it is also possible that the IC will have favourable interactions with the template, and thus be located primarily on pore surfaces.

The Chang group were the first to report the synthesis of a MIMO (MCM-41-type) with the IC designed to create imprint sites inside the pore walls.\textsuperscript{99} The imprint molecule, diethylstilbestrol (DES), was bound at two points to ICPTES via a carbamate linker (Figure 2-17a).\textsuperscript{vi} The imprint

\textsuperscript{vi} No doubt the popularity of the carbamate bond for semicovalent imprinting in sol-gel silica is caused at least in part by the early success it enjoyed, and a desire to introduce as few uncertain variables into a new imprinting system as possible. The carbamate is easy to form in excellent yield, and just as easily liberates a hydrogen bonding 3-aminopropyl
molecule was one of four used in binding tests; the other three, bisphenol A (BPA), 4,4′-biphenol (BP), and hydroquinone (HQ), were chosen for their structural similarity but different phenol separation distances (Figure 2-17b). Compared to a control nonimprinted mesoporous organosilica (NIMO) prepared with APTES instead of the IC, the MIMO showed large uptake capacity and selectivity for DES, while the control showed low capacity and little selectivity for all four targets (Figure 2-17c). In a kinetic binding test using DES, the both the MIMO and NIMO reached 95% of equilibrium binding in approximately 5 minutes (the MIMO was slightly faster), and complete equilibrium in less than 10 minutes (Figure 2-17d). This binding speed is close to that of surface-imprinted PMOs, which is reasonable given the very short diffusion distances in PMS/PMO materials. In the spherical particles produced in this report (Figure 2-17e), the authors estimated the wall thickness to be 2.3 nm. Given the length of the DES molecule (1.4 nm), they reasoned that the imprint sites were most likely embedded in the walls of the imprinted PMO particles. However, in the absence of a binding test using a molecule with a larger size than DES, which could confirm a closed size-selective cavity, it is also possible that the imprint sites were on or only partially embedded in the surface of the pores. In a follow-up report, the Chang group prepared a BPA-imprinted MCM-41-type PMO in which CdSe quantum dots were used to detect binding of targets by fluorescence quenching. The imprinted PMO showed excellent binding for BPA but boor binding for the larger DES and smaller targets BP and HQ (Figure 2-17e), which confirmed that a size-selective cavity had indeed been produced.

We recently reported the synthesis and characterization of SBA-15-type MIMO, where careful characterization of the porous structure of the material did indeed offer evidence of buried imprint sites. The complete report is reproduced in Chapter 4 of this thesis. Using BPA as an imprint molecule, the semicovalent imprinting strategy with ICPTES as the functional monomer shown in previous examples discussed here (IC formed by a carbamate bond) and TEOS as the crosslinker, we functionality. The prevalence of this bond in silica imprinting is akin to the extensive use of propranolol as an imprint molecule in imprinted organic polymers, simply because it has come to be an archetypal imprint molecule.
prepared a series of three SBA-15-type materials: MIMO (I) containing the IC, a control non-imprinted mesoporous organosilica (NIMO, II) prepared with ICPTES (which was reduced to a primary amine during the synthesis) and no BPA, and control blank SBA-15 (III), synthesized from only TEOS (Figure 2-18a). Based on careful assessment of the dimensions of the porous structure (pore diameter and wall thickness) found by gas sorption and small-angle X-ray scattering, and the likely interactions that the molecular precursors had with the pore template, we concluded that the imprint cavities in I were most likely embedded in the pore walls, the 3-aminopropyl groups in II were on the surface of the pores, and III contained the typical surface silanol and residual ethoxy groups found in this class of materials, which allowed it to demonstrate the effect of nonspecific binding only. In solid-phase extraction tests (flow rate ~0.2 mL/min) of four competing targets in aqueous solution (phenol (Ph), resorcinol (R), 4,4′-biphenol (BP), and bisphenol A (BPA), Figure 2-18b), a clear imprinting effect was observed (Figure 2-18c). Both BP and BPA were strongly bound in I, while Ph and R, which are significantly smaller, could not participate in interactions with
both amine in the imprint cavities. Some weak binding of BP and BPA was shown in both II and III, but the rinse step removed most of the bound molecules. Despite the absence of imprint functional groups, III showed similar binding behaviour to II, which, coupled with the strong binding observed for I, confirms the creation of imprint cavities with precisely arranged primary amine groups. Static binding of the large dye bromothymol blue (BTB) served to confirm that I contained imprint cavities that were fairly size selective (Figure 2-18d). II bound 98% of the dye, while I only bound 71%. Approximately 50% of the amount could be accounted for by nonspecific binding (as found from the dye bound to III). Overall the results confirmed that the easily accessible surface amines in II were different from the at least partially buried amines in the imprint cavities of I. In a porous material with walls less than 5 nm thick, the depth at which imprint cavities are buried is likely not sufficient to completely exclude bulkier molecules like BTB, which still contains the same spatial arrangement and separation of the phenol groups.

These two examples served as the first confirmation that successful molecular imprinting inside the walls of the two most commonly used classes of PMS materials (MCM-41 and SBA-15) was possible. As opposed to surface imprinting techniques, wall-imprinting has the potential to produce MIMO materials with a high degree of size and shape selectivity that is similar to imprinting in bulk MIO materials, but still show the very fast kinetic binding profiles made possible by templated mesopores.

### 2.5 Outlook for Imprinting in Silica

Clear advantages have been demonstrated moving from bulk monoliths to particles to thin films to templated mesoporous materials. The importance of diffusion length for target binding in an imprinted material is evident in the kinetics of target binding, and an obvious improvement exists when controlled porosity in the form of uniform channels is created. Three years ago we asked ourselves the question: why PMO? Molecular imprinting imbues any polymer with tremendous functionality and utility, and the intersection of the fields of molecular imprinting and supramolecular pore templating offers a truly elegant answer to this question. The extensive library of molecular sol-gel precursors that are commercially available makes careful tuning of the imprinting method easy. The wide range of processing conditions that are possible with sol-gel chemistry (including non-aqueous methods) make virtually any imprinting system possible: noncovalent, semicovalent, ionic, and coordination imprinting have all been successfully achieved in
silica, and it is likely that covalent imprinting has been overlooked so far simply because of the energetic cost of binding a target relative to the semicovalent approach. The ease with which the morphology of sol-gel materials can be controlled gives them a significant advantage over many organic polymer systems used for molecular imprinting, and allows for fine control over diffusion distances down to the nanometre scale. Finally, the development of hierarchical imprinting/templating approaches in highly porous MIMO materials, both surface- and wall-imprinted, opens the door to the production of imprinted materials that possess excellent starting material economy from the first step of synthesis to the final imprinted material, a greener chemical system that uses water and alcohol instead of toxic organic solvents, and the ability to exploit virtually all of the successes of more than eight decades of molecular imprinting and four decades of sol-gel science and technology. It is certain that imprinting in organic and inorganic polymers will continue to travel parallel paths. However, the renaissance of imprinted silica and the birth of imprinted mesoporous organosilica have demonstrated silica’s excellence as not just the passive solid support used throughout the organic polymer field, but a truly imprinted polymer matrix, able to resolve fine molecular detail through delicate selectivity and tremendous versatility.
Works Cited


3 Characterization Techniques

Because this thesis straddles a number of disciplines within chemistry and materials science, it relies on a number of characterization techniques that do not necessarily cross between disciplines or that are used in a less conventional manner. As such, it is of value to provide an overview of three techniques in particular, which are gas sorption, solid-state nuclear magnetic resonance spectroscopy (SSNMR), and small-angle X-ray scattering (SAXS). Gas sorption is widely used but some of the fine points that affect results are frequently overlooked in so-called “routine” analysis using the most popular methods. This oversight can produce results that are, in fact, erroneous. Alternatively, a limited understanding of the information that can be obtained from gas sorption experiments means that they simply are not performed. SSNMR is the less frequently used cousin of solution-state NMR, and is subject to a number of experimental and theoretical limitations. SAXS is a form of X-ray diffraction that obeys the same diffraction laws as regular X-ray crystallography, but, like SSNMR, is limited in its precision due to the nature of mesoporous materials.

Chemical characterization was achieved using SSNMR and FTIR; physical characteristics of the materials were determined using gas sorption, electron microscopy, and SAXS. These methods are used to complement one another and fill information gaps. None of these techniques should be used without a good understanding of the working principles. It should not be acceptable to use a technique simply because it appears in the literature, and therefore at least a basic discussion of the fundamentals of each method is necessary.
3.1 Characterization of Porous Solids by Gas Sorption

The following is a brief overview of the important concepts required for correctly interpreting the results of gas sorption experiments. A more comprehensive discussion of the theory upon which these concepts are based, including derivations, examples of experimental results and their interpretation, and alternative approaches to determining various properties of porous solids, can be found in several excellent texts on the subject.¹-⁴

Gas sorption is one of many experimental techniques available for characterizing porous solids.⁵ Others include stereology (such as electron or optical microscopy, depending on the size range), radiation scattering (light, X-ray, or neutron), pycnometry (including mercury porosimetry and ellipsometric porosimetry), fluid flow, calorimetry, size exclusion chromatography, and xenon NMR. Among the factors used to select the characterization method are the expected size range of the pores, the intended use of the material (specifically the interactions that occur in the application), and the morphology and dimensions of the material (powder, thin film, pellet, monolith, etc.).

For many reasons, gas sorption is the most popular method for characterizing porous solids.⁵ It is possible to analyze pore sizes from ~0.35 nm to more than 100 nm, which covers the complete range of micropores and mesopores, and extends into macropores. Coupled with mercury porosimetry, it is possible to obtain a pore size profile between 0.35 nm and ca. 400 μm. Gas sorption is convenient, inexpensive, and non-destructive. Automatic instrumentation and specialized software allow users to quickly and easily collect and process data using a variety of experimental methods and theoretical approaches to calculations.

No method provides absolute values for parameters such as porosity, pore size, surface area, and surface roughness. In all methods, the values obtained are affected by the principles upon which the method is based. It is not possible to check the validity of obtained values by comparing results from different methods and looking for perfect agreement. While this agreement may occur, it is not necessarily proof of the correctness of the results. Instead, the focus should be on understanding how different techniques are complementary to one another.⁵
Figure 3-1. Schematic representation of the types of pores that may be present in a porous solid. Closed pores may be spherical (a), though this shape is rarely of interest as these pores are not accessible and only affect the overall density of the porous solid; open pores are channels with at least two openings; blind pores have only one opening; open and blind pores may be cylindrical (b), branched (c), interconnected channels (d), ink bottle-shaped (e), or funnel-shaped (f).

It is common to use a combination of techniques when characterizing a single sample. In this thesis, gas sorption is used together with SAXS (see Section 3.3) and electron microscopy (scanning electron, SEM, and transmission electron, TEM) to establish an understanding of the different physical characteristics of the materials prepared. These techniques were combined in order to gain a more complete understanding of each sample, but are not intended to serve as checks of one another.

3.1.1 Classification of Pores

A porous material is any solid that contains cavities, channels or interstices, and there are three main types of pores: closed, open, and blind (Figure 3-1). To be classified as a pore, a feature must be deeper than it is wide; therefore, surface roughness is not considered porosity. True pores may be spherical (a), cylindrical (b), branched (c), interconnected (d), slit-shaped (not shown), ink bottle-shaped (with a narrower mouth, e), or funnel-shaped (with a wider mouth, f). In order to understand the distinctions between different types of porous materials, and to recognize the significance of the physical length scales that are present in these materials, it is first necessary to clarify the key attribute that distinguishes the three main classes of porous materials. The IUPAC has articulated the crossover length scales for porous materials. A microporous material contains pores with diameters smaller than 2 nm; a macroporous material contains pores with diameters larger than 50 nm; a mesoporous material fits in the middle, with pores ranging in diameter from 2 to 50 nm. Pore sizes are most frequently reported as diameters, but the choice of parameter (diameter or radius) should always be specified. The choice of units is also important to specify, as nanometres and Ångstroms appear equally frequently in the literature, the choice generally depending on the field of study.
A porous material may contain more than one kind of pore (for example, mesoporous materials prepared from molecular precursors almost always contain micropores); in order to determine to which class a material belongs, the type of pore that contributes the largest fraction of the total pore volume (or void volume in the material) is used.

3.1.2 Adsorption at the Gas-Solid Interface

Adsorption at the gas-solid interface occurs between a solid (adsorbent) and a gas (adsorptive). The fluid that is adsorbed to the surface is called the adsorbate. The amount of gas adsorbed depends on the absolute temperature, \( T \), the pressure, \( P \), and the interaction potential \( E \) between the adsorbate and the adsorbent. The temperature is kept constant in a typical adsorption experiment, so at any given equilibrium pressure, the weight \( W \) of gas adsorbed on a unit weight of adsorbent is given by

\[
W = F(P, E)
\]

A plot of \( W \) as a function of \( P \) at constant \( T \) is called the sorption isotherm for a given experiment. In a real experiment, the volume of gas adsorbed, \( V_{\text{ads}} \), rather than the weight, is typically plotted as a function of relative pressure, \( P/P_0 \), measured between the sample cell and a reference cell, which is held at constant pressure (saturation pressure \( P_0 \), close to atmospheric pressure).

The interaction energy depends on the strength of the interactions between adsorbent and adsorptive. When strong interactions are present, the process is called irreversible adsorption, or chemisorption. This is because interactions are of a strength that approximates a chemical bond. This type of adsorption is typically used to study catalysts, in order to determine the number of active sites on a sample.

In physisorption, weak interactions dominate. The van der Waals forces between species result in the adsorption of successively more layers of gas as pressure increases. Because the interactions are weak, it is also possible to remove adsorbed gas molecules by lowering the pressure. Physisorption is characterized by the following:

1. Low heats of adsorption with no accompanying structural changes to the surface of the adsorbent.
2. The possibility of surface coverage by more than one layer of adsorbate.
3. Complete pore filling, which is useful in determining pore volume and pore size distribution.
4. Rapid equilibrium, though diffusion may slow adsorption in very small pores.
5. Complete reversibility, which allows for the study of both adsorption and desorption in a non-destructive manner.
6. No restriction of adsorbed molecules to specific sites (as with chemisorption), which allows for complete surface coverage.

When considering the thermodynamics of gas adsorption, there are a number of assumptions that are required to reduce the problem to a manageable scope. The process of adsorption is exothermic. The entropy, $\Delta S_a$, is negative, since adsorption results in the loss of one degree of translational freedom as molecules go from the gaseous state to the condensed state. It is reasonable to assume that the adsorbent’s entropy remains effectively constant (or at least does not increase more than $\Delta S_a$ decreases), so the overall $\Delta S$ is negative. Because adsorption is spontaneous, the Gibbs free energy, $\Delta G$, is also negative. Therefore, the enthalpy $\Delta H$ of the adsorption process, as defined by

$$\Delta H = \Delta G - T\Delta S$$

is negative, which indicates an exothermic process.

As previously stated, the dominant interactions in physisorption are the van der Waals interactions. London dispersion forces, which are present regardless of the chemical nature of interacting species, have an interaction energy $U_s(z)$ between a gas molecule and a planar surface of

$$U_s(z) = C_1z^{-9} - C_2z^{-3}$$

where $C_1$ and $C_2$ are constants and $z$ is the distance of the gas molecule from the surface. The first term describes Born repulsive forces, which occur when a molecule is too close to the surface, and the second term describes the attraction between the fluid and the wall. This attractive force at the minimum of the interaction potential is more than ten times greater than $k_B T$, where $k_B$ is the Boltzmann constant, which means that gas molecules will accumulate close to the surface. When $T$ is around the boiling point of the adsorptive gas, a dense monolayer of molecules forms at $P$ values well below the saturation pressure $P_0$, and a multilayer of increasing thickness and liquid-like density builds up as $P$ increases toward $P_0$. During this process, gas molecules are in dynamic equilibrium between the vapour phase and the adsorbed fluid phase.
3.1.3 Sorption Isotherms

3.1.3.1 Adsorption Potentials in Pores of Different Sizes

Each surface has its own interaction potential with an adsorptive molecule. Adsorption is governed by the combination of adsorptive–adsorbent (fluid–wall) and adsorptive–adsorptive (fluid–fluid) interactions, and confinement effects caused by narrow pores within the adsorbent (Figure 3-2). The three classes of pores (macropores, mesopores, and micropores) exhibit distinct adsorption behaviour because of the different dominant interactions that occur. Macropores can essentially be considered flat surfaces. Micropores are so small that the potentials of opposite walls overlap, and fluid-wall interactions dominate adsorption processes. In mesopores, fluid-wall interactions dominate near the walls, while fluid-fluid interactions dominate near the core. The attractive forces between gas molecules near the core can result in capillary condensation of the adsorptive gas at pressures well below the saturation pressure P₀. The interplay between fluid-wall and fluid-fluid interactions affects the shape of the sorption isotherm of a given material.
3.1.3.2 Classifying Sorption Isotherms

As a result of extensive literature study, IUPAC was able to provide a classification system for adsorption isotherms. There are six classes, and they represent materials that display different combinations of the fluid-wall and fluid-fluid interactions discussed in Section 3.1.3.1. In each of the six isotherms (Figure 3-3), a distinct shape occurs as a result of specific conditions.

In a Type I isotherm, a concave curve with respect to the relative pressure (P/P₀) axis is observed and the amount of gas adsorbed approaches a limiting value as P/P₀ → 1. This type of isotherm is most commonly observed in chemisorption, where monolayer coverage occurs and reaches a maximum value before saturation pressure. In physisorption, this isotherm shape occurs on microporous materials, where micropore filling occurs at low relative pressures, and the uptake limit is due to accessible micropore volume rather than internal surface area.
In a Type II isotherm, unrestricted monolayer–multilayer adsorption occurs. This is typical of nonporous or macroporous solids. The “knee”, or inflection point, at B indicates the point at which monolayer coverage is complete and multilayer coverage commences.

In a Type III isotherm, the convex shape of the curve over the whole P/P₀ range indicates very weak adsorbate–adsorbent interactions. In this case, adsorbate–adsorbate interactions are more prevalent. This is a very uncommon isotherm shape.

In a Type IV isotherm, the distinct presence of a hysteresis loop coupled with a plateau in the isotherm at high P/P₀ is characteristic of mesoporous materials. The hysteresis loop occurs as a result of capillary condensation within mesopores (this will be discussed further in Section 3.1.3.4), and the plateau indicates the point at which complete pore filling has occurred. As with a Type II isotherm, B indicates the point at which monolayer coverage is complete and multilayer coverage commences.

In a Type V isotherm, pore condensation and hysteresis indicate the presence of mesopores, but the convex shape of the isotherm before condensation points to weak adsorbate–adsorbent interactions as seen in Type III isotherms.

The Type VI isotherm is a special case: stepwise multilayer adsorption on a uniform, nonporous surface. The shape of the steps depends on the adsorptive gas, the temperature, and the homogeneity of the adsorbent surface.

### 3.1.3.3 Adsorption in Mesopores

In an ideal pore, with smooth, solid walls and a perfectly cylindrical shape, it is possible to model shape of the full sorption isotherm. As mentioned in Section 3.1.3.1, both fluid-wall and fluid-fluid interactions are present during adsorption in mesopores. Initially, fluid-wall interactions result in multilayer coverage of the adsorbent surface. However, the multilayer cannot grow indefinitely in a cylindrical pore, and the stability of the adsorbed film depends on a combination of fluid-wall interactions and the surface tension and curvature of the liquid-vapour interface. When the adsorbed film becomes thick, the chemical potential is dominated by the curvature of the interface, which depends on the core radius. This radius depends on the radius of the pore and the thickness of the
film; when the film thickness reaches a critical value, fluid-fluid interactions result in capillary condensation (also known as pore condensation). This phenomenon is a first-order phase transition from vapour-like to liquid-like states, and occurs at \( P/P_0 < P_0 \). The major phases in sorption in a single ideal pore can be distinguished from one another by the dominant process occurring at each phase (Figure 3-4).\(^3\) At point A, monolayer adsorption is complete, and multilayer adsorption proceeds through point B. This is the range in which the surface area of a material can be determined using Brunauer, Emmett, and Teller (BET) theory, which will be discussed in Section 3.1.5.1.

Between points A and F, adsorption is fully reversible. As multilayers build up, the adsorbed film eventually reaches a critical thickness at point C. At this point, the metastable film is at the limit of its stability. As \( P/P_0 \) continues to increase, spontaneous condensation occurs in the core of the pore, resulting in a steep rise in the isotherm. At point D, pore filling is complete, and the isotherm displays a plateau. The liquid inside the pore is separated from the bulk gas outside it by a hemispherical meniscus. As \( P/P_0 \) decreases during desorption, pore evaporation starts at point E and proceeds via a receding meniscus. The pressure at which this occurs is less than the condensation pressure, and represents an equilibrium transition. The hysteresis loop closes at point F, which represents a return to the equilibrium between the adsorbed multilayer film and the vapour in the core of the pore (and the
bulk gas phase). At this point, the three phases of the system (multilayer, condensed fluid, and gas) all coexist.\textsuperscript{2}

The ideal case of a single cylindrical pore is useful for establishing the source of the key features of a type IV isotherm, but the likelihood of a real isotherm having exactly this ideal shape is very small. A real sample will have a pore size distribution, not a single pore size, and this will result in a pore condensation step that is less sharp and almost certainly not vertical. The broader the pore size distribution in a sample, the shallower the slope of the condensation step, and the shorter the step is likely to be. Likewise, the hysteresis loop in the ideal case is perfectly symmetrical, whereas in a real isotherm it will likely be at least somewhat asymmetrical.

3.1.3.4 Isotherm Hysteresis

Hysteresis is observed in numerous systems, and indicates that the current state of a system also depends on its previous state. In the case of gas sorption, hysteresis is observed around the pore condensation step in an isotherm of a mesoporous material.

3.1.3.4.1 Types of Hysteresis

In addition to classifying the six types of isotherms, IUPAC has also empirically classified the four major shapes of hysteresis loops (Figure 3-5).\textsuperscript{8} Just as the slope of the condensation step can give insight into the narrowness of the pore size distribution in a real mesoporous sample, so can the shape of the hysteresis loop give information about the texture (including the pore size distribution, the geometry of the pores, and the connectivity of the pores) of the sample.

A type H1 hysteresis loop is observed for samples that closely resemble the ideal single pore case: they consist of well defined, ordered cylinder-like pore channels with uniform diameter. Alternatively, a sample of agglomerates of uniform spheres could also display this type of hysteresis, again due to well-defined uniform pores produced between the spheres.
Figure 3-5. IUPAC classification of the commonly encountered hysteresis loops. H1: porous materials with well-defined cylinder-like pore channels or agglomerates of uniform spheres. H2: disordered pores, large pore size distribution, ill-defined pore shape. H3: non-rigid aggregates of plate-like particles that produce slit-shaped pores. H4: narrow slit pores and micropores. Adapted from ref. 8.

Type H2 hysteresis is typically observed in disordered samples in which the pore size distribution and pore shape are poorly defined. This also occurs in cases where pore necks are narrower than the channels inside them (Figure 3-1e).

Type H3 hysteresis appears in isotherms with no upper limit of adsorption, and occurs in samples containing movable aggregates of plate-like particles that create slit-shaped pores in the sample cell.

An isotherm showing type H4 hysteresis is similar to the case with H3, but the sample is microporous. The parent isotherms for H3 are likely Type II and for H4 are Type I (Figure 3-3). The forced closure of the hysteresis loops in H3 and H4 (and sometimes in H2) is a result of the tensile strength of the adsorbate gas (typically nitrogen). This will be discussed in more detail in Section 3.1.3.4.2.2.

The dashed lines showing low-pressure hysteresis for all four isotherms represent either of two possible scenarios: non-rigid pores that swell during adsorption, or irreversible adsorption of adsorbate in ultramicropores (of about the same size as the adsorbate molecule). Chemisorption also produces open hysteresis loops (ones that do not close at all).
Figure 3-6. a) Real isotherm of argon at 87 K on MCM-41 compared with NLDFT isotherm calculated for a single ideal pore of diameter 4.8 nm (corresponding to approximate pore size of real sample). The experimental adsorption branch corresponds with spinodal condensation, while desorption is an equilibrium transition. Reproduced from ref. 11, copyright 2001, with permission from Elsevier. b) NLDFT isotherm at 77 K in a spherical cavity of diameter 15.5 nm, where $p_{sl}$ is the liquid-like spinodal pressure, $p_{e}$ is the equilibrium pressure, and $p_{sv}$ is the vapour-like spinodal pressure corresponding to the transitions shown in a). The capillary condensation pressure $p_{c}$ corresponds to $p_{sv}$, while the desorption pressure $p_{d}$ range covers three regions of evaporation with borders at $p_{e}$ and the cavitation pressure $p_{cav}$. Reproduced with permission from ref. 13. Copyright 2002 American Chemical Society.

### 3.1.3.4.2 Origin of Hysteresis

Three models exist that account for the observation of hysteresis in isotherms of mesoporous materials: the independent pore model, the network model, and the disordered porous material model. There still exists some disagreement as to the precise mechanism(s) responsible for hysteresis, but each of the first two models shows good agreement with primarily one kind of hysteresis observed for mesoporous materials. Understanding how these models explain different kinds of hysteresis is of great value when qualitatively interpreting isotherms and choosing data for calculations.

#### 3.1.3.4.2.1 Independent Pore Model

In this model, H1 hysteresis is observed for the phase transition occurring in the single, ideal pore discussed in Section 3.1.3.3. The standing theory, confirmed by theoretical Non Local Density Functional Theory (NLDFT) studies,\textsuperscript{10,11} is that hysteresis is caused by the existence of metastable states of the pore fluids around the pore condensation step. Pore condensation does not occur until the critical point at which the liquid phase is nucleated. If both the adsorption and desorption processes proceeded through the same metastable state, both would be associated with spinodal transitions, with
the adsorption displaying spinodal condensation, and desorption spinodal evaporation. However, real systems have pores of finite length, which allow evaporation to proceed by a receding meniscus (Figure 3-4), meaning that metastability does not occur for desorption. Using NLDFT, it is possible to predict the position of the possible transitions on the isotherm using real parameters (Figure 3-6a). An experimental isotherm, when overlaid with the model isotherm, indicates which branch corresponds to which transition in the system: adsorption occurs by spinodal condensation from a metastable state, and desorption proceeds by an equilibrium transition well before the point at which spinodal evaporation would occur. Understanding which branch corresponds to which kind of transition is critical for selecting the correct method to calculate pore size distribution in a sample, which will be discussed further in Section 3.1.5.2.

3.1.3.4.2.2 Network Model and Tensile Strength Effect

When a sample displays type H2 hysteresis, it is most likely composed of a wide distribution of pore sizes that are interconnected throughout the material. This include necks in the walls of pores that connect channels, and pore blocking or narrowing effects that occur at outlets or connections. Ink-bottle pores (either open or blind) with narrow necks but wider interiors exhibit this kind of hysteresis: the large interiors cannot empty at high pressure because they are blocked by a narrower opening, which controls the relative pressure at which evaporation can begin. This pressure also depends on the interconnectivity between pores and the state of neighbouring pores (which may or may not be similarly constricted at their openings). Desorption therefore cannot proceed by an equilibrium transition, and instead goes via a percolation mechanism.

Another aspect of this kind of hysteresis is the pressure at which rapid desorption is observed. The percolation mechanism can account for delayed or more gradual desorption, but when it is very sharp, almost vertical, and precisely located, another phenomenon is also occurring. Cavitation, a diffusion mass-transport process whereby cavities can be emptied via the necks while the necks remain filled, corresponds to spinodal evaporation from a metastable state (Figure 3-6b). It is observed at the lower limit for hysteresis in an isotherm using a given gas and experimental temperature, and is correlated to the tensile strength effect: when the relative pressure reaches a particular value, the meniscus of the condensed gas in that system no longer has sufficient tensile strength, and spontaneous spinodal
evaporation occurs. This event is independent of the texture or pore size distribution of the material being analyzed, and, if incorrectly interpreted, can result in a false peak in the pore size distribution. This will be discussed further in Section 3.1.5.2.3.

### 3.1.4 Collecting an Experimental Isotherm

Gas sorption experiments can proceed either by volumetric analysis or gravimetric analysis. The two approaches differ in cost and capability. While more expensive, gravimetric analysis is generally more precise and accurate. However, provided the limitations of the volumetric approach are recognized and taken into account, volumetric analysis is more than sufficient for most routine work. In this thesis, volumetric adsorption of nitrogen gas is the method of choice, and has been conducted on a Quantachrome Instruments Autosorb 1-C (Figure 3-7). The adsorption apparatus is fully automatic, and is controlled by AS1Win, software also from Quantachrome.
In a typical experiment, there are three steps:

1. Sample pretreatment by degassing
2. Isotherm collection
3. Data processing

In the pretreatment step, a clean sample cell is weighed on an analytical balance, and then a small amount of sample is added to the bulb of the cell. The cell is connected to one of the two degassing ports, and a heating mantle is attached around the bulb. Using the instrument software, the sample is first placed under vacuum via a controlled pump down of the manifold pressure. When high vacuum is achieved, the sample is heated using the heating mantle, while still under active vacuum, in order to drive off physisorbed material from the surface of the sample. This creates a clean surface in a reproducible initial state, and allows for accurate measurement of the sample surface area. Degassing is done at the highest temperature the sample can withstand without undergoing structural changes, and degassing time decreases with increasing degassing temperature. For mesoporous organosilica samples, an degassing temperature of between 110 °C and 120 °C for at least 12 hours is typically sufficient. After sufficient degassing has occurred, the degassed sample weight is found by difference by weighing the sample cell again.

Sorption experiments are carried out with the sample held at or near the boiling point of the gas. Nitrogen is generally the adsorptive of choice, mostly because it is readily available both as gas and as the liquid coolant, which has resulted in the richest library of experimental data available for comparison and verification. The automated sorption instrument records a point-by-point (discontinuous) isotherm by adding small amounts of gas to the system manifold, which is of precisely known volume. This allows the amount of gas to be precisely known, and at regular intervals, this gas is admitted to the sample cell, the effective void volume of which is also known. Thus, the sample cell is dosed with controlled amounts of adsorptive gas. By convention, all quantities are converted to

---

1. The effective void volume is the volume of the sample cell that is not occupied by the sample, which is the additional volume available to the adsorptive when the manifold is opened to the sample cell. It is measured during the instrument initialization by dosing the cell with a precise volume of helium gas while the sample cell is at room temperature, and again after the sample cell is immersed in the coolant. Helium is essentially not adsorbed at 77 K, and exhibits approximately ideal behaviour at this temperature. For a discussion of how the void volume is affected by nonideality exhibited by real gases in an actual experiment, and the method for correcting this error, see Section 14.2 in ref. 3.
standard temperature and pressure (STP, where $T_{Std} = 273.15 \text{ K}$ and $P_{Std} = 760 \text{ torr}$). Standard conditions are used because they allow for simple conversion (by dividing the volume in cm$^3$ by 22414 cm$^3$, the volume that one mole of ideal gas at STP occupies) to the number of moles of adsorbed gas, which is the required unit for determining the specific surface area of a sample (see Section 3.1.5.1). The volume of dosed gas, $V_d$, is given by

$$V_d = \left( \frac{P_m V_m}{T_m} - \frac{P_e V_m}{T_{me}} \right) \times \frac{T_{Std}}{P_{Std}} \quad (3-4)$$

where $P_m$ is the pressure of the manifold before the dose, $V_m$ is the volume of the manifold, $T_m$ is the manifold temperature before the dose, $T_{me}$ is the manifold temperature when the system reaches equilibrium (this will be the same as $T_m$ if the manifold temperature is regulated), and $P_e$ is the pressure in the system when it reaches equilibrium (the same as $P$ in $P/P_0$ in the rest of this discussion). At each dose, the pressure drops as some adsorptive gas is physisorbed to the surface of the sample. The volume sorbed, $V_{si}$, after the ith dose is

$$V_{si} = V_d - \left( \frac{P V_{ef}}{P_{Std}} \right) \quad (3-5)$$

However, because real gases deviate from ideality by a non-trivial amount at the cryogenic temperature used in a typical physisorption experiment (77 K for nitrogen), a correction to this equation is necessary. The corrected $V_{si}$ is given by

$$V_{si} = V_d - \left( \frac{P V_{ef}}{P_{Std}} + \frac{P_e^2 V_{vc} \alpha}{P_{Std}} \right) \quad (3-6)$$

where $V_{vc}$ is the volume of adsorptive immersed in the cooling bath and $\alpha$ is the “non-ideality factor”, which is $6.58 \times 10^{-5}$ for nitrogen if $P$ is expressed in torr.$^{14}$ During an experiment, the level of coolant must be kept constant to within 1–2 mm, otherwise the value of $V_{vc}$ will change. On the Autosorb 1-C, this is achieved using a thermistor coolant level control that adjusts the level of the Dewar automatically.
Adsorptive gas is added in regular increments until saturation pressure is achieved. To generate a complete isotherm with both adsorption and desorption branches, the process is then reversed, with the instrument’s vacuum pump used to progressively remove gas from the system in controlled increments. This is achieved by isolating the manifold, evacuating it, and then adding adsorptive to a lower pressure than in the sample cell before opening the system and allowing it to reach equilibration.

3.1.5 Analyzing Experimental Isotherms

In a typical sorption experiment, a total of 79 data points are collected: 40 on the adsorption branch, and 39 on the desorption branch (Figure 3-8). As stated in the introduction to Section 3.1, no method provides absolute values of the parameters of a material. In the case of gas sorption, any result involving the amount of gas sorbed depends on the mass of sample present in the experiment. Thus, all related quantities are divided by the sample mass to yield a value that allows comparison between samples.

The shape of an isotherm can give a lot of qualitative information about a sample, but the quantitative information is of equal importance. The two major quantitative results from gas sorption examined in this thesis are specific surface area by the Brunauer, Emmett and Teller (BET) theory and the pore size distribution by NLDFT. Additionally, the $\alpha_s$ plot empirical method of isotherm analysis can provide additional supporting information for interpreting isotherms.
Figure 3-9. Schematic representation of the surface of an adsorbent exposed to an adsorptive gas as seen by Langmuir and BET theories. In Langmuir theory, a complete monolayer of adsorbed gas forms at sufficient relative pressure. By contrast, BET theory does not assume monolayer coverage, and instead postulates the existence of stacks of adsorbed molecules of varying height (thickness), depending on inhomogeneities in surface sites. The top molecule in each stack is in dynamic equilibrium with the gas phase, and all surface sites are not necessarily filled.

3.1.5.1 Determining Specific Surface Area by BET Theory

In 1938, Brunauer, Emmett and Teller published the derivation of what has become known as the BET equation for determining specific surface area of a solid from gas adsorption data.\(^\text{15}\) They expanded upon the Langmuir model, which describes type I isotherms in terms of the kinetic formation of a complete monolayer on a smooth, uniform surface.\(^\text{16}\) While Langmuir theory is quite useful for type I isotherms and chemisorption, it cannot satisfactorily describe adsorption past one complete layer. In BET theory,\(^\text{ii}\) surface inhomogeneities and textural features result in an uneven distribution of affinity for adsorptive molecules. The first sites to be occupied at low pressures are the most energetic ones. As more gas is adsorbed to the surface, it does not necessarily adsorb to available surface sites. Instead, it can also stack on top of already adsorbed molecules (Figure 3-9). At any point, BET theory assumes that the top molecules in any stack are in dynamic equilibrium with the vapour phase, and though the exact location of molecules within layers may vary, the number of molecules in each layer is constant at constant relative pressure.

\(^{\text{ii}}\) For the derivation of the BET equation, see ref. 15 and Section 4.1.2 of ref. 5. Section 5.2 in ref. 5 and Section 6.2 in ref. 2 give detailed discussions of the application of the BET equation and its limitations.
In order to use BET theory to find the specific surface area, the volume of gas adsorbed must be converted to weight. BET theory is valid for type II and type IV isotherms in the relative pressure range from $0.05 \leq P/P_0 \leq 0.30$, which is the range in which experimental and theoretical data show the best agreement. Most monolayers are completed in this range, and most applicable isotherms are linear across most of this range.

In order to determine the specific surface area by BET theory, the appropriate data points are first selected. The typical approach in a routine BET analysis of a mesoporous sample showing a type IV isotherm is to select five ascending points on the isotherm starting at $P/P_0 \sim 0.1$ (Figure 3-10a). These data points, in terms of volume of gas adsorbed per unit mass, $V_{ads}$, are converted to weight adsorbed per unit mass, $W$, by

$$W = \left( \frac{V_{ads} \times M}{22414 \text{ cm}^3\text{mol}^{-1}} \right)$$

where $M$ is the molecular weight of the adsorptive gas. This conversion is necessary in order to use the BET equation, which is

$$\frac{1}{W \left[ \frac{P_0}{P} \right]} = \frac{C - 1}{W_m C \left( \frac{P}{P_0} \right)} + \frac{1}{W_m C} \quad (3-8)$$

where $W_m$ is the weight of a complete monolayer and $C$ is the BET constant, which depends on the experimental conditions (sample and adsorptive). The BET equation above is written in the form of the linear equation $y = mx + b$, where
\[ y = \frac{1}{W[P_0/P-1]} , \quad m = \frac{C-1}{W_mC} , \quad b = \frac{1}{W_mC} \]  

and rearranging gives the following expressions for \( C \) and \( W_m \):

\[
C = \frac{m}{b} + 1 , \quad W_m = \frac{1}{m + b}
\]

where \( C \) is unitless and \( W_m \) is in units of \( g/g \). In the BET plot (Figure 3-10b), then, a simple linear regression of the points yields values for the slope \( m \) and the intercept \( b \), which can be used to solve for \( C \) and \( W_m \). Because this method relies on linear regression, it is best to find the best fit set of data points from the isotherm, which may be anywhere in the validity range for BET theory (not necessarily starting at \( P/P_0 \sim 0.1 \)). For a given adsorptive with average molecular cross-section \( A_x \), (16.2 \( \times \) 10\(^{-20} \) \( m^2 \) for nitrogen gas) the surface area can be found by

\[
S = \frac{W_m N_A A_x}{M}
\]

where \( N_A \) is Avogadro’s number. This surface area is given in units of \( m^2/g \), and when determined by BET theory in this way, the surface area of a material is always referred to as the BET specific surface area.

BET theory is a remarkable example of a mathematical treatment, derived from classical kinetics without the aid of modern computing power or advanced automated sorption instruments, that is still widely used by scientists after more than seven decades.

### 3.1.5.2 Determining Pore Size Distribution

The approaches to calculating pore size distribution can be divided into two categories: classical macroscopic methods and modern microscopic methods.
3.1.5.2.1 The Barrett, Joyner, and Halenda Method

The classical methods are based on the Kelvin equation for an ideal cylindrical pore,

$$\ln \left( \frac{P}{P_0} \right) = \frac{-2\gamma \bar{V}}{rRT} \quad (3-15)$$

where $\gamma$ is the surface tension of the liquid, $\bar{V}$ is the molar volume of the condensed liquid contained in a pore of radius $r$, and $R$ is the gas constant. The most popular classical method, which is still widely used today despite its limitations, is based on the theory developed by Barrett, Joyner, and Halenda (BJH) in 1951.\textsuperscript{17} It assumes rigid, well-defined pores that behave identically, regardless of their location in the pore network, that the thickness of the multilayer inside the pores is the same as it would be for a flat surface in the same environment, and an equilibrium transition by the successive thinning of the adsorbed multilayer on the surface. Therefore, the desorption branch of the isotherm is selected.

Starting at the upper plateau of the isotherm (normally close to $P/P_0 = 0.95$), and working down the relative pressure values, each data point is converted to a cumulative pore volume as a function of the mean pore size (Figure 3-11a, either pore radius or pore diameter is valid, but the choice must be specified). Taking the first derivative of this plot yields the pore size distribution (Figure 3-11b). In this case, BJH theory on the desorption branch gives a mode pore diameter of 5.45 nm.
A modified BJH method has been developed that makes corrections to the Kelvin equation and can be used with the adsorption branch.\(^\text{18}\) This method takes into account the fact that the Kelvin equation provides the core radius, which differs from the actual pore radius by the thickness of the adsorbed multilayer. The corrected pore radius can be found by

\[
r_{(P/P_0)} = \frac{2\gamma V}{RT \ln(P_0/P)} + t(P/P_0) + 0.3 \text{ nm}
\]

where \(t\) is the statistical film thickness on the pore walls at a given \(P/P_0\), and the correction of 0.3 nm is used to account for a slight underestimation of pore size. This method has been applied to MCM-41-type and SBA-15-type materials (with pore diameters up to \(~7 \text{ nm}\) with success.\(^\text{19,20}\)

### 3.1.5.2.2 Non-Local Density Functional Theory

The modern microscopic approach is most commonly based on NLDFT and a library of reference isotherms used to fit the experimental data. This approach is better able to describe the thermodynamics of adsorbate in a mesoporous sample because of the wealth of reference materials that have been analyzed, and it can address both spinodal condensation and equilibrium transition desorption. Based on the experimental isotherm (in this case, the adsorption branch), NLDFT generates a matching curve by fitting the data (Figure 3-12a). The fit shows steps rather than a smooth curve because the model assumes a chemically and geometrically smooth surface pore wall,
Figure 3-13. Isotherms and corresponding BJH pore size distributions from the desorption branch for three different mesoporous samples. Sample A shows H1 hysteresis and narrow pore size distribution at ~5.5 nm with a slight rise at 3.7 nm. Sample B shows H1 hysteresis that closes at low relative pressure (around \( P/P_0 = 0.45 \)), and a pore size distribution with two peaks, one at ~6 nm and one at 3.7 nm. Sample C shows asymmetric hysteresis with a sharp drop closing the loop around \( P/P_0 = 0.45 \), and a pore size distribution with one small broad peak at ~5 nm and one large sharp peak at 3.7 nm.

which would yield a stepwise type VI isotherm (Figure 3-5) if it were a simple flat surface. Based on the fit, a cumulative pore volume plot is generated and the first derivative of this plot gives the NLDFT pore size distribution (Figure 5-12b). This method gives a mode pore diameter of 6.79 nm. NLDFT is also used to determine the total pore volume of the sample, using the second-to-last adsorption data point.
3.1.5.2.3 Reasons to Use NLDFT and the Adsorption Branch

Until the middle of the 1990s, BJH theory was the dominant choice for determining pore size distribution. However, these are two key reasons that BJH theory is not the best choice for determining pore size distribution in a real mesoporous sample. The first reason is based on deviation from ideal H1 hysteresis. Real experimental data are more useful than generalized drawings for understanding how different hysteresis loops affect the reliability of BJH theory (Figure 3-13). The application of classical BJH theory is limited to the desorption branch. As confirmed by NLDFT studies, the adsorption branch is a spinodal condensation transition from a metastable state, and BJH theory uses the Kelvin equation, which describes an equilibrium transition. In the example in Section 3.1.5.2.1, which corresponds to sample A above, the experimental isotherm has near-perfectly symmetrical H1 hysteresis. Based on this example alone, using the desorption branch is not problematic. However, as shown in Section 3.1.5.4.1, the hysteresis loop may have a different shape. For mesoporous materials with pores that contain any combination of narrow regions, interconnections, and blockages, the hysteresis loop may be broader, asymmetrical, or open below the end of desorption from the dominant mesopores.

In sample A, H1 hysteresis is present in the isotherm, which appears to close just above $P/P_0 = 0.6$. The pore size distribution shows a sharp peak around 5.5 nm, and a small amount of smaller mesopores with a slight rise showing at 3.7 nm. This rise is unremarkable until this pore size distribution is compared with that of sample B. Here, the hysteresis loop does not close completely, and the final drop in the desorption branch at $P/P_0 = 0.45$ results in a second minor peak in the pore size distribution at 3.7 nm. This peak is small, however, and the dominant pores according to this plot have a mode diameter of ~6 nm. Finally, in sample C, the hysteresis loop is a mixture of H1 and H2, and closes sharply at $P/P_0 = 0.45$. In the pore size distribution, the dominant peak is at 3.7 nm, with a smaller, broad peak around 5 nm.

In all three examples, the peak (large or small) at 3.7 nm in the pore size distribution is an artifact, and does not correspond to real pores in the sample. This artifact is the result of the tensile strength effect (see Section 3.1.5.4.2.2), in which spontaneous evaporation of remaining condensed nitrogen occurs when the meniscus of the liquid reaches its limit of stability. In all three isotherms, the desorption branch is affected by this in some way, while the adsorption branch is not.
When reporting the pore size distributions of a sample, it is necessary to indicate the method and branch of the isotherm used to find it. Comparisons between samples are only valid if the pore size distributions have all been found by the same method. Thus, in cases where some samples display the tensile strength effect on the desorption branch, it is better to use the adsorption branch. Since BJH theory is not valid for the adsorption branch, NLDFT becomes the obvious choice of the two.

The question of which method provides a more accurate pore size distribution sheds light on the second reason that NLDFT is the better choice is that BJH theory. Using sample A again, the BJH and NLDFT pore size distributions from the desorption isotherm show significant deviation (Figure 3-14). Conversely, the pore size distributions found by NLDFT from both isotherm branches show good agreement. Based on a series of comparisons between pore size distributions found by various methods both dependent on the position of the pore condensation and evaporation steps and independent of them, NLDFT has been shown to give the best agreement in pore size distributions.\textsuperscript{21,22} BJH theory has been shown to significantly underestimate pore sizes, while modified BJH theory overestimates the diameter of large pores.\textsuperscript{21,23} In general, the validity of BJH theory is limited to a narrower pore size range than NLDFT by its use of bulk properties, which do not apply for very small mesopores and micropores, and modified BJH theory is limited by its inability to accurately describe the capillary condensation transition. However, it is critical to note that NLDFT calculations must be performed using a compatible kernel in the software’s reference library. AS1Win,
the software used with the Autosorb 1-C, has kernels for nitrogen gas at 77 K adsorbed on silica for both the spinodal condensation and equilibrium evaporation transitions. Thus, the pore size distributions found by NLDFT for both isotherm branches of sample A give reliable results, provided the tensile strength effect is not observed on the desorption branch, as NLDFT is equally limited by this phenomenon.

Overall, given the sensitivity of the desorption branch to the tensile strength effect and the proven reliability of NLDFT for pore size distribution calculations, the best method to use when determining the pore size distribution of a mesoporous silica-based sample is NLDFT on the adsorption branch of the isotherm. This allows for consistent data treatment across all samples, which makes it possible to compare pore size distributions in a meaningful way.

### 3.1.5.3 Additional Empirical Assessment: the $\alpha_s$ Method

It is not always easy to classify an isotherm according to the IUPAC recommendations, especially if the capillary condensation step is short relative to the total adsorption volume of the sample. An empirical method of gaining additional insight into the porosity of a sample is known as the $\alpha_s$ method, which does not rely on any *a priori* assumptions about the mechanism of adsorption. Instead, it allows for a comparison of a test isotherm to a reference isotherm of a chemically similar material using the same adsorptive. For each point on the isotherm’s adsorption branch (Figure 3-15a), the amount of gas adsorbed is normalized by a fixed reference, usually the amount at $P/P_0 = 0.4$ on a

---

**Figure 3-15.** a) Raw adsorption isotherm for a mesoporous sample that also contains micropores. b) $\alpha_s$ plot from experimental isotherm, showing linear regression of selected points in the first linear region of the plot. The intercept (in units of mol·g$^{-1}$) of the first linear portion is used to calculate the micropore volume, and the slope can be used to calculate the specific surface area of the primary mesopores. The intercept of the second linear portion is used to find the mesopore volume, and the slope to find the external specific surface area.
nonporous reference material of the same chemical composition using the same adsorptive, to obtain the $\alpha_s$ value:

$$\alpha_s = \frac{n}{n_{n,i}}$$  \hspace{1cm} (3-17)

A plot of the number of moles adsorbed as a function $\alpha_s$ gives the reduced isotherm (Figure 3-15b). A nonporous sample will show a linear reduced isotherm. Upward deviation at high $P/P_0$ (or $\alpha_s \sim 1.1$, in this example) at indicates capillary condensation, and downward deviation at low $P/P_0$ indicates the presence of a micropore filling step. From the shape of the plot, it is possible to determine the types of distinct pore sets present in a sample. In the plot shown, micropores are present, and are distinct from mesopores.

The slope of the first linear portion of the reduced isotherm (data points shown in green) is used to find the mesopore specific surface area by

$$A_{\alpha_s} = 6.45 \times 10^4 \times m_{\alpha_s,\text{linear}}$$  \hspace{1cm} (3-18)

for nitrogen adsorption, using a nonporous hydroxylated silica surface at 77 K as the reference standard material. The mesopore specific surface area found by this method is valid for samples that show a microporosity contribution (positive intercept of the first linear portion) in the $\alpha_s$ plot.

The micropore volume can be calculated by extrapolating the first linear portion of the plot to the y-axis to obtain the micropore capacity, $n_p(mic)$, which is equal to the intercept found by linear regression. The micropore volume, $v_p(mic)$, can be found simply by converting this value to liquid volume:

$$v_{p(mic)} = n_{p(mic)} \times \frac{M_{ads}}{\rho_{ads}}$$  \hspace{1cm} (3-19)

where $M_{ads}$ is the molecular weight of the adsorptive gas, and $\rho_{ads}$ is its liquid density at its boiling point (28.01 g/mol and 0.808 g/cm$^3$, respectively, for nitrogen). If the linear fit passes through the origin, there is no distinct micropore filling step in adsorption. If the intercept has a negative value, it indicates that the sample has weaker interactions with the adsorptive than the reference material does.
The second linear portion (data points shown in magenta) is used to find the external specific surface area (for macropores and large mesopores that do not participate in the capillary condensation step). The intercept of this line gives the mesopore volume by the same calculation shown above for the micropore volume, which, if it is in good agreement with the cumulative pore volume (found by NLDFT), indicates that the total pore volume is entirely due to mesopores. Conversely, if the values differ, then other contributions to the total pore volume exist. These include micropores, if the first linear portion has a positive intercept, and larger mesopores and macropores, especially if more than one linear portion is observed for high P/P₀ values, or if the slope of the α plot continues to increase.

3.1.6 Final Thoughts on Gas Sorption

The goal of this section was to provide a clear and accessible overview of the main aspects of the theoretical and practical aspects of gas sorption. Though lengthy, this section is important for later discussion of experimental results, and will hopefully serve as a useful point of reference. Gas sorption seems to be particularly poorly understood in the literature, with many publications reporting gas sorption results that are not, strictly speaking, correct. Selecting experimental data “by convention” seems to be the cause for confusion, especially in reporting pore size distributions. The use of the BJH method can be especially problematic if, for example, the original BJH method is used with a desorption branch displaying the tensile strength effect, or instead of the modified BJH method with the adsorption branch of an isotherm. The inconsistency in selected methods overall makes meaningful comparison to literature difficult to do.
When a nucleus of non-zero spin \( m \) is placed in a magnetic field \( B_0 \), it will align itself parallel or anti-parallel to that magnetic field (Figure 3-16a). The difference in energy or Zeeman energy \( \Delta E \) between the two states is given by

\[
\Delta E = E_{m,\uparrow} - E_{m,\downarrow} = \gamma \hbar B_0 m
\]  

where \( \gamma \) is the gyromagnetic ratio of the spin, \( \hbar \) is the reduced Planck constant, and \( m \) is the value of the spin. This spin will precess around that magnetic field (Figure 3-16b) with a frequency \( \omega \) given by

\[
\omega = -\gamma B_0
\]  

Figure 3-16. a) Schematic representation of the energy levels associated with a nucleus with magnetic spin \( 1/2 \) aligning itself parallel \( (m=1/2) \) and anti-parallel \( (m=-1/2) \) to an external magnetic field \( B_0 \). The difference in energy of the spins is \( \Delta E \). b) A nucleus of spin \( \mu \) placed in an external magnetic field \( B_0 \) will precess around the field with frequency \( \omega \). This is known as the Larmor precession rate. c) Two nuclei of spins \( I \) and \( S \) placed in a magnetic field along the \( z \)-axis, where \( \theta \) is the angle between the internuclear vector and \( B_0 \).
This frequency is known as the Larmor precession rate, and is normally in the radio frequency (RF) range. Resonant absorption occurs when electromagnetic radiation of the correct frequency $\nu_0$ is applied to a spin inside a constant magnetic field:

$$\nu_0 = \frac{\gamma B_0}{2\pi}$$

(3-22)

Differences in the resonant frequency occur as a result of the electron environment surrounding a spin, which either shield or amplify the effect of the applied magnetic field. Therefore, the effective field that a spin experiences is the sum of the external and local fields.

When a broad-spectrum RF pulse is applied to a spin in a magnetic field along the z-axis, the spin tips to the xy-plane. This creates an oscillating magnetic field in the xy-plane as the spin precesses, and the field diminishes over time as the spin relaxes back. Fourier transform of this so-called free-induction decay (FID) yields a standard NMR spectrum that shows signals for all nuclei in the given molecule that are of the magnetic spin being interrogated.

A standard NMR spectrum is plotted with the chemical shift, $\delta$, in parts per million on the x-axis. The value of $\delta$ is given by the difference between the resonance frequency of a particular nucleus and a standard reference material, normalized to the frequency of the spectrometer.

### 3.2.1 Solid-State versus Solution Phase

There are three key interactions that characterize the distinction between solution phase NMR and SSNMR: heteronuclear dipolar coupling, homonuclear dipolar coupling, and chemical shift anisotropy. In the solution phase (used in conventional NMR experiments), molecules are free to tumble rapidly. These isotropic motions average out the interactions between nuclei in the molecule and with external field gradients, to give an average interaction $\sigma_{avg}$ of

$$\sigma_{avg} = \frac{1}{3}(\sigma_{xx} + \sigma_{yy} + \sigma_{zz})$$

(3-23)

where the individual interactions in the x, y, and z directions are distinct.

In the solid state, this tumbling does not occur. Interactions between spins in nuclei are anisotropic, and depend heavily on the orientation of the surrounding electron clouds relative to $B_0$. The dominant
interaction in the solid state is dipole-dipole coupling between nuclei. This can be either homonuclear or heteronuclear.

### 3.2.1.1 Heteronuclear Dipolar Coupling

Heteronuclear coupling occurs between nuclei of different spins, typically an abundant spin such as $^1$H, labeled I by convention, and a rare spin such as $^{13}$C, labeled S. Each spin will have an independent Zeeman interaction with the external field $B_0$, and will feel the additional effect (additive or subtractive) of the other spin provided it is nearby ($<10$ Å away). The strength of this effect in an external field applied along the z-axis is determined by the degree of dipolar coupling, which is given by the Hamiltonian:

$$H_{IS} = -d \left( 3 \cos^2 \theta - 1 \right) I_z S_z$$  \hspace{1cm} (3-24)

where $I_z$ and $S_z$ are the z components of the nuclear spin angular momentum, $\theta$ is the angle between the internuclear vector and the z-axis (Figure 3-16c), and $d$ is the dipolar coupling constant:

$$d = \left( \frac{\mu_0}{4\pi} \right) \frac{h \gamma_I \gamma_S}{r_{IS}^3}$$  \hspace{1cm} (3-25)

where $\mu_0$ is the permeability of free space, $\gamma_I$ and $\gamma_S$ are the gyromagnetic ratios of the two nuclei, and $r_{IS}$ is the internuclear distance. This interaction falls off rapidly as the internuclear distance increases. The coupling is also strongly affected by the orientation relative to the external field, an effect that is significantly more pronounced in a solid-state material than in rapidly tumbling solution-phase molecules.

### 3.2.1.2 Homonuclear Dipolar Coupling

Homonuclear dipolar coupling occurs as a result of resonance between nearby nuclei of the same spin, which can undergo an energy-conserving “flip-flop” transition where two spins exchange magnetization. This adds an additional term to the Hamiltonian:

$$H_{II} = -\frac{1}{2} d \left( 3 \cos^2 \theta - 1 \right) \left( 3 I_z I_{\perp} - (I_1 \cdot I_2) \right)$$  \hspace{1cm} (3-26)
This energy exchange can only occur when the Larmor rates (or resonance frequencies) of two nuclei are sufficiently close. For systems containing $^1$H, $^{13}$C, and $^{29}$Si, the resonance frequencies are so different that the exchange can only occur between nuclei of the same species. Because of the low abundance of the $^{13}$C and $^{29}$Si nuclei, the only nucleus that shows significant heteronuclear dipolar coupling in this system is $^1$H.

### 3.2.1.3 Chemical Shift Anisotropy

The third major interaction is the chemical shift anisotropy (CSA). As previously mentioned, the chemical shift is determined by the sum of external and local fields acting on a nucleus. In the solution phase, rapid tumbling averages out the local effects and renders them isotropic. In the solid state, this tumbling is not possible. A $^{13}$C atom in a carbonyl bond will display three different chemical shifts depending on which axis it is aligned (Figure 3-17). In this case, the Hamiltonian for CSA is given by

$$H_{\text{CSA}} = \gamma B_0 I_z \left[ \frac{1}{2} \hat{\delta}_{\text{iso}} + \frac{1}{2} \delta_{\text{CSA}} \left( 3 \cos^2 \theta - 1 \right) \right]$$  \hspace{1cm} (3-27)

where the isotropic chemical shielding factor $\hat{\delta}_{\text{iso}}$ is given by

$$\hat{\delta}_{\text{iso}} = \frac{1}{3} (\delta_{11} + \delta_{22} + \delta_{33})$$  \hspace{1cm} (3-28)

and the magnitude of the CSA $\delta_{\text{CSA}}$ is given by

$$\delta_{\text{CSA}} = \delta_{33} - \delta_{\text{iso}}$$  \hspace{1cm} (3-29)
The smallest shift occurs when the narrowest part of the electron cloud is oriented along the z-axis, and the largest shift occurs when the broadest part of the cloud is along the z-axis.

### 3.2.1.4 The Magic Angle

In all three interactions discussed above, the term \((3\cos^2 \theta - 1)\) appears in the Hamiltonians. This term goes to 0 when \(\theta = 54.74^\circ\). Thus, if all orientations in a given sample can be fixed at 54.74°, the anisotropic contributions of orientation-dependent terms would average to zero and a clean spectrum would result. However, especially in amorphous materials such as sol-gel silica, there is no possible way to achieve this. Considering a cube, the angle between the space diagonal and the z-axis is 54.74° (Figure 3-18a). This is known as the magic angle. The solution in an amorphous solid sample is simply to spin the sample rapid at the magic angle (Figure 3-18b), which causes all three axes to average out. In a real experiment, magic angle spinning (MAS) is achieved by high pressure air, which floats and spins the sample to the desired frequency inside the sample chamber.

### 3.2.2 Experimental Method

A solid-state NMR spectrum is more complicated to obtain than a solution NMR spectrum. The specifics of the procedure vary with instrumentation and sample. A general overview of the method for an organosilica powder sample will be presented here.
Table 3-1. Relevant SSNMR parameters for $^1$H, $^{13}$C, and $^{29}$Si nuclei.$^{24}$

<table>
<thead>
<tr>
<th>nucleus</th>
<th>$\gamma/2\pi$ (MHz/T)</th>
<th>$\omega$ (MHz)</th>
<th>relative nuclear signal</th>
<th>% abundance</th>
<th>rel. natural abundance signal</th>
<th>Standard SSNMR reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>42.57</td>
<td>200.00</td>
<td>1.0</td>
<td>99.98</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>10.71</td>
<td>50.29</td>
<td>0.032</td>
<td>1.1</td>
<td>$2.5 \times 10^{-4}$</td>
<td>Adamantane (28.46 ppm and 37.85 ppm)</td>
</tr>
<tr>
<td>$^{29}$Si</td>
<td>8.46</td>
<td>39.73</td>
<td>0.018</td>
<td>4.7</td>
<td>$8.5 \times 10^{-4}$</td>
<td>TTMS$^a$ (-9.9 ppm and -125.2 ppm)</td>
</tr>
</tbody>
</table>

$^a$ - TTMS = tetrakis(trimethyl)silyl silane

First, the sample is packed into the appropriate zirconia rotor. Good packing is essential, as it ensures the sample will spin evenly in the spectrometer and the cap will not pop off due to the buildup of internal pressure. It is often advisable to spin the sample at the desired rate in an external spinner prior to inserting it into the spectrometer, to avoid contaminating the instrument should the cap fly off, and to allow the sample inside the rotor to settle.

Unlike solution phase NMR, a SSNMR spectrometer requires manual tuning for the nuclei being interrogated to their individual Larmor resonance frequencies ($\omega$, Table 5-1). As seen from the relative natural abundance signals of $^{13}$C and $^{29}$Si, the signal to noise ratio is expected to be very low for these nuclei. In order to improve the signal to noise for rare nuclei, a technique called cross-polarization (CP) is used to transfer polarization from an a nearby abundant nucleus (in this case, $^1$H) to the rare nucleus and thereby enhance the rare nucleus’ signal. CP is caused by the tendency for magnetization to flow from highly polarized species to nearby species with low polarization. For heteronuclear pairs of nuclei, this flow must be driven by external RF fields (in contrast to homonuclear pairs, as discussed in Section 3.2.1.2).

The tuning is done to achieve what is known as Hartmann-Hahn matching, where two separate RF fields are applied at each of the resonance frequencies of the two nuclei. This allows each spin (I and S) to be rotated independently. When the frequencies of nutation of the two spins are equal, matching is achieved such that $\gamma_I \nu_I = \gamma_S \nu_S$ and cross-polarization energy transfer is possible (Figure 3-19a). Experimentally, this is achieved by a specific pulse sequence (Figure 3-19b): first, a $\pi/2$ pulse is applied along the x-axis to rotate the proton spin into the xy plane. Then, appropriate RF fields are applied to both spins for the cross-polarization contact time $\tau_{CT}$, which caused magnetization transfer...
Figure 3-19. a) Schematic diagram of the resonance frequencies of $^1$H and $^{13}$C before and after the application of appropriately selected RF pulses. In the lab frame, the frequencies are mismatched. In the doubly rotating frame where RF pulses are applied to both spins (causing both of them to rotate), the frequencies are matched and polarization transfer can occur. b) Schematic pulse sequence of a typical CP MAS experiment.

from the I spin to the S spin (in this case, $^1$H to $^{13}$C). The S spins are then detected while the I spins are decoupled. The relaxation delay at the end of one sequence is necessary in order to ensure the system has fully relaxed before the next pulse sequence is initiated.

As with conventional solution-phase NMR, Fourier transform of the acquired FID yields the spectrum. However, unlike solution NMR, the spectrum acquired in the solid state has broad and/or overlapping peaks. Also, because of the complicated nature of cross-polarization energy transfers that occur, the integrated peak areas no longer correspond to stoichiometric amounts in the sample. It is possible to obtain quantitative stoichiometric information about a rare nucleus through a high-powered proton decoupling (HPDEC) pulse sequence that allows for no CP energy transfer. The downside of this approach is that the acquisition time increases drastically to obtain a satisfactory signal-to-noise ratio.

### 3.2.3 Interpreting $^{13}$C and $^{29}$Si SSNMR Spectra

Although the solid state has no effect on average chemical shifts in a NMR spectrum, the line broadening that occurs as a result of imperfect averaging can cause peaks to overlap and spectra to become convoluted.

---

iii. Proton decoupling is necessary in order to control the length of $\tau_{CT}$ and thus the amount of energy transfer allowed between spins. It is achieved by another strong RF pulse only on the proton channel while the S spins are detected on the S channel.
Figure 3-20. Experimental HPDEC $^{29}$Si SSNMR spectrum showing deconvolution of overlapping peaks using Dmfit, and structures of different silicon species present in an organosilica sample.

The overlap is generally not a problem in $^{13}$C spectra of organosilica materials, as the carbon chemical shifts are spread farther apart than typical line broadening. For $^{29}$Si, however, the distinction between the chemical shifts of the different silicon species present in organosilica is smaller than the line broadening, and peak overlap occurs. In order to correctly interpret a $^{29}$Si SSNMR spectrum, it is necessary to know the expected chemical shifts and to deconvolute the spectrum using software such as Dmfit. This freely available program works with the common NMR-active nuclei, and can be used to create and optimize deconvoluted spectra (Figure 3-20). While overlap occurs for silicon species in the same parent group (Q or T), the groups do not overlap. Q species are silicon atoms bonded to four oxygen atoms, and T species are silicon atoms bonded to one carbon and three oxygens. D and M species (bonded to two carbons and two oxygens, and three carbons and one oxygen, respectively), are located farther downfield.
3.3 Small Angle X-Ray Scattering

Under the umbrella of X-ray diffraction, SAXS is useful for interrogating length scales greater than \( \sim 1 \) nm (whereas wide angle scattering looks at the atomic scale in crystal lattices). Detailed theory and practical information on X-ray diffraction and specifics on SAXS can be found elsewhere.\(^{28}\) Presented here is an overview of the basics of diffraction, the experimental SAXS method, and interpreting the results.

Diffraction of waves (light, X-rays, neutrons) in a lattice is governed by Bragg’s law:

\[
2n\lambda = d \sin \theta
\]  

(3-30)

where \( \lambda \) is the wavelength, \( d \) is the distance between diffraction planes, and \( \theta \) is the incident angle of the waves (Figure 3-21a). Destructive interference occurs unless the Bragg equation is satisfied, which allows \( d \) to be determined when \( \lambda \) and \( \theta \) are known. In a 2D hexagonal system composed of overlapping equilateral triangles (like in typical ordered mesoporous silica prepared by surfactant templating) (Figure 3-21b), a simple trigonometric relationship exists between the separation of diffraction planes and the centre-to-centre distance between adjacent pores (Figure 3-21c). For the 2D hexagonal lattice, the first diffraction peak occurs in the (100) direction. Diffraction planes exist because of the difference in electron density between rows of pores and adjacent pore walls.
In any diffraction method, it is necessary to have a single wavelength incident on a sample. For SAXS, monochromatic X-rays are generated by firing a high-voltage electron beam at a metal source. This beam causes an electron in the K shell of the atom to be ejected (Figure 3-22a). When an electron in a higher level fills the vacancy, an X-ray is emitted. Copper, the metal of choice for SAXS, emits K$_\alpha$ X-radiation with a wavelength of 1.5418 Å. This radiation is passed through a sample and diffracts at 2\(\theta\) (Figure 3-22b). A detector integrates the intensity of the diffraction pattern and generates a one-dimensional spectrum (Figure 3-22c). From this spectrum and the peaks present, it is possible to determine the lattice parameter \(a\) and obtain additional information about the degree of ordering present in the mesopores.$^{29}$ Using \(d(100)\) found by the Bragg equation (Figure 3-22d), the lattice parameter is given by

\[
a = \frac{2d(100)}{\sqrt{3}} \tag{3-31}
\]

and using the mean pore diameter found by gas sorption, it is then possible to calculate the wall thickness of the sample by difference. The degree of ordering is evaluated by the number of diffraction peaks observed; higher order results in more peaks. SAXS data can be used to fingerprint mesoporous structures if they show enough diffraction peaks (typically three or more).$^{30}$
3.4 Other Characterization Methods

The other characterization methods used in this thesis are more straightforward, both in employment and interpretation. Fourier transform infrared (FTIR) spectroscopy is a rapid technique used to gather evidence of organic functional groups present in a sample, and can be used to complement SSNMR characterization of organosilica materials. Detailed theory can be found elsewhere.\textsuperscript{31} Electron microscopy, both scanning (SEM) and transmission (TEM), are regularly used to image mesoporous materials and complement data obtained by gas sorption and SAXS. In some cases, SEM and TEM can resolve uncertainties in data interpretation, such as determining the main cause of H2 hysteresis.\textsuperscript{32} Detailed discussions of theory and instrumentation for various types of electron microscopes can be found elsewhere.\textsuperscript{33,34}

3.5 Conclusions

It is customary for publications to report the results of characterizations such as those described here with only a brief description of the key parameters used and any assumptions made. For techniques that are taught regularly in university courses, such as FTIR, conventional NMR, and SEM/TEM, correctly understanding the results reported is a simple thing. However, when the techniques used are more specialized and open to a variety of experimental and mathematical methods, such as with gas sorption, the expectation is that the researcher has studied the appropriate resources before selecting the method, and made informed experimental decisions during both data collection and data processing.
Works Cited


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4
Molecularly Imprinted Mesoporous Organosilica


This chapter is reproduced from the article published in *ACS Nano* in 2011. It represents the proof-of-concept work that followed the early results discussed in the Preface. As the lead author of this publication, I was responsible for material synthesis, characterization, and testing. The second author, Igor Moudrakovski, is the researcher at the National Research Council of Canada who assisted me with obtaining solid-state NMR (I travelled to Ottawa and collected the spectra myself). Because the incorporation of a large bridging organic group into the walls of PMS/PMO by cocondensation is a nontrivial problem, the main goal of this study was to obtain evidence of molecular imprinting in the walls. Therefore, the main focus of material testing was on the differences in binding behaviour between the imprinted, nonimprinted, and blank control materials.

**ABSTRACT:** We have prepared molecularly imprinted mesoporous organosilica (MIMO) using a semicovalent imprinting technique. A thermally reversible covalent bond was used to link a bisphenol A (BPA) imprint molecule to a functional alkoxysilane monomer at two points to generate a covalently bound imprint precursor. This precursor was incorporated into a crosslinked periodic mesoporous silica matrix via a typical acid-catalyzed, triblock copolymer-templated, sol–gel synthesis. Evidence of imprint sites buried in the pore walls was found through careful characterization of the imprinted material and its comparison to similarly prepared non-imprinted mesoporous organosilica (NIMO) and pure periodic mesoporous silica (PMS). After thermal treatment, the imprinted material (M) removed more than 90% of appropriately sized bisphenol species from water, yet showed significantly lower binding for both smaller and larger molecules containing phenol moieties. Identically treated N showed much poorer retention behaviour than M for the same bisphenol species, and behaved only slightly better than P.

4.1 Introduction

Molecular imprinting can be defined as the assembly of a crosslinked polymer matrix around an imprint molecule that is held in place, either covalently or noncovalently, by judiciously chosen functional monomers. The removal of the imprint molecule yields an imprint cavity of a specific size. The surface of the imprint cavity contains functional groups that are able to interact, either covalently or noncovalently, with certain moieties on an appropriately sized target molecule. This concept was first demonstrated in silica in 1951, yet the majority of the literature to date is focused on molecular imprinting in organic polymers; it is only in the past decade that interest in molecular imprinting in silica has seen a revival. This could be due to a major difference in the swellability of commonly used organic polymers compared to silica. While organic polymers can easily be customized to swell and shrink by controlled amounts in response to stimuli such as solvent exposure and applied voltage, silica is more rigid, and this can cause a problem for molecular imprinting. Slow diffusion through bulk silica can severely hamper access to imprint sites, which could be why the molecular imprinting
community first moved away from silica as a matrix. A simple way to overcome this diffusion problem is to reduce the diffusion distance. Several reports of molecular imprinting in thin films of silica have confirmed that significantly improved target interaction can be achieved by reducing the diffusion length.\textsuperscript{4,8–10} Alternatively, surface imprinting on silica eliminates the need for diffusion altogether,\textsuperscript{11,12} but in the case of organic molecules this method does risk sacrificing the size and shape selectivity that can be achieved by creating a closed imprint cavity.

Thin films may be useful for sensing and other analytical applications, but if molecularly imprinted silica is to be used for preparative applications or produced in larger quantities for industrial use, a better choice of morphology is silica powder; it can easily be packed into columns or other vessels, suspended in various solvents and then isolated by centrifugation or filtration, and washed and dried for reuse. In this case, the way to overcome the diffusion problem is to make the silica powder highly porous. Specifically, the solution is to create a close-packed network of nanometre-sized channels in the silica matrix, thereby reducing the diffusion distance to the order of a few nanometres. The choice of channels instead of closed pores increases the likelihood that they will be open at the outer surface of individual particles, thus further reducing the amount of silica through which a molecule must diffuse to reach an imprint site and allowing for easier flow through the material.

This type of porosity is easily created using a sol-gel approach involving a micelle template in aqueous media, to generate a mesoporous material. Two common periodic mesoporous silicas (PMSs), MCM-41\textsuperscript{15} and SBA-15\textsuperscript{,14} prepared using an alkoxy silane sol-gel precursor with ionic surfactant and non-ionic copolymer templates, respectively, have the same hexagonal close-packed channel pore structure, but differ in surface area, pore diameter, and wall thickness. Periodic mesoporous organosilica (PMO) can be prepared in exactly the same way as PMS, but has bridging organic groups covalently bound to at least two silicon atoms each in the silica matrix. Organosilica can refer to any silica-based material that contains silicon-carbon covalent bonds. We report herein the synthesis, characterization and utility of a molecularly imprinted mesoporous organosilica (MIMO). Reports on ionic and molecular imprinting in MCM-41 have shown good target response,\textsuperscript{15,16} yet to our knowledge there has not yet been reported a thorough study of the mesoporous material itself at all stages of the material synthesis to state with confidence the actual location of imprint sites in the
material and the preservation of the material’s pore morphology throughout. We believe this is essential to proving successful molecular imprinting in (not on) mesoporous materials, as most large silane-functionalized organic moieties are difficult to definitively incorporate into the pore walls of micelle-templated mesoporous silica, preferring instead to occupy surface sites or extend into the pores. For this study, a covalently bound imprint molecule was incorporated into SBA-15-type mesoporous silica, as it possesses larger pore diameters, thicker walls and better hydrothermal stability than MCM-41. This provides both better access to imprint sites through wider channels and a more robust silica matrix due to thicker pore walls that are better able to maintain the shape of the imprint cavity compared to MCM-41, while still offering the necessary high surface area and short diffusion distances for better access to imprint sites than in bulk silica. The chosen imprint molecule was bisphenol A (BPA), a well-known endocrine disruptor that has received significant attention in recent years due to concern over its presence in a variety of consumer products including food packaging, food storage containers and baby bottles. BPA is not only a relevant choice of imprint; it comes from a large family of phenol-containing molecules, the bisphenols, which offers the opportunity for a systematic study of the relationship between small, stepwise changes in structure and a BPA-imprinted material’s response to the corresponding target molecule.

4.1.1 Material Synthesis

In order to create a well defined imprint site yet achieve rapid target molecule interaction, a semicovalent imprinting approach was used. The imprint was covalently bound to the functional monomers through a thermally reversible carbamate bond that was formed by a direct coupling between phenol moieties on BPA and isocyanate groups on (3-isocyanatopropyl)triethoxysilane (ICPTES) (Figure 4-1). The imprint–functional monomer complex (IC), BPAP, was produced nearly quantitatively (90% by NMR) from the reaction of a 1:2 stoichiometric mixture of BPA and ICPTES. This direct coupling reaction uses no catalysts or coreactants and requires only a solvent evaporation step to isolate the product. It is a simple synthetic method that is highly amenable to scale-up.
Figure 4-2. Synthesis of molecularly imprinted mesoporous organosilica (MIMO), imprint removal to yield M and interaction of a target molecule with the imprint site: a) the mixture of TEOS and BPAP cooperatively self-assembles around and between the hexagonal close-packed core-shell micelles of P123 in acidic aqueous media; b) stirring at room temperature for 24 hours followed by quiescent curing at 80°C for 24 hours; c) P123 template removal by Soxhlet extraction with ethanol for 20 hours; d) thermal cleavage of the imprint by heating in wet DMSO for 5 hours; e) sequestration of an appropriately sized target bisphenol molecule by hydrogen bonding between phenols and amines.

Molecularly imprinted periodic mesoporous organosilica (MIMO) powder was prepared by a triblock-copolymer-templated sol-gel method (Figure 4-2). A solution of 10 mol% Si from BPAP in 90 mol% Si from tetraethyl orthosilicate (TEOS) was prepared and completely dissolved to ensure uniform distribution of BPAP throughout the TEOS cross linker. This solution was added to a solution of Pluronic P123 (PEO\textsubscript{20}PPO\textsubscript{70}PEO\textsubscript{20}) and NaCl in aqueous HCl with a Si:HCl:P123:H\textsubscript{2}O:NaCl molar ratio of 1:6.15:0.022:228:0.006. A non-imprinted mesoporous organosilica (NIMO) with a terminal organic group instead of a bridging one was similarly prepared using the same molar amount of Si from ICPTES in place of BPAP. A control PMS was also prepared by the same method using only TEOS. In order to cleave the carbamate bonds in MIMO and produce the final imprinted material, M, a portion of the powder was suspended in wet dimethyl sulfoxide (DMSO) and stirred at 160 °C. Identical treatments were done to NIMO and PMS samples, yielding N and P respectively.
4.2 Material Characterization

In order to state that MIMO does indeed contain molecular imprint sites, and that M does indeed function as an effective molecularly imprinted mesoporous organosilica material, simple rebinding tests alone are not sufficient. An inherent challenge in the generation of a new PMO species is the limitation of the types of organic bridging groups that can be used. It is generally accepted that large flexible organic bridging groups are difficult, if not impossible, to incorporate in large proportions into the pore walls of a PMO, as they either disrupt the self-assembly to such a degree that a disordered or nonporous material is produced, phase separate to yield a mixture of dense organosilica and PMS, or interact preferentially with the pore template and reside on the pore surfaces instead of within the pore walls. Even if a given PMO is successfully synthesized, it is difficult to state unequivocally the location of the organic bridging groups. Thus, the introduction of a new organosilica precursor of the size used here carries with it the concern that one of the above three situations will arise during the material synthesis, and careful examination of MIMO and its comparison to NIMO and PMS (and their thermally treated counterparts) is therefore of the utmost importance. This comparison relies on a combination of common characterization techniques for periodic mesoporous materials and the behaviour of M, N and P in size- and shape-selective target rebinding studies.

In order to ensure a valid comparison, significant care was taken to control the distinctions between the three materials, from the molar ratios of the different precursors in the material synthesis to the post-synthetic treatment steps. This was necessary to reduce the number of variables in the system, and consequently the chemical composition, organic loading, pore structure and physicochemical properties were carefully determined for all materials at each step. Rebinding tests were not performed until all parameters were evaluated and a satisfactory and controllable degree of variation between materials was demonstrated.
Figure 4-3. Fourier transform infrared spectra of MIMO (solid trace), M (dot-dashed trace), NIMO (dashed trace) and N (dotted trace). All spectra show no trace of the isocyanate peak at 2270 cm$^{-1}$.

4.2.1 Chemical Composition of the Materials

To confirm the successful cleavage of the carbamate bond in M, the solid materials were characterized by Fourier transform infrared spectroscopy (Figure 4-3). The carbamate C=O stretch at 1720 cm$^{-1}$ is clearly visible for the as-synthesized MIMO sample, and is eliminated after thermal treatment. No isocyanate (NCO) stretch appears at 2270 cm$^{-1}$ in the M spectrum, showing that the thermal bond cleavage does not regenerate the original NCO group and indicating that the carbamate is indeed converted to a primary amine. The NH$_2$ bending mode at 1670 cm$^{-1}$ overlaps with an OH bending mode at 1640 cm$^{-1}$, but it can still be identified by the asymmetry of the peak. NIMO shows no isocyanate stretch, indicating that the NCO group was converted to an amine during heating in the presence of water and acid in the synthesis solution. The asymmetric peak at 1640 cm$^{-1}$ can again be assigned to overlapping NH$_2$ bending and OH bending in silica. N shows no difference, which was anticipated as no further chemical modifications were expected to occur for this sample (for full spectra of all samples, see Appendix A, Figure A-1).
To obtain a more comprehensive characterization of the composition of the materials, solid-state nuclear magnetic resonance (SSNMR) spectroscopy was employed. $^{13}$C cross-polarized magic-angle spinning (CP MAS) spectra (Figure 4-4a) were obtained for all samples to confirm the presence of the expected organic groups. In the MIMO spectrum the aromatic carbons (120 – 160 ppm) and the methyl carbons (50 ppm) of the BPA imprint are clearly present, as well as the anchoring propyl groups (8 ppm, 22 ppm and 42 ppm). Residual ethoxy moieties (16 ppm and 58 ppm) and some residual P123 template (70 – 80 ppm) are also present. P123 is eliminated after thermal treatment, due to the extra washing of the material. The aromatic and methyl carbon peaks also disappear into the baseline after thermal treatment while the relative intensity of the carbon signals in the remaining aminopropyl (AP) groups is unchanged, indicating that most of the imprint molecules
Figure 4-5. $^{29}$Si HPDEC spectra of a) M and b) N (grey traces) and fitting (black traces) showing individual Gaussian peaks and Si species assignments. Insets: integration of the individual Gaussian peaks for each plot and the corresponding Si species.

were removed without affecting the anchoring groups. A slight peak at 59.5 ppm indicates the presence of some residual DMSO from the thermal treatment, and residual ethoxy groups are also still present. In the NIMO spectrum, the propyl carbons of the AP groups are clearly present, as are some residual P123 and surface ethoxy groups. The P123 signals disappear after thermal treatment, while a residual DMSO peak appears and the AP and ethoxy carbon signals persist. As expected for PMS, the only carbon signals are those of residual ethoxy groups and a small amount of P123, which after thermal treatment remain unaffected. These data confirm that M and N contain the same carbon species and therefore the same AP groups, which makes a comparison of their performance in bisphenol extraction valid. Conversely, P contains only residual ethoxy carbon species, making it possible to use this sample to determine how the unmodified mesoporous silica matrix interacts with the chosen targets.

$^{29}$Si CP MAS spectra were taken of all samples to identify the silicon species present in each material (Figure 4-4a). For MIMO, M, NIMO, and N, five $^{29}$Si species can be identified. Two T species, T$^2$ (RSi$_x$OR’, -52 ppm) and T$^3$ (RSi$_y$OR$_z$, -65 ppm), correspond to the RSiO$_{x}$OR’$_{y}$ sites containing the AP functional groups (R = BPAP organic bridge or AP anchor, see Figure 4-2, R’ = OH or OCH$_2$CH$_3$), while the remainder of the matrix is composed of Q sites, SiO$_{x}$OR’$_{y}$, namely Q$^2$ (SiO$_2$(OR’)$_z$, -92 ppm), Q$^3$ (SiO$_3$OR’$_z$, -102 ppm) and Q$^4$ (SiO$_4$, -110 ppm). As expected, PMS and P show only Q species.
4.2.2 Quantification of the Imprint Sites

In order to quantify the number of T sites and so estimate the number of imprint sites or non-imprinted amine groups in the M and N materials, respectively, quantitative $^{29}$Si high-power decoupling (HPDEC) MAS spectra were taken of these two samples (Figure 4-5). The peak positions of the different Si species in these samples were determined by fitting Gaussian peaks to the $^{29}$Si CP MAS spectra; these positions were subsequently used to fit the HPDEC MAS spectra with Gaussian peaks that were then integrated. Both M and N had a higher proportion of T species than expected from the original synthesis conditions (13.4% and 15.8%, respectively, compared to 10% in the original synthetic mixture). While a small fraction of this discrepancy may have arisen from the peak fitting, a more likely reason is that the hydrolysis and polycondensation (or gelation) rates of $(R'O)_3SiRSi(OR')_3$ type silanes where the bridging R is long and flexible (as with BPAP) are known to be faster than those of TEOS. It is therefore likely that less BPAP would remain unreacted than TEOS, some of which would be more likely to remain in solution. However, these results are still below the established 25 mol% threshold for the successful incorporation of non-rigid or terminal organic groups into a mesoporous silica material that does not collapse upon removal of the template. This indicates that the AP sites are likely to be homogeneously distributed throughout the materials, and that the imprint sites are likely buried in the pore walls, not dangling on the internal pore or external particle surfaces. The low loading of imprint sites was designed with this in mind, in order to reduce the possibility that the overall mesopore structure would collapse following thermal removal of the imprint molecules, as this step effectively breaks crosslinks in the material.

From the fraction of T species, the number of imprint sites in M and non-imprinted amine sites in N were determined. M and N contain exactly the same functional groups, so their empirical formulae can be directly compared. M contains 13.4 mol% T, which yields an approximate empirical formula of $\text{SiO}_{1.95}(\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2)_{0.15}$. Each imprint site contains two T species, so for every gram of M, there are $2.0 \times 10^{-3}$ mol AP groups, or $1.0 \times 10^{-3}$ mol imprint sites. N contains 15.8 mol% T, so its empirical formula is $\text{SiO}_{1.92}(\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2)_{0.16}$. For every gram of N, there are $2.3 \times 10^{-3}$ mol AP groups, or the equivalent of $1.1(5) \times 10^{-3}$ mol “imprint” sites or pairs of AP groups. In other words, N has approximately 15% more AP groups than M per gram of material.
4.2.3  The Effect of the Organic Groups on the Pore Structure

The preservation of the mesoporous structure was confirmed visually by transmission electron microscopy (TEM) of all three materials before and after thermal treatment (Figure 4-6). There is no discernible difference in pore structure or size in any of the materials before and after the imprint removal treatment. However, there is a clear difference in the long-range order of the mesopores going from MIMO to NIMO to PMS: PMS/P show long-range ordering both perpendicular and parallel to the channels; NIMO/N show good ordering and parallel channels, but the domains are noticeably smaller; MIMO/M show relatively parallel channels and some evidence of a hexagonal pore structure, but the pores are significantly more disordered. This trend is also evident in the small-angle X-ray scattering (SAXS) data obtained for each of the samples (Figure 4-7). The broadest and least intense (100) reflections were obtained for MIMO and M; those of NIMO and N are slightly sharper and more intense. Only PMS and P exhibit any higher order diffraction peaks ((110) and (200)). The large two-point-attached imprint precursor, BPAP, was expected to have a somewhat disruptive effect on the cooperative self-assembly of TEOS with the P123 micelles in solution, so it is logical that MIMO and M show the poorest pore ordering. (3-Aminopropyl)triethoxysilane (APTES)
Figure 4-7. Small-angle X-ray scattering plots for all samples before (dotted traces) and after (solid traces) thermal treatment, showing the (100) peak. All plots are to the same scale. Inset: enlargement of second-order (110) and (200) diffraction peaks for P, which shows evidence of long-range 2D hexagonal ordering.

has been shown to have a significant disruptive effect on the structural ordering of SBA-15 when synthesized in acidic media due to the protonation of the amines. However, this effect was not as pronounced in the synthesis of NIMO, since the AP groups were produced by the reduction of NCO groups during the curing step; amine groups were not introduced in significant amounts until after the micelle self-assembly and initial condensation of the silica matrix had occurred. As such, a small amount of disorder due to the incorporation of non-rigid ICPTES can be expected, which accounts for the moderate ordering observed in NIMO and N. The best ordering is observed for PMS and P because TEOS facilitates micelle self-assembly and is known to yield excellent long-range ordering when used to prepare P123-templated PMS.

4.2.4 Determining the Location of the Imprint Sites

Nitrogen gas sorption was performed on each material to determine surface area, pore size and pore volume. All materials exhibited Type IV isotherms with H1 or mixed H1/H2 hysteresis typical of SBA-15-type mesoporous materials containing ordered cylindrical pores (see Appendix A, Figure A-5). The pore diameter was found by NLDFT using the adsorption branch of each isotherm and a spinodal condensation transition, and was used along with the d-spacing found from SAXS to calculate the pore wall thickness (Table 4-1). The surface areas, pore diameters and pore volumes of all materials fall within the expected range for this class of material, yet there are noticeable differences in the precise values. NIMO displays a larger than typical pore diameter and a smaller
Table 4-1. Physicochemical properties of the prepared mesoporous materials determined from nitrogen adsorption and X-ray diffraction.

<table>
<thead>
<tr>
<th>Sample</th>
<th>BET specific surface area (m²/g)</th>
<th>NLDFT mode pore diameter (nm)</th>
<th>NLDFT cumulative pore volume (cm³/g)</th>
<th>d(100) (nm)</th>
<th>Wall thickness* (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIMO</td>
<td>552</td>
<td>6.32</td>
<td>0.66</td>
<td>9.71</td>
<td>4.90</td>
</tr>
<tr>
<td>M</td>
<td>398</td>
<td>6.79</td>
<td>0.49</td>
<td>10.04</td>
<td>4.80</td>
</tr>
<tr>
<td>NIMO</td>
<td>629</td>
<td>7.59</td>
<td>0.92</td>
<td>10.76</td>
<td>4.83</td>
</tr>
<tr>
<td>N</td>
<td>552</td>
<td>6.79</td>
<td>0.66</td>
<td>10.04</td>
<td>4.80</td>
</tr>
<tr>
<td>PMS</td>
<td>594</td>
<td>6.79</td>
<td>0.69</td>
<td>10.33</td>
<td>5.13</td>
</tr>
<tr>
<td>P</td>
<td>661</td>
<td>6.79</td>
<td>0.66</td>
<td>10.47</td>
<td>5.29</td>
</tr>
</tbody>
</table>

* calculated by thickness = 2 × d(100)/√3 – pore diameter.

wall thickness. This suggests that the ICPTES was mostly localized at the micelle surface during the material synthesis, causing swelling of the template, which resulted in an expanded pore structure with the AP groups on the pore surface. The overall mesostructure (pore diameter and d spacing) is smaller for MIMO than for PMS, but the cumulative pore volume is very similar. Attractive interactions between BPAP and the poly(ethylene oxide) blocks of P123 would result in smaller templated pores and smaller spacing between hydrophobic micelle cores (poly(propylene oxide) blocks) in solution. This indicates that the BPAP resided between micelles during the material synthesis, causing the PEO blocks to penetrate into the condensing inorganic phase and resulting in a compressed mesopore network. Thus it is reasonable to conclude that the imprint sites are indeed buried in the walls of MIMO/M and not located on the surface of the pores. The lower pore volume and specific surface area in M is the result of additional condensation during the imprint removal step, which is also indicated by the increase in pore diameter and accompanying decrease in wall thickness. Likewise, N shows a decrease in all porosity parameters, which is consistent with additional condensation during thermal treatment. In all three thermally treated materials, the pore diameter is the same, which removes it as a variable in the comparison of the target binding behavior of the materials.
4.3 Target Binding Tests

4.3.1 Confirming Imprinting by Solid-Phase Extraction Tests

To determine whether M was actually molecularly imprinted, target binding tests were performed using two sets of phenol-containing compounds (Figure 4-8a). Series 1 contained three targets (BPF, BPA and BPAF), which have similar size and arrangement of phenol moieties but increasing hydrophobicity due to substitution at the bridging carbon atom (indicated by an asterisk in Figure 4-8a, Series 1); series 2 contained four targets (Ph, R, BP and BPA), which possess varying numbers and arrangements of phenol groups and aromatic rings.

Solid-phase extraction (SPE) is a useful separation technique that can be used to isolate desired compounds from an impure or mixed solution; as in liquid chromatography, separation is achieved as a result of differences in affinity between compounds in a solution and the stationary phase through which the solution flows. M, N and P were evaluated using a simplified SPE method (Figure 4-8b). First, 80 mg of powder (M, N or P) was sandwiched between filter paper in a polyethylene syringe to create a simple SPE cartridge. To this cartridge was added 1.0 mL of an aqueous stock solution (either
Figure 4-9. a) stacked solid-phase extraction plots for Series 1: the concentration of BPF, BPA and BPAF relative to an aqueous stock solution of BPF, BPA and BPAF, each at a concentration of $1 \times 10^{-4}$ M; b) stacked solid-phase extraction plots for Series 2: the concentration of Ph, R, BP and BPA relative to an aqueous stock solution of Ph, R, BP and BPA, each at a concentration of $1 \times 10^{-4}$ M. Numbers at the top of each column indicate the total percent of each target recovered.

Series 1 or Series 2). This load volume was allowed to flow through the syringe by gravity and collected in a vial. As soon as the load solution had fully passed into the cartridge, a rinse of 1.0 mL water was added to wash out any weakly adsorbed molecules. As the rinse flowed through, the remainder of the load solution was forced through the cartridge and collected, and in a second vial the rinse solution was then collected. Once the rinse had flowed into the cartridge, a total of 6.0 mL acetonitrile (ACN) were used to elute any strongly bound molecules from the cartridge. The remainder of the rinse fraction was forced through by the eluent and collected. Finally, a total of 6.0 mL of ACN was collected in a third vial. This eluent fraction was evaporated to dryness and the residue was redissolved in 1.0 mL ACN. The load, rinse, and redissolved elute fractions were analyzed immediately by HPLC to find the concentration of each target and compared to the corresponding stock solution.

In the first test, Series 1 was used. As expected for a molecularly imprinted material, M removed most of all three species from Series 1 (Figure 4-9a). Greatest retention (lowest residual concentration in the load fraction) was observed for the most hydrophobic target, BPAF, followed by the smallest target, BPF, with the smallest retention observed for the imprint molecule, BPA; overall, however, all three targets were more than 85% retained after the load and rinse steps. This can be explained by considering that the most favourable binding is for the most hydrophobic target, and that the smallest
target would most easily diffuse into the imprint site. Being non-imprinted, N and P showed an interesting and logical trend: BPF, the least hydrophobic molecule, was the least retained, and BPAF, the most hydrophobic, was the most retained; in fact, more BPAF was retained after loading in N than BPA was retained in M. N retained approximately 5% more of each target than did P after loading, suggesting that silica alone does have some ability to retain bisphenol species in solid-phase extraction from aqueous solution, and that only a small enhancement of this retention is achieved through the incorporation of 15% AP groups. Rinsing was sufficient to recover more than 80% of BPF and BPA from N and P, indicating that the major contributor to their moderate retention is weak interactions with surface functional groups on the mesoporous materials. BPAF was more strongly retained in both N and P, which is likely due to its significantly larger hydrophobic character. Rinsing with water, a poor solvent for BPAF, does little to disrupt the nonspecific binding interactions between BPAF and the (organo)silica matrix to which it is adsorbed.

In Series 2, which contained Ph, R, BP and BPA, the importance of the arrangement and spacing of phenol groups for binding is clearly demonstrated (Figure 4-9b). Although Ph and R are very small molecules and possess the appropriate functional groups, they were poorly retained in M. BP and BPA, on the other hand, were retained very well; BP was better retained than BPA, which can again be explained by its smaller size and greater ease of diffusion into the imprint sites. N and P displayed very similar retention behaviour for Series 2, with the general trend corresponding approximately to the hydrophilic/hydrophobic nature of the targets. The retention behaviour of M for smaller molecules than the imprint is encouraging: higher retention than N indicates that there are indeed imprint cavities in the material which are able to trap some molecules as they flow through the SPE cartridge, yet low retention after rinsing confirms that good binding can only be achieved when the target is of a large enough size that two-point binding within the imprint site is possible.

Solid-phase extraction relies on rapid interaction between the solid phase material and the target molecule. A concern with the use of mesoporous materials was that most of the load solution would pass around the M particles instead of entering the mesopore channels, thus negating the imprinting altogether. If this was the case, it was expected that M and P would show similar behaviour in retention of the targets in Series 1 and 2 since the outer surfaces of particles of these two materials are
similar: since the imprint sites are mostly buried within the pore walls of M, the outer surface of M particles contains almost no organic functional groups, and as such it very closely resembles unmodified silica. The few imprint sites that may exist on the outer surface of M particles can only account for a tiny difference from P in retention behaviour. However, as a significant difference was observed in the extraction properties of M and P, it is possible to conclude that the load solution is indeed penetrating into the mesopores of the materials, and therefore able to access imprint sites buried in the walls. Likewise, if the binding were simply due to interactions with amine imprint sites on the surface of the pores or the outer surfaces of the particles, it was expected that N would show better binding than M, due to its larger concentration of AP groups and the localization of these groups on the pore walls. This, too, was not the case, indicating conclusively that although buried in the pore walls, the imprint sites are accessible to aqueous solutions. Additionally, binding in the imprint site must occur rapidly, since the load solutions were flowed through each cartridge by gravity in five minutes or less. If binding were slow, only very low flow rates would yield good extraction. Overall it is clear that for the removal of bisphenol species from water, the addition of AP groups alone to silica is not sufficient; in other words, the arrangement and spacing of these groups, achieved in this case by molecular imprinting, are necessary to achieve good target binding.

4.3.2 Confirming Size-Selectivity by Static Binding Tests

To confirm that a size-selective imprint cavity had been created in M, a static binding test using an aqueous solution of bromothymol blue (BTB) was used (Figure 4-10a). BTB possesses the correct number and arrangement of phenol groups, but is significantly larger than BPA and has more
crowded phenol groups. Similar binding for these two molecules should be observed if the imprint sites are either not buried in the walls or are not size-selective. Qualitatively, the solutions suspended with P (2) and M (3) still retain most of their colour (Figure 4-10b), while the solution suspended with N (4) is colourless. This somewhat counterintuitive result can be explained by considering the nature of the pore surfaces of each of the three materials. Surface silanol groups are present on all three species, as indicated by the $Q^2$ and $Q^3$ signals observed in $^{29}$Si CP MAS NMR (Figure 4-4b). Additionally, N and M possess primary amine groups, with N having about 15% more amines per gram of material than M. BTB is only slightly soluble in water, so it is to be expected that it would readily be desolvated by appropriate interactions with the surface of the materials. In this case, 31% of the BTB is adsorbed from solution 2 by P, indicating that there is some interaction but not enough to completely desolvate the dye (Figure 4-10b). M removes 71% of the dye from solution 3, slightly less than half of which can be attributed to silica interactions, indicating that only 40% of the dye is removed from solution by interactions with amine groups. In contrast, 98% of BTB is removed from the solution exposed to N (indicated both by the colourless solution and the very low relative absorbance in the UV-Vis spectrum for this sample). Based on the trends observed in the SPE experiments, a more hydrophobic target molecule is better removed from aqueous solution than a less hydrophobic one; however, in a truly imprinted material, size selectivity also exists. In this case, size selectivity is confirmed by observing that even though M is better able to sequester appropriately sized bisphenol molecules than N, it does not remove a significantly larger and sterically bulkier bisphenol analogue such as BTB from solution, despite the proper arrangement of AP groups in the imprint sites. This could be partially due to the size of the molecule and partially due to the substitution at both carbons ortho to the phenols; however, since near quantitative removal of the dye is observed for N, the steric environment of the phenol is not too crowded for primary amines, so size selectivity is more likely. Some BTB is removed by M, which can be accounted for by considering that a fraction of imprint sites are likely present at or very close to the surface of the pores, and so are at least partially accessible to larger targets like BTB.

4.4 Conclusions

In conclusion, we have demonstrated the successful molecular imprinting of BPA into SBA-15-type mesoporous organosilica using a thermally reversible semicovalent imprinting strategy. Careful
comparison of the properties of MIMO and M to those of NIMO/N and PMS/P provides evidence of a mesoporous organosilica material with the BPAP organic bridging group buried in the pore walls. Low loading of the imprint precursor ensured the retention of the mesoporous structure after imprint removal, yet M still showed excellent binding of appropriate bisphenol species. An interesting study would probe the loading limit of a MIMO material that retains its pore structure after imprint removal; it is not likely that the loading could exceed 25%, but even this number could produce an excellent material. Shape selectivity was demonstrated in the poor binding of smaller phenol molecules, as was size selectivity, evinced by the poor sequestration of BTB in M compared to N; this highlights the difference in behaviour of AP groups buried in the walls from AP groups located specifically on the surface of the pores. We have also observed an unexpected trend in the affinity of N and P for bisphenol species of different hydrophobicity, which could be used to screen for new imprint molecules for silica. An interesting study would compare materials imprinted with a range of bisphenol species of varying size and hydrophobicity, and their extraction properties for different bisphenol molecules. Based on the affinities demonstrated in this study, a BPAF-imprinted MIMO should show the greatest binding properties with preference for BPAF. A recent report of molecular imprinting in MCM-41-type materials has prompted us to wonder whether a nanometre-scale change in wall thickness can result in improved or diminished target interaction, and whether there is a lower limit to the thickness of the enclosing matrix, below which the structural integrity and thus the selectivity of the imprint site is compromised. This is of particular concern in the synthetic method described herein, as it involves a hydrothermal treatment to remove the imprint.

4.5 Experimental Methods

4.5.1 Materials

(3-Isocyanatopropyl)triethoxysilane (ICPTES), tetraethyl orthosilicate (TEOS), phenol (Ph), resorcinol (R), 4,4'-biphenol (BP), bisphenol F (BPF), bisphenol A (BPA), hexafluorobisphenol A (BPAF), bromothymol blue (BTB), and Pluronic P123 (P123, $M_n \sim 5750$ g/mol) were purchased from Aldrich and used without further purification.
4.5.2 Imprint Precursor Synthesis

In a typical batch, BPA (2.751 g, 12 mmol) and ICPTES (5.97 mL, 24 mmol) were added to 25 mL of dry tetrahydrofuran (THF) in a round-bottomed flask and allowed to react with stirring under N₂ at 65 °C for 20 hours. The solvent was removed by rotary evaporator and the resultant oily liquid was characterized by FTIR, ¹H NMR and ¹³C NMR. The yield, estimated from ¹H NMR, was 90%. For FTIR and NMR spectra of the precursor, see Appendix A, Figure A-1.

4.5.3 Mesoporous Material Synthesis

A stock template solution was prepared by mixing P123 (8.4 g, 1.5 mmol), NaCl (24.4 g, 0.418 mol), water (69.6 g, 3.86 mol) and 2 M HCl (208.8 g, 11.6 mol H₂O, 0.42 mol HCl) and stirring until complete dissolution was achieved.

Molecular imprinted mesoporous organosilica (MIMO) was prepared by adding a predissolved solution of BPAP (0.347 g, 0.48 mmol) in TEOS (1.800 g, 8.6 mmol) to 44 g of stock template solution with stirring. Non-imprinted mesoporous organosilica (NIMO) was prepared by adding a predissolved solution of ICPTES (0.2565 g, 0.96 mmol) in TEOS (1.800 g, 8.6 mmol) to 44 g of stock template solution with stirring. Periodic mesoporous silica was prepared by adding TEOS (2.000 g, 9.6 mmol) to 44 g of stock template solution with stirring. Each mixture was stirred at room temperature for 24 h, then transferred to an 80 °C oven and cured quiescently for 24 h. The resultant powders were isolated by filtration, rinsed and then washed free of P123 by Soxhlet extraction with ethanol for 20 h. The washed powders were characterized by FTIR, ¹³C CP MAS solid-state NMR, ²⁹Si CP MAS solid-state NMR, SAXS, nitrogen sorption and transmission electron microscopy.

4.5.4 Imprint Removal

MIMO (1 g) was suspended in dimethylsulfoxide (DMSO) in a round-bottomed flask. Several drops of distilled water were added, and the suspension was heated to 160 °C for 5 hours with stirring. The imprint-removed material, M, was isolated by filtration, rinsed three times with alternately distilled water and ethanol, twice more with ethanol, and then oven dried. The same treatment was carried out on NIMO to generate N and PMS to generate P. The powders were characterized by FTIR, ¹³C CP MAS solid-state NMR, ²⁹Si CP MAS solid-state NMR, X-ray diffraction, nitrogen adsorption and
transmission electron microscopy. M and N were also characterized by $^{29}$Si HPDEC MAS solid-state NMR.

### 4.5.5 Solid-Phase Extraction and Static Adsorption Experiments

Solid-phase extraction (SPE) cartridges were prepared by packing 80 mg each of M, N and P into respective 3 mL polyethylene syringes between circles of extra-thick cellulose filter paper. In Series 1, an aqueous stock solution of BPF ($1.1 \times 10^{-4}$ M), BPA ($1.1 \times 10^{-4}$ M) and BPAF ($1.0 \times 10^{-4}$ M) was prepared and analyzed by HPLC. Each SPE cartridge was loaded with 1.0 mL of stock solution, which was allowed to flow through by gravity (~0.2 mL/min) and collected. Each cartridge was then rinsed with 1.0 mL of deionized water, which was allowed to flow through and collected. Each cartridge was then eluted with 6.0 mL of acetonitrile (ACN), which was allowed to flow through, collected, evaporated to dryness and redissolved in 1.0 mL ACN. Each fraction, load, rinse and elute, respectively, was analyzed by HPLC and compared to the stock solution. In Series 2, an identical solid-phase extraction procedure was used for a solution of Ph, R, BP and BPA in water, with all targets at a concentration of $1.0 \times 10^{-4}$ M. All solid-phase extraction tests were done in triplicate.

Static adsorption of BTB was performed by suspending 10 mg each of M, N and P in 10.0 mL of an aqueous solution of BTB (approximately $2.5 \times 10^{-5}$ M) and briefly sonicating each sample to fully disperse the powders. The suspensions were then allowed to stand undisturbed for 48 hours. Samples of each solution were collected and alkalized with a few drops of concentrated NaOH$_{aq}$. The absorbance at 615 nm of each solution (corresponding to the maximum absorbance of BTB at basic pH, as well as the maximum absorbance of the alkalized stock) was taken and compared to the stock dye solution. Static adsorption was performed in triplicate.

### 4.5.6 Instrumentation

Fourier transform infrared spectroscopy was carried out on a Perkin-Elmer Spectrum BX FT-IR system. BPAP was characterized as a liquid film on KBr windows. All powders were prepared as KBr pellets.

Solution $^1$H and $^{13}$C NMR spectra of BPAP in CDCl$_3$ were obtained on a Mercury 500 spectrometer.
All solid-state NMR experiments were carried out on a Bruker Avance 200 MHz spectrometer with powder samples packed into a 7 mm zirconia rotor and spun at a frequency of 5 kHz. \(^{13}\)C CP MAS experiments were carried out using a 1 ms contact time, composite pulse proton decoupling and ramp cross-polarization, with an average of 5000 scans acquired per sample. \(^{29}\)Si CP MAS experiments were carried out using a 10 ms contact time with an average of 6000 scans acquired per sample. \(^{29}\)Si HPDEC MAS experiments were carried out using high-power decoupling with a 5 \(\mu\)s 90° pulse, a 50 s recycle delay and a 5 \(\mu\)s pre-scan delay, with 1550 scans acquired per sample.

Transmission electron microscopy images were obtained on a Hitachi H-7000 microscope with a 100kV accelerating voltage.

Small angle X-ray scattering was carried out on a Bruker AXS NanoStar SAXS diffractometer equipped with a high-power CuKα source and long, 670 mm, beam path to a GADDS™ area detector for 2D-images. A double Gobel-Mirror™ system was used for perfect collimation of the primary X-ray beam to a 0.35 mm spot, which passed through the sample in transmission mode. The sample’s camera and the whole beam path were kept under vacuum (10\(^{-8}\) mmHg) to eliminate air-scattering and to improve the resolution. The obtained 2D image of each sample was then integrated pixel-by-pixel to convert the whole image area to a conventional I/2θ scaled plot.

Nitrogen sorption was carried out on a Quantachrome AS1C-VP2 with a bath temperature of 77 K. All samples were degassed for at least 16 hours at 100 °C before being weighed. Surface areas were determined using Brunauer-Emmett-Teller (BET) theory and five adsorption points starting at \(P/P_0 = 0.1\), and pore size distributions were calculated using NLDFT with a spinodal transition from the adsorption branch.

HPLC experiments were carried out on a Phenomenex Gemini-NX 5µ C18 110 Å column with dimensions 150 × 4.6 mm, using a Perkin-Elmer Series 410 LC pump, a Perkin-Elmer Series 200 autosampler, a Shimadzu CTO-6A column oven and a Shimadzu SPD-10A UV-Vis detector. Sample injections of 50 \(\mu\)L, a flow rate of 1.0 mL/min, a column temperature of 35 °C, UV detection at 280 nm and a mobile phase of varying ratios of acetonitrile (ACN) and aqueous 0.01M H$_3$PO$_4$ (PA) were used. Series 1 targets were run using isocratic elution with 50:50 ACN:PA. Series 2 targets were run with a mixed pump program with the following eluent compositions and times: 2.5 min at 55:65
ACN:PA, 1.5 min at 80:20 ACN:PA, 6 min at 35:65 ACN:PA. Integrated peak areas for each target were obtained using TC4 software and normalized to the corresponding stock solution.

UV-Vis spectra were obtained on a Cary 100 Bio UV-Vis spectrometer in a quartz cuvette with 1 cm path length. The absorbances were normalized to that of the stock solution at its maximum absorbance (at 643 nm).
Works Cited


25. This screening concept was originally proposed for organic polymers by Claudio Baggiani of the University of Turin at the MIP2010 conference in New Orleans in August 2010, where he reported a connection between the affinity of blank polymers and their effectiveness as MIPs for given imprint molecules. Our results suggest a similar strategy would be valid for silica and possibly other inorganic species.
5 The Effect of Crosslinker on Binding Behaviour in Molecularly Imprinted Mesoporous Organosilica

ABSTRACT: We have prepared two series of molecularly imprinted mesoporous organosilica materials from two different crosslinkers, tetraethoxysilane (T series) and 1,2-bis(triethoxysilyl)ethene (E series) in imprinted (M), nonimprinted (N), and blank control (P) forms. Based on careful characterization of the chemical composition of the materials to ensure consistency, and evaluation of the pore structure of each material to rule out anomalous effects of porosity (or lack thereof) on binding behaviour, we evaluated the materials for binding in aqueous solid-phase extraction and cyclohexane static binding tests. Overall, the T series demonstrated the best selectivity based on the original structure of the imprint molecule, while the E series showed the best size selectivity for structural analogues of the imprint. Overall, evidence has been found for an enhancement of certain types of binding when an organic bridging group (ethene) is quantitatively incorporated into the crosslinker molecule, but universally superior binding behaviour for the E series over the T series was not observed.

5.1 Introduction

The uniform covalent incorporation of organic functional groups into surfactant-templated mesoporous sol-gel materials was considered to be a tremendous discovery at the end of the twentieth century. Since their discovery in 1999,1–3 periodic mesoporous organosilica (PMO) materials have been the subject of a wide variety of research pursuits. Initially, the focus was largely on exerting control over the physicochemical properties of PMOs, where pore size distributions, morphologies, crystallinity, and chemical composition were varied and manipulated to a variety of ends. In recent years, however, there has been a shift in focus to emerging applications of PMO materials. Many of the most common PMO precursors contain small groups (methylene, ethylene, ethenylene, and phenylene being the most ordinary) that are relatively inert, and difficult to activate to enable interesting organic chemistry. Post-synthetic modification of the organic bridging groups, such as the functionalization of ethene1,4,5 and various benzene6–8 PMOs, have achieved success in imparting chemical reactivity on PMOs. PMOs have also been used for host-guest applications such as assisted protein refolding,9 controlled drug release,10 and chromatography.11

Another approach to creating functional PMO materials with interesting organic content is to employ a hierarchical templating strategy using both small molecules and micelles. The use of small molecules to template pores in a crosslinked polymer matrix is known as molecular imprinting. The functional groups on a selected small imprint molecule and complementary functional groups on an
appropriate functional monomer interact favourably to form an imprint–functional monomer complex (IC), which is polymerized with a compatible crosslinker to produce a solid polymer with the imprint molecule bound inside. Removal of the imprint molecules yields cavities with walls containing specifically arranged functional residues. These groups can then favourably interact with an appropriate target, and if the imprinting strategy is successful, selectively and tightly bind that target. We and others have demonstrated successful molecular imprinting by cocondensation of an IC and crosslinking sol-gel precursors in SBA-15-type silica, MCM-41-type silica, and molecularly imprinted pure PMO materials have also been prepared with such bridging groups as benzene and diethylbenzene.

Recently, Baggiani et al. investigated the relationship between the performance of a molecularly imprinted polymer (MIP) as a selective matrix for an imprint molecule and that polymer's inherent affinity for the same imprint molecule. Their proposal was that the presence of an imprint molecule in the synthesis of a MIP enhances the existing inherent binding properties of that polymer, which are a consequence of the presence of nonspecific binding sites within the matrix. Upon examining a combinatorial library of nearly 100 candidates, their results indicated that when a nonimprinted polymer (NIP, chemically identical to the MIP, but prepared in the absence of an imprint species) showed strong binding for a given imprint molecule, the corresponding MIP performed well. Conversely, a NIP that had poor binding for a given imprint molecule performed poorly when prepared as a MIP.

A common theme in the PMO literature when discussing applications is the advantage that the integrated organic bridging groups offer. In light of this, we wondered whether the same phenomenon that Baggiani and coworkers observed for organic polymers would hold true for silica-based MIPs in general, and MIMO materials in particular. If organic bridging groups truly do impart enhanced functionality on PMO materials, as compared to their inorganic PMS counterparts, would MIMO materials prepared entirely from organosilica species then demonstrate superior performance to analogous silica/organosilica hybrids?
In this chapter, we present the synthesis of a series of MIMO materials, with corresponding nonimprinted mesoporous organosilica (NIMO) and blank PMO control materials. After confirming the successful synthesis of the desired materials by detailed chemical and morphological characterization, and establishing a satisfactory degree of similarity between the materials, we tested the binding properties of the prepared materials, in both aqueous and nonpolar media, with a variety of targets with structural similarity to the imprint molecule, bisphenol A (BPA): phenol (Ph), 4,4'-biphenol (BP), biphenyl (biphen), and 4,4'-methylenebis(2,6-dimethylphenol) (tBuBP) (Figure 5-1a). To the best of our knowledge, this is the first time a direct comparison has been made between the binding behaviour of a complete set of imprinted organosilica materials (imprinted, nonimprinted, and blank control polymer), mesoporous or not, containing different organic bridging groups (as opposed to varying amounts of more than one bridging group).

### 5.1.1 Material Synthesis

Because the interactions that give rise to an imprinting effect are affected by the synthesis conditions under which the MIP is prepared, it is important to limit the number of experimental variables. In the case of the MIMO materials synthesized in this study, we chose to vary only the crosslinking species, keeping all other conditions identical. This way, the differences that might arise in the
binding behaviour of the different MIMOs should reasonably depend only on the presence of the organic bridging group both in the material synthesis and in target binding events, provided satisfactory mesoporous materials could be synthesized for all precursor combinations. Because silsesquioxane-type PMO precursors contain two equivalents of silicon per molecule, and are therefore able to form crosslinks at two sites, the molar amounts were adjusted for the total amount of Si in each system, and not the total moles of each precursor, because the relative amount of water and catalyst in a PMO synthesis is determined by the amount of Si atoms present in the system.

In a typical synthesis protocol, a covalently linked imprint-functional monomer complex (IC), designated BPAP, was formed via a carbamate bond between 3-isocyanatopropyltriethoxysilane (ICPTES) and bisphenol A (BPA). This precursor was dissolved in one of two crosslinkers: tetraethoxysilane (TEOS) or 1,2-bis(triethoxysilyl)ethane (BTEE). The IC:crosslinker molar ratio was controlled so that 10% of the Si atoms in the total solution were from BPAP and 90% were from the crosslinker. Long, flexible silsesquioxane precursors like the IC do not self-assemble to form physically stable PMOs, so it is necessary to use a rigid diluent in order to produce a robust material. At the same time, the BPA molecule in the IC also constitutes a crosslink, which means that a sufficiently large number of permanent crosslinks are needed to ensure that the imprint removal step does not cause the MIMO to collapse. The BPAP/crosslinker solution was added to a predissolved solution of Pluronic P123 in aqueous HCl with NaCl with a final Si:HCl:P123:H$_2$O:NaCl molar ratio of 1:6.13:0.022:228:0.006. The nonimprinted mesoporous organosilica (NIMO) was prepared with the same molar amounts, but using ICPTES instead of the IC. Finally, a control PMO material (or periodic mesoporous silica, PMS, in the case of TEOS) was prepared using 100% of the appropriate crosslinker. MIP systems typically involve the formation of the MIP and a corresponding NIP, but do not always include the blank, unfunctionalized polymer as well. We chose to include this to gain some understanding of potential nonspecific matrix binding that might contribute to the overall target binding behaviour, which we considered a distinct possibility in the case of the E series. Following

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i. 3-aminopropyltriethoxysilane has been shown to disrupt the self-assembly of PMO materials, so ICPTES was chosen instead. The isocyanate undergoes reduction at elevated temperature during the hydrothermal curing step of the PMO synthesis to produce a primary amine.
removal of the template by Soxhlet extraction with ethanol, a thermal treatment step using wet DMSO was used to cleave the carbamate bond in the MIMO to produce the final material, M, with 3-aminopropyl (AP) groups covalently attached to the matrix by Si–C bonds. The same treatment was performed on the NIMO to produce N (also with AP groups), and the PMO (or PMS) to produce P (with no added functional groups).

### 5.2 Characterization Results

In total, a library of six materials (M, N, and P for the two crosslinkers) was synthesized (Table 5-1). These materials, in sets of three, were characterized chemically and physically in order to ensure an acceptable degree of similarity. The interactions that are responsible for creating a MIP are sensitive to the synthetic conditions used. Although the semicovalent imprinting strategy does ensure control over the main imprinting interaction (the formation of the covalently bound IC), the formation of the imprint cavity walls around the IC during polymerization, which involves nonspecific interactions between other fragments of the IC and the crosslinker, may be affected by the synthetic conditions. Likewise, the interactions that cause cooperative self-assembly of a templated mesoporous material depend heavily on the presence of catalyst, water, template, and additives. Chemical characterization by FTIR and solid-state NMR (SSNMR) confirmed the presence of the desired organic groups, while morphological characterization by nitrogen sorption and small-angle X-ray scattering (SAXS) provided information about the pore structure of the materials and confirmed that they were sufficiently similar to proceed with testing.
Figure 5-2. Solid-state NMR spectra of T and E series: a) T series $^{13}$C CP MAS, b) T series $^{29}$Si CP MAS, c) E series $^{13}$C CP MAS, d) E series $^{29}$Si CP MAS. $^{13}$C spectra show spinning side bands (indicated by *) for E series. Peaks between 68 and 74 ppm are attributed to a small amount of residual template. Sharp peaks at 39 ppm are from residual DMSO.

5.2.1 Chemical Characterization

Fourier transform IR spectra were obtained for the imprinted materials before and after thermal imprint removal treatment (see Appendix B, Figure B-1). As discussed in Chapter 4, the presence of the C=O mode at $\sim$1720 cm$^{-1}$ was used to confirm the successful incorporation of BPAP into the MIMOs with the carbamate bond intact. This peak disappeared after thermal treatment, confirming the cleavage of the carbamate bond.

Cross-polarized magic angle spinning (CP MAS) solid-state NMR was used to identify the different carbon and silicon species present in each sample (Figure 5-2). In the T series, $^{13}$C CP MAS spectra confirmed the presence of the organic fragments of BPAP in MIMO$_T$: BPA aromatic signals at 120, 126, 148, and 154 ppm, and CH$_3$ signal at 29.5 ppm; propyl CH$_2$ signals at 9, 25, and 41 ppm; residual ethoxy groups at 15 and 58 ppm (Figure 5-2a). The successful removal of BPA after thermal treatment (disappearance of BPA aromatic and CH$_3$ signals), and the preservation of the propyl
carbons in the imprint cavities are clearly shown. The \(^{29}\)Si CP MAS spectra for the T series clearly show Q signals from silica species (Q\(^4\), Si(OSi)\(_4\), -114 ppm; Q\(^3\), Si(OSi)\(_3\)(OH), -105 ppm; Q\(^2\), Si(OSi)\(_2\)(OH)\(_2\), -96 ppm) and T signals from BPAP (T\(^3\), (SiO)\(_3\)SiR, -70 ppm; T\(^2\), (SiO)\(_2\)SiR(OH), -60 ppm) for MIMO, M, and N, and only Q signals for P (Figure 5-2b). The \(^{13}\)C CP MAS spectra of the E series confirm the presence of all relevant organic species present in the T series, as well as the ethylene carbons from the crosslinker at 146 ppm (Figure 5-2c).\(^1\) \(^{29}\)Si CP MAS spectra of the E series confirm that all Si atoms are T species, which indicates no Si–C bond cleavage occurred (Figure 5-2d). However, in addition to the T species from the BTEE precursor (at -86, -77, and -68 ppm), there are also T signals farther downfield (around -60 ppm) in MIMO, M, and N, which correspond to the IC or AP groups. Based on chemical characterization, the three series differ only in the type of organic bridging group present in the crosslinker.

5.2.2 Porosity Characterization

Nitrogen sorption is a powerful tool for determining the porous characteristics of mesoporous materials. From the shape of the isotherm and its hysteresis, it is possible to deduce the shape and organization of templated pores. The pore size distribution indicates the fidelity of the templating, and the \(\alpha\) method yields information about the microporosity, pore volumes, and surface areas of the sample. Using the T series as a benchmark, criteria for isotherm shape, pore size distribution, and \(\alpha\) plots were established. Type IV isotherms with either H1 or mixed H1/H2 hysteresis were observed for all six samples (Figure 5-3).

The pore size distributions for all samples were found by Non-Local Density Functional Theory (NLDFT) from the adsorption branch using a spinodal condensation transition. All samples show a dominant mesopore peak, narrow size distribution, and little or no presence of large mesopores (Figure 5-4). Overall, \(N_T\) and \(N_E\) appear to contain the largest proportion of smaller mesopores and/or micropores among the samples in their respective series. This can be explained by considering the favourable interaction that likely occurs between the PEO blocks of P123 (or even the PPO blocks) and the propyl chain in ICPTES. This interaction causes the PEO blocks to swell and penetrate into the (organo)silica matrix as it is condensing, resulting in the formation of a sort of porous corona. While the addition of NaCl to the synthesis mixture\(^19\) and hydrothermal aging\(^20\) are intended to
Figure 5-3. Nitrogen sorption isotherms for a) T series and b) E series. All are Type IV isotherms with a distinct pore condensation step in the mesopore relative pressure range, and show either H1 hysteresis ($M_T$, $M_E$) or H1/H2 mixed hysteresis ($N_T$, $P_T$, $N_E$, $P_E$).

Figure 5-4. Pore size distributions found by NLDFT using the adsorption branch and a spinodal condensation transition: a) T series, b) E series. All samples show a narrow pore size distribution with a clearly dominant mesopore size. Smaller mesopores and micropores are evident in most cases, while larger mesopores are either entirely absent or present in minimal amounts. $N_T$ and $N_E$ show the largest relative contribution of pores smaller than the dominant mesopore.
reduce the degree to which PEO penetrates into the silica by reducing PEO’s water solubility, thus producing denser walls and more well-defined pores, the competing positive interaction with ICPTES seems to at least partially overwhelm the effect of both of these strategies.

The contribution of microporosity and mesoporosity to the overall pore volume were determined by the \( \alpha_s \) method.\(^{21}\) Based on the methods described in Chapter 3, Section 3.1.5.3, the first linear portion of the \( \alpha_s \) plot was used to find the microporosity contribution and mesopore surface area (if a micropore contribution was observed); the second linear portion was used to find the mesopore volume and external surface area for each sample (Table 5-2, see Appendix B, Figure B-2). SAXS was used to obtain information about the degree of order in each sample, and to estimate the \( d \) spacing of the (100) diffraction planes in the 2D hexagonal pore structure. In both series, P showed the highest relative degree of ordering, with (110) and (200) diffraction peaks clearly visible (see Appendix B, Figure B-3). From the position of the first diffraction peak, the value of \( d(100) \) was determined for each sample, and used in conjunction with the mode pore diameter from NLDFT to estimate the wall thickness of each sample (Table 5-2). As expected from the postulated interactions between ICPTES and P123, both N samples show evidence of microporosity. The pore diameters of all materials are in the typical range for P123-templated (organo)silica materials, as are the BET specific surface areas.\(^{22}\) Further discussion of any particularities in the porosity of the materials appears in relation to their target binding behaviour.
5.3 Binding Studies

The main motivation for this study was to determine whether the presence of certain organic bridging groups in a MIMO enhances that MIMO’s performance in target binding events, and to check for a correlation between the performance of the MIMO and the corresponding nonimprinted material or the blank control PMO. Specifically, we chose to investigate the effect of complementary hydrophobic interactions from the organic groups in target binding in aqueous media, and the possibility of enhanced selectivity in nonpolar media. Aqueous binding was carried out using a set of three targets: Ph, BP, and BPA, all of which contain the same key functional group, but have different overall structures and molecular geometries. Static binding in nonpolar media was carried out in cyclohexane using a set of four targets: BP, BPA, biphen, and tBuBP, which are paired according to the same skeleton (BP and biphen, BPA and tBuBP), but are differently functionalized (biphen has no hydrogen bonding groups, tBuBP is more sterically hindered).

BPA as a pollutant causes concern in a wide variety of aqueous environmental systems. As such, it is natural to first test a BPA-imprinted material in aqueous media. We prepared simple solid-phase extraction (SPE) cartridges by packing 100 mg of each material into a polyethylene syringe, sandwiched between circles of thick cellulose filter paper. In the aqueous SPE binding test, 1.0 mL of a mixed solution of Ph, BP, and BPA, each at a concentration of $1.0 \times 10^{-5}$ M, was loaded into each cartridge (Figure 5-5a). This was followed by a rinse of 1.0 mL of deionized water. Finally, the
cartridge was eluted with 6.0 mL of ACN. Each fraction was collected quantitatively, analyzed by HPLC, and the concentrations of each target were compared to the stock solution.

In a typical static binding (SB) experiment in nonpolar media (cyclohexane), 10 mg of material was suspended in 5.0 mL of a solution of BP \( (1 \times 10^{-4} \text{ M}) \), BPA \( (8 \times 10^{-5} \text{ M}) \), biphen \( (1 \times 10^{-4} \text{ M}) \), and tBuBP \( (8 \times 10^{-5} \text{ M}) \) in cyclohexane (Figure 5-1a). The amount of each target remaining in solution after 3 hours was determined by HPLC and compared to the stock solution to find the fraction of each target bound by each sample (Figure 5-5b).

5.3.1 Solid-Phase Extraction in Aqueous Media

The aqueous SPE in normalized stacked plots show the amount of each target present in each fraction of the SPE experiment (Figure 5-6). The T series shows binding behaviour nearly identical to what was observed in Chapter 4: Ph is less than 50% retained in the load fraction, and almost quantitatively collected after the rinse. More BP is retained than BPA, which is likely due to its narrower structure, which can facilitate diffusion into both mesopores and imprint cavities. Less of each target is retained in N\text{T} than in M\text{T}, and less still in P\text{T}.

The E series displays different binding behaviour. While M\text{E} shows excellent binding, with better binding for BPA than BP in both load and rinse fractions (which suggests enhanced selectivity in this sample), P\text{E} retains more of all three targets than N\text{E}. This could be due to hydrophobic interactions with the ethene bridging groups and the targets, which are less accessible in the case of N\text{E} with its
surface AP groups. Ethene PMO is more hydrophobic than PMS,\textsuperscript{24} which likely enhances the nonspecific binding in $P_E$ (as compared to $P_T$).

A concern that arises when working with mesoporous materials is that narrow pore openings or bottlenecks in the channels will adversely affect flow through the pores of a material. This challenge presents itself for any material displaying H2 or mixed H1/H2 hysteresis in the sorption isotherm (Figure 5-3). Although there is more than one possible cause of this asymmetric hysteresis (see Chapter 3, Section 3.1.3.4 for a discussion of sorption hysteresis), a common one is the presence of constrictions in the pores, either at the opening or within the channels. However, despite very pronounced H2 hysteresis, $N_T$ showed better target binding in aqueous SPE than $P_T$. Therefore, it is reasonable to conclude that any narrowing of the pores that may be present does not adversely affect the ability of the aqueous stock solution to access the channels of materials in this study, or that the hysteresis in this system is caused by another factor such as percolation through connections between pores.

5.3.2 Static Binding in Nonpolar Media

Although aqueous binding behaviour is most useful for understanding how the imprinted materials would behave with real environmental samples, water as a solvent is capable of hydrogen bonding with both the phenol groups on the target molecules and the primary amines and silanols in the sample materials. Additionally, hydrophobic interactions for slightly soluble species like BP (0.26 g/L in water) and BPA (0.071 g/L in water) as compared to Ph (96 g/L in water) could possibly account for a significant amount of binding in aqueous media (though if water solubility alone determined binding, the trend should be different from what we have observed, with BPA instead of BP being the most retained in all cases, and Ph barely retained at all). Using a nonpolar solvent instead makes it possible to examine the binding behaviour solely due to hydrogen bonding between functional groups present on the target molecules (phenols) and the sample materials (primary amines and silanols).
Near-quantitative binding is shown for BP and BPA for all samples, M, N, and P alike (Figure 5-7), which can be partially attributed to the very low solubility of these two targets in cyclohexane:ii hydrogen bonding with available amine and silanol groups on the samples is much more favoured than nonpolar solvating interactions with the solvent, which causes these targets to adsorb to any polar surface they can access. This makes these two targets poor candidates for demonstrating selectivity of the imprint sites based on preferential hydrogen bonding between the primary amines of the imprint sites and phenols on the targets.

By contrast, the highly cyclohexane-soluble biphen and tBuBP are good candidates to demonstrate the role of hydrogen bonding in this system. The tBu groups on tBuBP improve the solubility of this target in cyclohexane, but are also bulky and create a sterically crowded environment around the phenol moieties. These three factors (solubility, crowding around the phenol, and overall molecular size) can account for the relatively low overall fraction of this target bound, as compared to BPA, in all samples. However, sufficient binding of it and biphen are observed to demonstrate preference for tBuBP over biphen, which correlates with tBuBP’s ability to hydrogen bond. M_{T} shows the greatest overall binding of tBuBP. The small amount of biphen bound to materials in the T series can be

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ii. Solubility data are not reported for BP, BPA, or tBuBP in cyclohexane, but qualitative observations indicate that the relative solubilities of BP and BPA, as compared to biphen and tBuBP, are very low. While 10 mg of biphen or tBuBP dissolved easily and completely in ~10 mL cyclohexane in a quick solubility test, 5 mg of BP or BPA showed little visible dissolution in 10 mL cyclohexane. Complete dissolution of 4–5 mg each of all four targets (depending on the desired final concentration) was achieved in a total of 200.0 mL cyclohexane for the static binding stock.
explained by the presence of propyl carbons on the AP groups of M_T and N_T, and by residual ethoxy groups present in all materials (cf. Figure 5.2a). M_E appears to show better size selectivity than M_T, based on the total amount of tBuBP bound compared to BPA (which is less for M_E). Finally, despite having the lowest hydrophobicity, the T series shows the highest binding of the highly hydrophobic tBuBP target. This suggests that hydrogen bonding, and not hydrophobic interactions, are the dominant reason for target binding from solution.

We anticipated that there would be an increase in nonspecific binding due to favourable hydrophobic interactions between the aromatic rings on the targets and the bridging ethene groups in the E series materials. While M_E shows lower overall binding than M_T, P_E binds more of both biphen and tBuBP than P_T does, which suggests that hydrophobic interactions to play some role in target binding. The binding of biphen and tBuBP in cyclohexane in the E series follows the same trend as overall target binding in aqueous SPE in the E series, where M_E binds the largest fraction of all targets, and N_E binds the smallest fraction. This, too, indicates that nonspecific binding as a result of interaction with the bridging organic groups in the matrix enhances target binding: randomly placed AP groups on the surface of the pore of N_E restrict access to surface ethene groups, while precisely organized and separated AP groups in imprint do not.

5.3.3 Quantifying the Imprinting Effect

Visual representations of binding behaviour are useful for rapidly identifying trends in sets of materials, but numerical quantities allow for more precise comparisons between samples. The partition coefficient \( K_p \) is the ratio of bound substrate (target molecule) to the original amount of substrate in solution (see Chapter 2, Section 2.2.4 for a complete discussion of evaluating the binding behaviour of a MIP). The ratio of partition coefficients of two targets is the selectivity factor, \( \alpha \), which represents the difference in binding between two substrates on the same material. The imprinting factor, IF, for a given target molecule is the ratio of partition coefficients for the MIP to the corresponding NIP (or blank control polymer), and is a measure of how many times better the binding of a target molecule is to a MIP than to a NIP or blank control polymer. This value provides a means of numerically removing nonspecific binding, leaving only the imprinting effect (the arrangement of functional monomers inside a specifically shaped imprint cavity). The higher the value of IF, the more pronounced the imprinting effect in that system.
Based on the normalized amounts bound, values of IF and $\alpha$ were determined for all relevant sample and target combinations (Table 5-3). Based on cases where IF > 1, the T series and the E series show a clear imprinting effect for M vs. both N and P in aqueous SPE. The presence of AP groups in N_T also shows an enhancement of target binding for all three targets in water. Conversely, the presence of AP groups in N_E seems to have a detrimental effect on target binding, as indicated by IF < 1 for N_E vs. P_E.

In cyclohexane, only the IF values for tBuBP were calculated, since this target was the only hydrogen-bonding target that showed significant variation in binding. As with the aqueous system, the T series showed the greatest binding enhancement for M compared to both N and P, as well as for N vs. P.

Interestingly, while the presence of AP groups as pairs in the imprint sites in the E series resulted in a high value of IF, AP groups on the pore surfaces of N resulted in IF < 1 as compared to P. Likewise, the value of IF was higher for M vs. N than it was for M vs. P, which points to nonspecific binding with the ethene bridging groups as a factor in the overall binding performance of M_E. In a true binding cavity, the walls contain exposed ethene bridging groups, and they are not blocked by dangling AP groups as is the case with the surface AP groups in N_E. This is also consistent with the binding behaviour of the E series observed for aqueous SPE, where P_E had better binding of the targets than N_E.

The selectivity factors in aqueous SPE (Table 5-5b) show clear preference for both BP and BPA over Ph, which is to be expected for a targets that can form two hydrogen bonds with the imprint site, rather than only one. The values of $\alpha$ are highest in the T series. No samples show significant selectivity for BPA over BP, with M_E being the only sample with $\alpha > 1$ (but only slightly). The trend in $\alpha$ values in cyclohexane SB for the T series points to a true imprinting effect, with M_T showing the
best selectivity for the appropriately shaped target (despite its bulky tBu groups). In the E series, N shows greater selectivity than M; this could also be due to the enhanced size selectivity of the imprint cavities in M_E, which allows for lower overall binding of tBuBP, while surface AP groups in N_E can more easily come into contact with tBuBP’s phenols. This reversal of binding is also consistent with the static binding test performed with bromothymol blue in Chapter 4, where the greatest binding of the dye was observed for N, with AP groups on the surface of the pores.

5.3.4 Do Porosity and Surface Area Influence Binding Patterns?

The question of whether the specific surface area plays a role in the binding behaviour of MIMO materials can be answered by comparing the values for each series, and between series (for example, M_T with M_E). No clear correlation exists between high surface area and the total amount of any target bound, in either the aqueous SPE or cyclohexane SB trials. Likewise, no patterns emerge in the relative pore volumes (total, mesopore, or micropore), pore diameters, or wall thicknesses of the materials. Therefore, it is reasonable to conclude that provided significant surface area and pore volume are present, and approximately equal mesopore sizes exist for all materials, the difference in binding behaviour of MIMO materials prepared with different crosslinkers can be attributed to the differences in those crosslinkers.

5.4 Conclusions

A small controlled library of organic MIPs has shown a clear correlation between the inherent interactions between imprint molecules and the NIP and the performance of the corresponding MIP. Ethene-bridged MIMO shows good evidence of greater size-selectivity in nonpolar media, and in aqueous SPE trials, it displays better target retention and perhaps also selectivity than MIMO prepared with TEOS as the crosslinker. Based on a lack of correlation between the binding behaviours observed and the physicochemical properties of the materials, the ethene bridging group in the E series is responsible for this result. However, selectivity for a hydrogen-bonding target over a target that cannot hydrogen bond is best in the T series in nonpolar media, as is selectivity for BP over Ph in aqueous SPE. Therefore, it is reasonable to conclude that binding enhancement due to improved nonspecific interactions between the target and the polymer matrix can be achieved in a MIMO, but universally superior performance is not yet proven. As a first investigation of this question, the results shown here point to further studies in a variety of directions: binding studies with a wider variety of
targets containing different functional groups, saturation investigations to determine the binding
capacity as it relates to the presence of nonspecific binding events, and synthesis of a larger library of
MIMO materials containing other organic bridging groups (including mixtures of different groups) to
establish a broader trend and confirm the patterns observed here. PMO materials have not yet proven
themselves to be superior to silica/organosilica hybrids for molecular imprinting applications, but
these early results do indeed suggest that, with the correct optimization, they can outperform their
PMS counterparts.

5.5 Experimental Methods

5.5.1 Materials

(3-Isocyanatopropyl)triethoxysilane (ICPTES), Tetraethyl orthosilicate (TEOS), phenol (Ph),
biphenyl (biphen), 4,4'-biphenol (BP), bisphenol A (BPA), 4,4'-methylenebis(2,6-diterbutyl)phenol
(tBuBP), and Pluronic P123 (P123, Mₙ ~ 5750 g/mol) were purchased from Aldrich and used without
further purification. 1,2-bis(triethoxysilyl)ethene (BTEE) was purchased from Gelest and used as
received.

5.5.2 Mesoporous Material Synthesis

BPAP was synthesized as described in Chapter 4. A stock template solution was prepared by mixing
P123 (8.4 g, 1.5 mmol), NaCl (24.4 g, 0.418 mol), water (69.6 g, 3.86 mol) and 2 M HCl (208.8 g, 11.6
mol H₂O, 0.42 mol HCl) and stirring until complete dissolution was achieved.

**T series.** Molecular imprinted mesoporous organosilica (MIMO, Mₜ) was prepared by adding a
predissolved solution of BPAP (0.547 g, 0.48 mmol) in TEOS (1.8000 g, 8.6 mmol) to 44 g of stock
template solution with stirring. Non-imprinted mesoporous organosilica (NIMO, Nₜ) was prepared by
adding a predissolved solution of ICPTES (0.2565 g, 0.96 mmol) in TEOS (1.8000 g, 8.6 mmol) to 44 g
of stock template solution with stirring. Periodic mesoporous silica (Pₜ) was prepared by adding TEOS
(2.000 g, 9.6 mmol) to 44 g of stock template solution with stirring. Each mixture was stirred at room
temperature for 24 h, then transferred to an 80 °C oven and cured quiescently for 24 h. The resultant
powders were isolated by filtration, rinsed and then washed free of P125 by Soxhlet extraction with
ethanol for 20 h. The washed powders were characterized by FTIR, ¹³C CP MAS solid-state NMR, ²⁹Si
CP MAS solid-state NMR, small angle X-ray scattering, and nitrogen sorption.
E series. M_E, N_E, and P_E were prepared as above, but using BTEE instead of TEOS: M_E and N_E were prepared with 1.407 g (4.3 mmol) BTEE; P_E was prepared with 1.693 g (4.8 mmol) BTEE.

5.5.3 Imprint Removal

MIMO (1 g) was suspended in dimethylsulfoxide (DMSO) in a round-bottomed flask. Several drops of distilled water were added, and the suspension was heated to 160 °C for 5 hours with stirring. The imprint-removed material, M, was isolated by filtration, rinsed three times with alternately distilled water and ethanol, twice more with ethanol, and then oven dried at 100 °C. The same treatment was carried out on NIMO (1 g) to generate N and PMS (1 g) to generate P. The powders were characterized by FTIR, ^13^C CP MAS solid-state NMR, ^29^Si CP MAS solid-state NMR, small angle X-ray scattering, and nitrogen sorption.

5.5.4 Solid-Phase Extraction and Static Adsorption Experiments

Solid-phase extraction (SPE) cartridges for each series were prepared by packing 100 mg each of M, N and P into respective 3 mL polyethylene syringes between circles of extra-thick cellulose filter paper. In aqueous SPE, an aqueous stock solution of Ph (1.0 x 10^-4 M), BP (1.0 x 10^-4 M) and BPA (1.0 x 10^-4 M) was prepared and analyzed by HPLC. Each SPE cartridge was loaded with 1.0 mL of stock solution, which was allowed to flow through by gravity and collected. Each cartridge was then rinsed with 1.0 mL of deionized water, which was allowed to flow through and collected. Each cartridge was then eluted with 6.0 mL of acetonitrile (ACN), which was allowed to flow through, collected, evaporated to dryness and redissolved in 1.0 mL ACN. Each fraction, load, rinse and elute, respectively, was analyzed by HPLC and compared to the stock solution. Aqueous SPE was performed in triplicate.

In cyclohexane static binding, 10 mg of each material was weighed into a 20 mL scintillation vial. To this was added 5.0 mL of a stock solution containing BP (1 x 10^-4 M), BPA (8 x 10^-5 M), biphen (1 x 10^-4 M), and tBuBP (8 x 10^-5 M). The suspensions were sonicated briefly to break up aggregates, and allowed to stand with occasional shaking overnight. Aliquots were taken, filtered, and analyzed by HPLC and compared to the stock solution. SB tests were done in triplicate.
5.5.5 Instrumentation

Fourier transform infrared spectroscopy was carried out on a Perkin-Elmer Spectrum BX FT-IR system. BPAP was characterized as a liquid film on KBr windows. All powders were prepared as KBr pellets.

Solution $^1$H and $^{13}$C NMR spectra of BPAP in CDCl$_3$ were obtained on a Mercury 500 spectrometer.

All solid-state NMR experiments were carried out on an Agilent DD2 600 MHz spectrometer with powder samples packed into a 5.6 mm zirconia rotor and spun at a frequency of 8 kHz for $^{13}$C experiments and 7 kHz for $^{29}$Si experiments. $^{13}$C CP MAS experiments were carried out using a 1.3 ms contact time, composite pulse proton decoupling and ramp cross-polarization, with an average of 5000 scans acquired per sample. $^{29}$Si CP MAS experiments were carried out using a 12 ms contact time with an average of 2000 scans acquired per sample.

Small angle X-ray scattering was carried out on a Bruker AXS NanoStar SAXS diffractometer equipped with a high-power Gukα source and long, 670 mm, beam path to a GADDS™ area detector for 2D-images. A double Gobel-Mirror™ system was used for perfect collimation of the primary X-ray beam to a 0.35 mm spot, which passed through the sample in transmission mode. The sample’s camera and the whole beam path were kept under vacuum (10^{-8} mmHg) to eliminate air-scattering and to improve the resolution. The obtained 2D image of each sample was then integrated pixel-by-pixel to convert the whole image area to a conventional I/2θ scaled plot.

Nitrogen sorption was carried out on a Quantachrome AS1C-VP2 with a bath temperature of 77 K. All samples were degassed for at least 16 hours at 100 °C before being weighed. Surface areas were determined using Brunauer-Emmett-Teller (BET) theory and five adsorption points starting at P/P$_0$ = 0.1, pore size distributions were calculated using NLDFT with a spinodal transition from the adsorption branch, and α$_s$ plots were generated using the adsorbed volume at P/P$_0$ = 0.4 on hydroxylated amorphous silica at 77 K as the reference standard.

HPLC experiments were carried out on a Phenomenex Gemini-NX 5µ C18 110 Å column with dimensions 150 × 4.6 mm, using a Perkin-Elmer Series 410 LC pump, a Perkin-Elmer Series 200 autosampler, and a Shimadzu SPD-10A UV-Vis detector. Sample injections of 50 µL, a flow rate of 1.0
mL/min, UV detection at 280 nm and a mobile phase of varying ratios of acetonitrile (ACN) and aqueous 0.01M H₃PO₄ (PA) were used. Aqueous SPE targets were run using a mixed pump program with the following eluent compositions and times: 2.5 min at 35:65 ACN:PA, 2.0 min at 80:20 ACN:PA, 4.5 min at 55:65 ACN:PA. Cyclohexane SB targets were run using a mixed pump program with the following eluent compositions and times: 4.5 min at 45:55 ACN:PA, 5.0 min at 90:10 ACN:PA, 4.0 min at 45:55 ACN:PA. Integrated peak areas for each target were obtained using TC4 software and normalized to the corresponding stock solution.
Works Cited


6
The Effect of Pore Structure on the Kinetics of Target Binding in Molecularly Imprinted Mesoporous Organosilica

ABSTRACT: We have prepared a series of molecularly imprinted mesoporous organosilica materials with varying pore morphologies to examine the effect that porosity has on target binding kinetics. In time-dependent binding of only the imprint molecule, bisphenol A, approximate pore length was inversely related to the rate of binding. Competitive binding over time with two other structurally similar targets revealed the same trend, but lower overall binding rates and equilibrium binding capacities. The kinetics of binding show no correlation to specific surface area (total by BET or primary mesopore) or pore volume (total by NLDFT or primary mesopore), but are directly related to the ratio of the external specific surface area to the total specific surface area of the templated mesoporous materials, where a greater relative external specific surface area results in faster target binding kinetics. This confirms that when the overall diffusion pathways from the exterior of a particle to the imprint cavity are shortened, target binding is achieved more rapidly.

6.1 Introduction

When considering the choice of morphology for a molecularly imprinted polymer (MIP) with very high crosslink density, diffusion lengths become a primary concern. For a polymer that cannot swell in the presence of solvent, this concern is increased. Therefore, when considering silica as the matrix polymer for molecularly imprinted organosilica (MIO), control of the polymer morphology, at both the micrometre and nanometre scales, is of interest. In the past fifteen years, various approaches have been taken to adjusting or manipulating the morphology of MIO in order to optimize target binding times. As discussed in detail in Chapter 2, the micron-scale approaches include grinding monoliths and sieving to select particles sizes,\(^1\) synthesizing core-shell spheres with imprint sites located close to the surface,\(^2\) and creating thin films.\(^3\)–\(^5\) However, in all of these examples, the precise diffusion lengths remained somewhat uncertain as a result of the inherent porosity present in sol-gel (organo)silica materials, and the resulting disordered diffusion pathways between pores. The use of templated mesoporous sol-gel silica materials, including examples of surface grafting,\(^6\),\(^7\) in situ surface imprinting,\(^8\),\(^9\) and imprinting inside the walls,\(^10\),\(^11\) all point to the deliberate integration of templated mesopores to facilitate diffusion and improve binding kinetics.

The diffusion of substrates in porous silica-based sorbents has been the subject of study in a variety of contexts related to nonspecific adsorption. The use of mesoporous materials as heavy metal sorbents for environmental pollution applications\(^12\) has received much attention. In surface grafting of silica materials, metal ion diffusion kinetics have been shown to be superior in templated periodic
mesoporous silica (PMS) compared to amorphous silica gel, and both kinetics and capacity have been shown to be dependent on the pore size distribution. The diffusion of gas molecules in amorphous porous silica gel has been shown to depend on the average pore diameter, and in gas sensing applications, response time decreases with increased porosity. In recently developed functionalized PMS materials for CO₂ adsorption, the pore size was shown to be proportional to, and the pore length was determined to be inversely proportional to, the rate of CO₂ uptake. Piecing these results together, it stands to reason that a general conclusion could be as follows: larger, shorter, more uniform pore channels facilitate diffusion of substrates in sorbent materials for gas and metal ion adsorption. If the diffusion rates of smaller species (metal ions, di- or triatomic linear gas molecules) are affected by porosity, then the diffusion rates of larger species should be affected in the same way but to a greater extent. In organic dye adsorption studies on propyl-functionalized PMS, diffusion into the mesopores has indeed been shown to be the rate-limiting step. However, to our knowledge, no direct comparison has yet been made between different pore morphologies, both solvent-generated and templated, in MIO to determine whether the same kinetic factors that affect nonspecific surface adsorption also apply to target binding in molecular imprint cavities, or whether these factors would be distinct enough between mesoporous samples of the same pore diameter but different channel length to observe a significant difference in the kinetics of target binding.

In this chapter, we present the synthesis, characterization, and binding evaluation of bisphenol A (BPA)-imprinted mesoporous organosilica, containing either random solvent-generated or P123-templated mesopores. The MIO materials were prepared using TEOS as a crosslinker, and different inorganic salt additives were used to yield varying pore morphology and channel lengths. After confirming the chemical composition of the four materials was consistent, we evaluated the morphological characteristics of each sample. The kinetics of binding of BPA, both alone in solution and in competition with two other structurally similar targets, were evaluated and quantified using a pseudo-second order rate equation. Finally, the saturation capacities of the four materials were compared as a function of different morphological parameters, to determine if the binding followed any trends therein.
Our typical P123-templated MIMO synthesis yields random particles with diameters (and therefore channel lengths) ranging from 1–3 μm. We adopted two literature procedures for SBA-15 with controlled morphology to synthesize MIMO materials with shorter channel lengths, selecting ~1 μm-long rods with pores running along the long axis and ~300 nm-thick platelets with pores running along the short axis. These procedures differed from our typical synthesis in the inorganic salt used and the stirring and curing conditions used (Table 6-1). In addition, we prepared a non-templated “bulk” MIO by omitting the P123 from our typical MIMO synthesis. The IC was the same BPAP we have used in previous chapters, with thermal cleavage of the carbamate linker achieved by heating to 160 °C with stirring in wet DMSO for 5 hours, followed by exhaustive rinsing alternately with ethanol and water and drying overnight at 100 °C.

### Characterization Results

After confirming by FTIR and SSNMR that the chemical composition in each material was the same (see Appendix C, Figures C-1 and C-2), the approximate particle morphology of each material was determined by electron microscopy, and the pore morphology of each material was investigated by nitrogen sorption and small-angle X-ray scattering (SAXS).
6.2.1 Electron Microscopy

Of primary interest was the successful control over the particle morphology, and therefore the approximate pore length, of the different materials. Electron microscope images in both scanning and transmission mode were obtained for all four samples to determine the morphology and estimate the geometrical pore/diffusion length. Representative low-magnification images showing whole particles reveal the typical shape for each sample (Figure 6-1). B is composed of large particles (~6 μm-diameter particle shown here) with rough edges and irregular shape. T is composed of smaller, irregularly shaped particles (1–2 μm in length) with parallel channels up to 2 μm in length running through the length of the particles. R does not show the desired rod morphology, but does appear to be produced by the aggregation of wide rod-like or plate-like structures approximately 300 nm long with pore channels running along this axis. Finally, PL displays an unexpected foamlke particle
Figure 6-2. Scanning (first two in each row) and transmission (last one in each row) electron microscope images of a) B, b) T, c) R, and d) PL, taken from the same particles shown in Figure 6-1, showing surface texture and matching scanning/transmission images of the same spot of each sample. All images are shown to the same scale. Scale bar at top left is 200 nm.
Figure 6-3. Transmission electron micrographs of T, R, and PL, showing the difference in pore structure. Arrows point to dead-end pores in T. All images are shown to the same scale. Scale bar at bottom left is 100 nm.

morphology, rather than the desired platelets. The pore structure appears quite disordered, and no obvious channel length can be estimated. The failure of the procedures selected to produce MIMO materials with the expected particle morphologies can be attributed to the addition of BPAP in the synthesis solution. Large, flexible silsesquioxane precursors are known to disrupt the cooperative self-assembly of template micelles with sol-gel precursors in solutions with low template concentrations; spontaneous formation of precise morphologies of PMS/PMO particles in solution-phase synthesis is caused by control over the cooperative self-assembly and subsequent domain aggregation that occurs, so the addition of a second sol-gel precursor of the size and flexibility of BPAP would cause a different particle morphology to be produced.

High magnification scanning and transmission electron micrographs reveal more information about the pore arrangement within each material (Figure 6-2). B is clearly porous, but no order or uniformity is visible. Therefore, this sample is suitable as an example of random diffusion paths present in amorphous silica prepared in the absence of a mesopore template. T contains long, parallel channels that are open at the ends and run the length of particles. Therefore, this sample is suitable to demonstrate diffusion paths along long pores. R, though not rod-like in particle morphology, contains uniform pore channels that are oriented in the same direction throughout the particles. Therefore, this sample is suitable to demonstrate diffusion paths along shorter pores. Finally, the foamlike
morphology of PL contains pores running in random directions, in a wormlike configuration. The pores appear significantly shorter in PL (this is also evident in Figure 6-1d, where the particle has a very open structure), so this sample is suitable to demonstrate the shortest relative diffusion pathway in the set of materials presented here. Additionally, PL can potentially shed light on whether there is a benefit to random mesopore pathways (as opposed to the nontemplated random pores in B) for target diffusion in binding studies, and whether a particular advantage is gained by the presence of highly ordered mesopores (in comparison to R, for example). Additional TEM micrographs of T, R, and PL (Figure 6-3) show the difference in overall pore arrangement in the templated materials: T contains long channels that run approximately parallel, but are randomly interrupted with dead ends (indicated with arrows). The pores in R are parallel and run the width of particles. The pores in PL are randomly organized and very short. Overall, electron microscopy reveals that as a result of the particle morphologies, the pore structures of the four samples are distinct from one another.

6.2.2 Nitrogen Sorption and Porosity Parameters

Sorption isotherms for all materials were obtained in order to qualitatively assess the pore structure, and to quantitatively assess the specific surface area, pore size distribution, and pore volume. From the shape of the sorption isotherms (Figure 6-4), the degree of uniformity and connectivity of the pores can be determined. B displays a Type II isotherm with mixed H1/H2 hysteresis over a wide P/P\textsubscript{0} range. This is consistent with a random network of disordered pores. T and R display Type IV isotherms with H1 hysteresis. The isotherm for R has a steeper and sharper capillary condensation step, which indicates that it has more uniformly shaped pores than T. The symmetrical shape of the hysteresis loop indicates that there is limited connectivity between the pores in both T and R, which means that the primary diffusion pathway in both of these materials is within templated mesopore channels. PL also shows a capillary condensation step in the isotherm accompanied by H1 hysteresis, but the step is shorter and shallower, and is followed by additional adsorption and hysteresis at higher P/P\textsubscript{0}. This is consistent with a templated mesoporous material that also contains additional, larger mesopores between or surrounding the primary templated mesopores. The shapes of the isotherms are consistent with the morphological information displayed in the electron microscope images.
Figure 6-4. Nitrogen sorption isotherms for a) B, b) T, c) R, and d) PL. Hysteresis about the capillary condensation step for T, R, and PL is primarily H1.

Figure 6-5. NLDFT pore size distributions for a) B, b) T, c) R, and d) PL, using the adsorption branch of the isotherm and a spinodal condensation transition. The mode pore diameter for T, R, and PL is 6.79 nm.
The NLDFT pore size distributions from the adsorption branch of the four samples show the desired results: B has a broad, uneven pore size distribution with no dominant pore diameter, while T, R, and PL all show one narrow mesopore distribution with a mode value of 6.79 nm (Figure 6-5). Additional smaller mesopores are present in all three samples, and PL also has a very broad distribution of larger mesopores, which is consistent with both the shape analysis of the sorption isotherm and the results from electron microscopy. The identical mode pore diameter in the three templated materials indicates that although different salts used in the initial synthesis solution did contribute to the formation of MIMO particles with different morphologies, they did not have different effects on the aggregation number of P123 micelles in the templating of individual mesopores in the material synthesis. This removes pore diameter as a variable in the factors affecting the binding kinetics for the three templated materials, and allows for a comparison of the approximate channel lengths.

Information about microporosity, mesopore volume, and external surface area, as well as confirmation of the isotherm classification, was obtained from α₃ plots (Figure 6-6). While T, R, and PL show a
Table 6-2. Summary of physicochemical properties of the four materials.

<table>
<thead>
<tr>
<th>sample</th>
<th>BET SSA* (m²/g)</th>
<th>α_s primary mesopore SSA* (m²/g)</th>
<th>α_s external SSA† (m²/g)</th>
<th>fraction SSA_{ext} of SSA_{BET} (%)</th>
<th>NLDFT cumulative pore volume (cm³/g)</th>
<th>α_s primary mesopore volume‡ (cm³/g)</th>
<th>NLDFT mode pore diameter (nm)</th>
<th>d(100) spacing (Å)</th>
<th>wall thickness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>420</td>
<td>-</td>
<td>-</td>
<td>1.206</td>
<td>-</td>
<td>13.94</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T</td>
<td>398</td>
<td>374</td>
<td>24</td>
<td>6.0</td>
<td>0.486</td>
<td>0.439</td>
<td>6.79</td>
<td>100.38</td>
<td>4.80</td>
</tr>
<tr>
<td>R</td>
<td>683</td>
<td>610</td>
<td>73</td>
<td>10.7</td>
<td>0.872</td>
<td>0.735</td>
<td>6.79</td>
<td>96.01</td>
<td>4.29</td>
</tr>
<tr>
<td>PL</td>
<td>305</td>
<td>141</td>
<td>164</td>
<td>53.8</td>
<td>0.635</td>
<td>0.209</td>
<td>6.79</td>
<td>96.6</td>
<td>4.36</td>
</tr>
</tbody>
</table>

* SSA = specific surface area.
† found by BET SSA – α_s external SSA.
‡ from the upper linear portion of the α_s plot.
§ thickness = a - Ø, where a = 2d(100)/√3 and Ø is the NLDFT mode pore diameter.

distinct upward deviation around α_s = 1.3, consistent with a capillary condensation step, the α_s plot for B remains approximately linear over the entire α_s range. All plots show a zero intercept of the first linear portion (no downward deviation), which indicates that there is no significant micropore filling step in any sample. This is also consistent with the shape of the isotherms, as significant microporosity would give rise to pore connectivity, which would result in pronounced H2 hysteresis. This, too, serves to confirm that the major diffusion pathway in the templated mesoporous samples is through the mesopore channels. The external specific surface areas were found from the slope of the second linear portion of the plots for the templated mesoporous materials, and the primary mesopore volumes were found from the intercepts. The mesopore specific surface areas were found by difference using the external specific surface areas and the BET specific surface areas for each sample. Using the position of the first diffraction peak in the SAXS spectrum (see Appendix C, Figure C-3), the d(100) spacing of T, R, and PL was found. This was used to calculate the wall thickness of each sample.

From the summarized physicochemical parameters for the four samples (Table 6-2), degrees of similarity between materials can be established. In addition to having the same mode pore diameter, all three templated mesoporous materials have nearly the same wall thickness. T has the thickest pore walls, while R has the thinnest pore walls, but the difference of a few Ångstroms between individual samples is too small to cause a significant difference in the target binding kinetics, compared to the other factors present in this set of materials. PL possesses the largest external specific surface area, which is consistent with both electron microscopy data and shape analysis of the isotherm. R has an external specific surface area half as large as PL, but three times as large as T. This also is consistent with the shape and dimension differences between the three samples. Overall, B has the largest pores
and the greatest pore volume, but a BET specific surface area similar to those of T and PL. R has the largest BET specific surface area, and the largest primary mesopore volume. Although the different synthetic methods were not successful in producing particles with the desired morphology, they did yield materials with distinct porosity parameters.

6.3 Binding Studies

In order to evaluate the influence of particle and pore morphology on the target binding kinetics of molecularly imprinted organosilica, we conducted two binding experiments: imprint molecule binding using only BPA, and competitive binding using phenol (Ph), 4,4’-biphenol (BP), and BPA.

6.3.1 Kinetics of Target Binding

In BPA binding, 10 mg of each sample was suspended in 1.0 mL deionized water and sonicated briefly to break up aggregates. At time \( t = 0 \), 10.0 mL of an aqueous solution of BPA was added to each suspension. Aliquots were taken at designated time intervals, filtered immediately, and later analyzed by HPLC to determine the remaining concentration of BPA. The amount of BPA bound per gram of material at each time point was calculated by difference. From the binding plots, the influence of material morphology on the kinetics of binding is clear (Figure 6-7). While R and PL (which have the shortest relative channel lengths) reached equilibrium within 5 minutes, T did not reach equilibrium until close to 30 minutes, and B only reached equilibrium after 120 minutes.

The competitive binding plots show the difference in the kinetics of binding of the three targets used (Figure 6-8). BP reached maximum binding almost immediately in all four materials, yet the amount bound at \( t = 1000, A_{1000} \), was 55% of the maximum or less in all cases. This suggests that BP is bound very rapidly and in large amounts over the short term but weakly at equilibrium. Likewise, Ph reached a maximum amount bound very rapidly, yet \( A_{1000} \) was 10% or less of the maximum, indicating very weak (or negligible) equilibrium binding of Ph in all materials. BPA showed slower initial binding when competing with two other targets than when alone in solution, but overall a similar pattern in the binding is evident, with no decrease in amount bound going from \( A_{120} \) to \( A_{1000} \).
Figure 6-7. Binding plots of BPA only in solution as a function of time for a) B, b) T, c) R, and d) PL. N = 3.

Figure 6-8. Competitive binding plots as a function of time for a) B, b) T, c) R, and d) PL, with Ph and BPA plotted on the primary axis and BP plotted on the secondary axis. N = 3.
This demonstrates that although BPA is slower to diffuse into imprint cavities than BP or Ph, it is more tightly bound than either target at equilibrium, which points to a true imprinting effect in these materials. The solubility of the targets may also be partially related to the relative amount of each bound at equilibrium, as the more soluble Ph and BP targets are released over a long time period. However, were binding kinetics determined by solubility, we would expect to see BPA displaying the fastest binding kinetics, as it has the lowest water solubility of the three targets.

### 6.3.2 Binding Rate Constants

Adsorption of substrates in solution onto solids is frequently modeled using a pseudo-second order rate law, which takes into account external diffusion, internal diffusion, and actual adsorption, and considers multiple interactions in the binding event.\(^\text{21}\) The linear form of the pseudo-second order rate law is

\[
\frac{t}{A_t} = \frac{1}{A_e} t + \frac{1}{kA_e^2}
\]  

(6-1)

where \(A_t\) is the amount bound at time \(t\), \(k\) is the rate constant and \(A_e\) is the equilibrium capacity. Additionally,

\[
h = kA_e^2
\]  

(6-2)

gives the initial adsorption (or binding) rate, \(h\). The pseudo-second order plots of the kinetic data for BPA binding in both trials (BPA only and competitive) gave good linear agreement (see Appendix C,
Figure 6-9. Experimental (A_{1000}, blue) and calculated (A_{e}, magenta) equilibrium amounts bound for all four materials for a) BPA only and b) competitive binding trials.

Figure C-5), and made it possible to determine h, A_{e}, and k for BPA in both trials (Table 6-5). The h values correlate with the shape of the binding curves in the BPA only trial (which clearly show that PL has the highest initial binding rate) and clarify that the same general trend is present in the competitive trial as well (where the shapes of the curves do not show as obvious a trend). The rate constants in BPA only binding follow the expected trend, with shorter pore channel lengths resulting in the highest rate constant. However, competitive binding with more than one target results in a universal decrease in the rate constants, with R and not PL displaying the largest value of k. Both the initial binding rates and rate constants for BPA binding are adversely affected by competing target species. Were binding not the result of interactions with imprint cavities, and simply due to surface adsorption, the values of h and k for BPA should reasonably be the same for both systems.

The equilibrium capacities of all four materials for the BPA only trial are within less than 10% of one another, and indicate that under the experimental conditions used, approximately 1% of total imprint sites are occupied at equilibrium. Conversely, the A_{e} values in the competitive trial vary by up to 25%, with R having the lowest capacity and PL having the greatest capacity. Overall, the capacities in competitive binding are lower than in BPA only binding, which is consistent with multiple target species.

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i. Pseudo-first order plots of ln(A_{e} - A_{t}) vs. t were also prepared, in accordance with the linear form of the pseudo-first order rate law, ln(A_{e} - A_{t}) = ln A_{e} - kt. The plots did not give linear fitting, so this rate expression was ruled out.

ii. Based on the quantitative 29Si SSNMR results in Chapter 4, there are approximately 1 × 10^{-3} mol imprint sites per gram of MIMO prepared with TEOS as the crosslinker. Although the precise values may change, the orders of magnitude should be the same.
molecules competing for the same imprint cavity binding sites. Comparing the calculated values of $A_e$ to the experimental equilibrium amounts $A_{1000}$ (amount bound at $t = 1000$), we found good agreement (within experimental error) for both BPA only and competitive binding (Figure 6-9). This further confirmed that the kinetic model used gives a good fit to experimental data.

### 6.3.3 Ruling Out Binding Due to Porosity Differences

Based on nitrogen sorption data (Table 6-2), significant differences in binding kinetics and equilibrium capacity could reasonably be expected if binding were determined by such properties as pore volume (*via* sequestration inside pores) or specific surface area (*via* nonspecific adsorption to the surface). Comparing the kinetic values from the pseudo-second order fitting to the porosity properties, we are able to rule out several possible correlations. Although T, with the thickest pore walls, has the slowest binding for the templated mesoporous samples (which could be due to greater average diffusion distances to imprint sites buried in the walls), PL is faster than R, even though its walls are thicker. This rules out diffusion into the pore walls as the limiting step. The kinetic trends are also in disagreement with the trends in specific surface area (both total and primary mesopore, where applicable), which rules out nonspecific surface adsorption as the dominant process, and pore volume (both total and primary mesopore), which rules out simple sequestration within pores of nonspecific shape or size. However, the kinetics do correspond to the trend in the percent of the specific surface area that is external to the primary mesopores. This is consistent with the observed particle morphologies from electron microscopy, and with the estimated relative length of pore channels within each of the three templated mesoporous materials. Therefore, we can conclude that the approximate pore length, and not the presence or absence of 2D hexagonal ordering, has the dominant effect on the kinetic target binding behaviour of a MIMO material. However, we can confirm that the presence of uniform mesopore channels in the material does facilitate diffusion. Compared to diffusion pathways through random pores in amorphous silica, which can be 2.5 times the geometric path length of the overall particle, mesopores afford more direct access to randomly distributed imprint sites in the walls of the MIMO. Although the same increase in path length would apply to diffusion into the pore walls to reach imprint cavities, the geometric paths in P123-templated materials are on the order of only a few nanometres at most.
6.4 Conclusions

The successful synthesis of MIMO materials with varying approximate pore length has confirmed an inverse relationship between the kinetics of target binding in MIO materials and the diffusion distances from the outside of MIO particles to vacant imprint sites. Shorter mesopores provide the most rapid binding behaviour. Pseudo-second order kinetic fitting yields good agreement with experimental data, and confirms that binding is the result of a combination of interaction both with functional groups in the imprint cavity and nonspecific interactions with the surface. However, nonspecific interactions do not significantly contribute to the equilibrium binding capacity, as demonstrated by the similar values of $A_{1000}$ in all materials, despite different specific surface areas and pore volumes. The ordering of mesopore channels appears to be fairly insignificant to the overall binding kinetics, with the dominant factor in the templated mesoporous materials used here appearing to be the ratio of the external specific surface area to the BET specific surface area. The results obtained in this chapter are in agreement with the effects of pore morphology observed on diffusion behaviour of metal ions and gases in porous silica materials, despite the difference in the type of binding that occurs (nonspecific adsorption on random sites, rather than specific binding in imprint cavities). This study provides conclusive evidence that even compared to highly porous amorphous silica with a total pore volume exceeding 1 cm$^3$/g, templated mesopore channels with uniform diameter endow MIMO with superior target binding kinetics.

6.5 Experimental Methods

6.5.1 Materials

(3-Isocyanatopropyl)triethoxysilane (ICPTES), tetraethyl orthosilicate (TEOS), phenol (Ph), resorcinol (R), 4,4'-biphenol (BP), bisphenol F (BPF), bisphenol A (BPA), hexafluorobisphenol A (BPAF), bromothymol blue (BTB), and Pluronic P123 (P123, $M_n \sim 5750$ g/mol) were purchased from Aldrich and used without further purification.

6.5.2 Mesoporous Material Synthesis

BPAP was synthesized as described in Chapter 4.
B'. Molecular imprinted “bulk” organosilica was prepared by adding a predissolved solution of BPAP (0.521 g, 0.72 mmol) in TEOS (2.700 g, 12.9 mmol) to a predissolved solution of 44.28 g 2M HCl, 14.76 g distilled H$_2$O, and 5.175 g NaCl. The solution was stirred at room temperature for 24 h, then transferred to an 80 °C oven and cured quiescently for 24 h. The resultant powder was isolated by filtration, rinsed with water and ethanol, and then washed by Soxhlet extraction with ethanol for 20 hours.

T'. Standard molecularly imprinted mesoporous organosilica was prepared as described in Chapter 4.

R'. “Rod”-shaped MIMO was prepared according to a modified version of the reported literature procedure: to a predissolved solution of 1.6 g P123, 2.2 g KCl, and 60.0 g 2M HCl held at 38 °C was added a predissolved solution of BPAP (0.769 g, 1.07 mmol) in TEOS (3.78 g, 18 mmol). The solution was stirred vigorously for 10 minutes, left quiescent at 38 °C for 24 hours, then cured in an oven at 100 °C for a further 24 hours. The resultant powder was isolated by filtration, rinsed with water and ethanol, and then washed by Soxhlet extraction with ethanol for 20 hours.

PL'. “Platelet”-shaped MIMO was prepared according to a modified version of the reported literature procedure: to a predissolved solution of 1.0 g P123, 0.162 g ZrOCl$_2$•8H$_2$O, and 40.0 g 2M HCl held at 35 °C was added a predissolved solution of BPAP (0.388 g, 0.54 mmol) in TEOS (1.89 g, 9 mmol). The solution was stirred at 35 °C for 24 hours, and then cured quiescently at 80 °C for 24 hours. The resultant powder was isolated by filtration, rinsed with water and ethanol, and then washed by Soxhlet extraction with ethanol for 20 hours.

As-synthesized MIO/MIMO materials were characterized by FTIR and $^{13}$C CP MAS solid-state NMR.

6.5.3 Imprint Removal

The as-synthesized MIMO (1 g, B', T', R', or PL') was suspended in dimethylsulfoxide (DMSO) in a round-bottomed flask. Several drops of distilled water were added, and the suspension was heated to 160 °C for 5 hours with stirring. The imprint-removed material, B, T, R, or PL, was isolated by filtration, rinsed three times with alternately distilled water and ethanol, twice more with ethanol, and then oven dried at 100 °C. The final powders were characterized by FTIR, $^{13}$C CP MAS solid-state
NMR, $^{29}$Si CP MAS solid-state NMR, electron microscopy, small angle X-ray scattering, and nitrogen sorption.

6.5.4 Kinetics of Target Binding Experiments

In all experiments, 10 mg of each material was weighed into a 20 mL scintillation vial and suspended in 1.0 mL deionized water with brief sonication to break up aggregates.

BPA only binding was performed with a stock solution of BPA ($1 \times 10^{-4}$ M) in deionized water. The procedure for one sample was as follows: at time $t = 0$, 10.0 mL of the stock was added to the material suspension. At time intervals ($t = 1, 2, 5, 10, 30, 60, 120,$ and 1000 minutes), 0.5 mL aliquots were taken from the freshly shaken suspension, immediately filtered through cellulose filter paper, and collected in HPLC vials. All aliquots and the stock were analyzed by HPLC and the concentration in each aliquot, normalized to the precise mass of MIO/MIMO material in that sample, was found by difference from the stock concentration. All binding trials were performed in triplicate.

Competitive binding was carried out using the same procedure as above, but using a stock solution of Ph ($1.0 \times 10^{-4}$ M), BP ($1.0 \times 10^{-4}$ M) and BPA ($1.0 \times 10^{-4}$ M) in deionized water.

6.5.5 Instrumentation

Fourier transform infrared spectroscopy was carried out on a Perkin-Elmer Spectrum BX FT-IR system. BPAP was characterized as a liquid film on KBr windows. All powders were prepared as KBr pellets.

Solution $^1$H and $^{13}$C NMR spectra of BPAP in CDCl$_3$ were obtained on a Mercury 300 spectrometer.

All solid-state NMR experiments were carried out on an Agilent DD2 600 MHz spectrometer with powder samples packed into a 3.6 mm zirconia rotor and spun at a frequency of 14 kHz for $^{13}$C experiments and 12 kHz for $^{29}$Si experiments. $^{13}$C CP MAS experiments were carried out using a 1.3 ms contact time, composite pulse proton decoupling and ramp cross-polarization, with an average of 3000 scans acquired per sample. $^{29}$Si CP MAS experiments were carried out using a 12 ms contact time with an average of 2000 scans acquired per sample.
Electron microscopy samples were deposited on carbon-coated copper TEM grids. Scanning electron microscopy (with matching transmission electron images) was performed on a Hitachi S-5200 scanning electron microscope. Additional transmission electron microscopy was performed on a Hitachi H-7000 transmission electron microscope using the same sample grids.

Small angle X-ray scattering was carried out on a Bruker AXS NanoStar SAXS diffractometer equipped with a high-power Cukα source and long, 670 mm, beam path to a GADDS™ area detector for 2D-images. A double Gobel-Mirror™ system was used for perfect collimation of the primary X-ray beam to a 0.35 mm spot, which passed through the sample in transmission mode. The sample's camera and the whole beam path were kept under vacuum (10⁻⁸ mmHg) to eliminate air-scattering and to improve the resolution. The obtained 2D image of each sample was then integrated pixel-by-pixel to convert the whole image area to a conventional I/2θ scaled plot.

Nitrogen sorption was carried out on a Quantachrome AS1C-VP2 with a bath temperature of 77 K. All samples were degassed for at least 16 hours at 100 °C before being weighed. Surface areas were determined using Brunauer-Emmett-Teller (BET) theory and five adsorption points starting at P/P₀ = 0.1, pore size distributions were calculated using NLDFT with a spinodal transition from the adsorption branch, and αₛ plots were generated using the adsorbed volume at P/P₀ = 0.4 on hydroxylated amorphous silica at 77 K as the reference standard.

HPLC experiments were carried out on a Phenomenex Gemini-NX 5µ C18 110 Å column with dimensions 150 × 4.6 mm, using a Perkin-Elmer Series 410 LC pump, a Perkin-Elmer Series 200 autosampler, and a Shimadzu SPD-10A UV-Vis detector. Sample injections of 50 µL, a flow rate of 1.0 mL/min, UV detection at 280 nm and a mobile phase of varying ratios of acetonitrile (ACN) and aqueous 0.01M H₃PO₄ (PA) were used. BPA only solutions were run issuing isocratic elution at 65:35 ACN:PA. Competitive binding targets were run using a mixed pump program with the following eluent compositions and times: 2.5 min at 55:65 ACN:PA, 2.0 min at 80:20 ACN:PA, 4.5 min at 55:65 ACN:PA. Integrated peak areas for each target were obtained using TC4 software and compared to the corresponding stock solution.
Works Cited


7
Summary and Future Directions

7.1 Summary and Optimization

This thesis presents a proof-of-concept study of molecular imprinting in templated mesoporous organosilica materials, where hierarchical templating produces both molecule-sized imprint cavities, which can bind target molecules, and nanometre-sized mesopore channels, which provide diffusion pathways that facilitate target access. Evaluation of the chemical composition using different organic bridging groups indicates that there is indeed the potential for significantly enhanced MIMO performance if the nonspecific matrix interactions with the target are optimized. However, it appears that screening, and not hypothesis based on expected interactions, should be the driving force in selecting and optimizing the chemical composition of a MIMO. Morphological investigations confirm the intuitive hypothesis that shorter diffusion pathways through uniformly sized mesopore channels improves target binding kinetics, but also suggests that two-dimensional pore ordering might not be necessary for ideal material performance.

In the early stages of this project, we looked at the effect that loading of the flexible IC had on the overall meso ordering of the templated material, and observed, as we expected, that order decreased as IC loading increased (see Preface). Partly because of this observation, but partly also from a desire to preserve as much of the structural integrity of the materials as possible, we chose a relatively low molar loading of the IC (10%). This is in fact at the high end of typical IC loading in semicovalently imprinted organic polymers, which are typically prepared with IC molar loadings around 5%. Noncovalently imprinted polymers typically have much higher loadings of functional monomers (relative to the imprint molecule, often 4 or more functional monomers per complementary functional group on the imprint), as discussed in Chapter 2, Section 2.2.3.1, in order to shift the equilibrium in the system to favour the self-assembly of the IC. However, the overall molar loading of the imprint molecule in the prepolymerization mixture is also typically below 10%. It would be interesting to see exactly how high the loading can be in a MIMO that retains its structural integrity after imprint removal, and whether the increased loading would yield a corresponding (ie. by a linear relationship) increase in the partition coefficient of the target molecule.
Based on our results, we estimate that approximately 1 in 100 imprint sites are occupied at equilibrium for BPA alone in solution in our chosen binding conditions (neutral pH, deionized water). However, we have observed that the addition of sodium chloride solution to the target solution increases the binding capacity.\textsuperscript{1} This is consistent with other literature reports where the ionic strength of the binding solution is proportional to the binding capacity of the MIO.\textsuperscript{1,2} This is likely due to the fact that increased ionic strength in solution results in the original hydrogen bonding between the target and the MIMO being converted to ionic interactions (protonated AP groups in the imprint cavities, deprotonated phenols on the target molecules), which, as discussed in Chapter 2, Section 2.2.3.6, and shown in Table 2-1, have much larger binding energies. Silanols are deprotonated in high ionic strength solutions, which could hypothetically decrease nonspecific binding between the target(s) and the (organosilica) surface as a result of repulsion between anionic deprotonated silanols and deprotonated phenols.

The choice to use P123 in acidic media with a salt additive to synthesize the MIMOs presented here was an informed one, yet still somewhat arbitrary. Among the wealth of templates and synthetic conditions that exist, there could very well be a better system for producing MIMOs. It would be interesting to look at whether MIMOs prepared with different templates and under different experimental conditions have different binding behaviour. In particular, systems that are known to yield denser or more microporous PMOs, PMOS with crystalline walls as opposed to amorphous walls, and different pore structures could shed light on how the morphological attributes of the matrix actually affect target binding. The porosity characteristics of the materials prepared in this thesis are in the typical range of the SBA-15 class of templated materials, which are distinct from typical organic MIPs prepared with solvent porogens that possess broad pore size distributions and lower specific surface areas.

Our choice to use a semicovalent imprinting method was based on the assumption that noncovalent imprinting simply would not work in a templated mesoporous matrix. While the surface imprinting

\textsuperscript{i} In a rough experiment, 0.5 mL each of a $\sim0.9$ M aqueous solution of NaCl was added to the remaining volume of the suspensions of four materials (B, T, R, and PL) in aqueous BPA solution used in the BPA only kinetic study in Chapter 6. The amount of BPA bound at equilibrium increased from $\sim10$ μmol/g in deionized water to $\sim24$ μmol/g in the salt solution (see Appendix D, Figure D-1).
examples we discussed in Chapter 2 do indeed show that selectivity does not require closed cavities, the degree of size selectivity is not yet clear. It would be interesting to explore the possibility of forming a robust enough noncovalent IC to produce a MIMO with cavities inside the walls. It could be as simple as mixing, but no literature report we have found has yet convinced us that high-fidelity noncovalent imprinting in the walls of a truly templated mesoporous material is possible. It is likely that noncovalent imprinting inside the walls if MIMO would rely heavily on nonspecific attractive interactions between the imprint molecule and the crosslinker, which suggests that silsesquioxane precursors with organic bridging groups that are compatible with fragments of the imprint should have the best chance of success.

The performance of the MIMO materials prepared in this thesis is similar to that of other MIO materials discussed in Chapter 2. The imprinting factors obtained for our materials (as discussed in Chapter 5) are modest, but typical of MIO materials. Compared to organic MIPs, the values of IF in MIO materials for binding in solvents similar to those used in the MIP synthesis may be lower (organic MIPs have been reported with IFs as high as 75), but very few organic MIPs have been successfully synthesized and/or used in aqueous media. This makes it difficult to compare MIOs with organic MIPs, as the binding conditions and interactions involved are not the same. The binding capacities at equilibrium, compared to literature reports at corresponding concentrations for similar imprint loadings in MIOs prepared using the same semicovalent imprinting strategy, indicate that our materials are able to bind targets to the same order of magnitude (μmol/g). By comparison, the chiral surface-imprinted PMS described in Chapter 2, Section 2.4.3, binds the appropriate amino acid enantiomer in the millimole per gram range. This is not surprising, given the loading of imprint surfactant (100%) in the material synthesis. However, this imprinting strategy is distinctly different from our approach, a fact that must be considered when drawing comparisons. Finally, the kinetics of target binding in our materials, as discussed in Chapter 6, are similar to or better than (in the case of PL, which has a different pore morphology) other examples of MIMOs with imprints in the walls, but not as good as surface-imprinted MIOs (either prepared by cocondensation or grafting). This is not surprising, given our conclusion that our imprint cavities are at least partially buried in the walls, which means that the targets must diffuse both into the pores and then into the walls to reach the binding sites.
7.2 Future Directions, Beyond Optimization

The best chance that a body of work like this has for further development and any measure of success is if some small aspect of the results resonates with researchers in different fields. Working as I have, as a member of a research group whose foundation is an interdisciplinary approach, I firmly believe that the convergence of specialists to tackle a problem about which none of them is an expert has tremendous potential. And so, the directions that I imagine for this field, starting from the results presented in chapters of this thesis, extend well beyond my own expertise.

7.2.1 Tuning Chemical Composition

Tuning the chemical composition of the walls changes many PMO properties, including hydrophobicity, acidity/basicity, refractive index, and dielectric constant. Chapter 5 showed how the quantitative integration of organic bridging groups to create an all-organosilica MIMO could yield an imprinted material with better target retention and size/shape selectivity. Any good review of the PMO literature illustrates the variety of available precursors, and also highlights the potential that exists in the integration of organic and inorganic moieties. A very logical follow-up study to Chapter 5 would examine a larger library of precursors, and the number of different approaches possible is quite large. However, a few notable directions stand out.

Many reports exist in the literature of bifunctional or multifunctional PMOs synthesized from more than one silsesquioxane precursor. These include PMOs with mixed bridging ethane and terminal vinyl groups, mono-, di-, and trisubstituted benzene bridging groups, thiophene and benzene bridging groups, and ethane and isocyanaurate bridging groups, to name only a few. It is entirely possible that a mixture of different PMO precursors, including TEOS, could ultimately yield MIMOs with the best binding attributes. Preferential association of certain organic functionalities in the IC and PMO precursors, with different interactions between different precursors and different fragments of the imprint molecule or the IC, could endow MIMOs with a high degree of shape selectivity, including enantioselectivity, that might not be as pronounced in a MIMO prepared with only one kind of crosslinker, or a crosslinker with poorer noncovalent interactions with the imprint.
A challenge in creating MIP sensors is the fact that the binding event in its own does not necessarily produce a measurable response. Sol-gel MIP sensors frequently incorporate reporter species, including fluorophores,^{15,14} and quantum dots,^{15} which give a response that is detected spectroscopically. However, optical responses are also possible from photonic crystal structures, where the response to stimuli can be strong enough to yield a colour change visible to the naked eye.^{16} In a notable MIP example, an imprinted inverse opal structure that swells when it binds the correct enantiomer of its target has been shown to display a visible colour change from green to blue.^{17} While many organic polymers used in MIP systems are able to swell readily, (organo)silica generally is not. However, reports of sol-gels that swell in response to heat^{18} and solvents^{19} exist, and suggest that it should be possible to synthesize imprinted sol-gel photonic crystal structures that exhibit colour change response to an appropriate target.

7.2.2 Tuning Morphology

The degree of control that we can exert over the morphological features of sol-gel materials with very little effort is one of the great advantages of MIO systems of all forms (including thin films, spheres, and MIMOs of varying shapes and sizes). While examples of surface imprinting of sol-gel systems of many forms are numerous, the main advantage that PMS/PMO materials have is their incredibly large surface area. The sheer number of imprint sites that can be produced on materials with surface areas reaching up to 1000 m$^2$/g is truly impressive. The fact that flexible species like sol-gel ICs disrupt the self-assembly of many mesostructures is a strong argument for imprinting via post-synthetic grafting as opposed to cocondensation. However, additional steps are necessary if a shape- or size-selective imprint cavity is desired. Our group reported the well-defined stepwise addition of monolayers of PMO precursors to PMS, yielding so-called hybrid PMOs (HPMOs).^{20} A similarly prepared molecularly imprinted HPMO (MIHPMO – sick of reading these acronyms yet?) would offer a high degree of control over the depth of imprint sites, the degree of their enclosure, and the choice of pore morphology in the host material. Concerns over pore blocking as a result of the grafting could easily be allayed by using large-pore PMS materials, which are easily synthesized using either an appropriate large pore template or a swelling agent.^{21,22}

The morphologies of the materials prepared in Chapter 6 were not exactly as desired, as the original intention was to draw a comparison between samples with well-defined differences in pore length.
This is still an area of research that bears further investigation, as a more systematic study would allow for a more precise comparison. The MIHPMO approach, if proven successful, could facilitate this process, as it would enable molecular imprinting in essentially any sol-gel material. At the same time, it would allow for even more precise control over diffusion distances, which could shed more light on boundary conditions in MIO materials.

A very interesting discovery is that simple double helix DNA structures can be used to template mesoporous silica. Unlike surfactants and copolymers, whose micelle structures are limited, DNA origami makes it possible to design virtually any 2D or 3D structure, and have it self-assemble in solution. Combining these two concepts, the number of pore structures that could be created is essentially endless, and additional functionalization of the DNA template could be used for surface imprinting, to direct the location of other additives, or to create additional micropore channels as access points to deeply buried imprint sites. Silica’s proven compatibility with many biomolecules and biological systems should not be overlooked.

7.2.3 And Almost Anything Else…

Really. As a result of its biomimetics-motivated inception, the field of molecular imprinting contains so many minute aspects of optimization and variation. This thesis demonstrates the validity of the concept of molecular imprinting in templated mesoporous organosilica materials, and explores two avenues of research (chemical composition and morphology) along which further investigation should proceed. However, the wide array of concepts that converge to produce this relatively new field of study should indicate just how many options exist for its next steps: new imprints, new crosslinkers, new templates, new shapes, new applications, new approaches to integrated systems. The crossover between MIPs and PMOs is only beginning, but with the cooperation and multidisciplinary collaboration of researchers in science, engineering, and medicine, it could very well see tremendous breakthroughs in the years to come.
Works Cited


Appendix A
Supporting Information – Chapter 4

Figure A-1. a) $^1$H NMR, b) $^{13}$C NMR, and c) FTIR spectra of the imprinting precursor BPAP after solvent removal. Asterisks in the proton spectrum indicate signals from unreacted BPA. Integration of the aromatic signals from BPA and BPAP was used to determine the yield based on the stoichiometry in the original reaction mixture. Some unreacted ICPTES is indicated by the presence of some residual NCO stretching at 2270 cm$^{-1}$ in the FTIR spectrum. However, the C=O mode at 1730 cm$^{-1}$ demonstrates the successful formation of the carbamate bond.
Figure A-2. Fourier transform infrared spectra of i) MIMO, ii) M, iii) NIMO, iv) N, v) PMS, and vi) P. The C=O stretching mode at 1720 cm$^{-1}$ is clearly visible in MIMO, and eliminated after thermal treatment to produce M.
Figure A-3. Nitrogen sorption isotherms and NLDFT pore size distributions from the adsorption branch using a spinodal transition for all materials before (solid data points) and after (open data points) thermal imprint removal treatment. All sorption isotherms show a clear pore condensation step. MIMO/M and NIMO/N show H1 hysteresis, while PMS/P show mixed H1/H2 hysteresis.
Figure B-1. Fourier transform infrared spectra before and after thermal treatment for: M<sub>T</sub> a) before and b) after, and M<sub>E</sub> c) before and d) after. The C=O mode at 1720 cm<sup>-1</sup> is clearly visible in all before samples, and eliminated in the after samples, providing evidence of the successful incorporation of the carbamate bond into the original materials, and its successful cleavage after thermal treatment.
Figure B-2. αs plots for all samples in a) T series and b) E series, showing linear regression for the first and second linear portions. ME shows two linear portions above the capillary condensation transition, corresponding to larger mesopores than the primary templated mesopores that are separate from the external surface of the sample. The intercepts of the first and second linear portions were used to calculate the micropore (for NT and NE) and mesopore volumes, respectively. The slopes of the first and second linear portions were used to calculate the mesopore (for NT and NE) and external specific surface areas, respectively. Calculations were done in accordance with the method presented in Chapter 3, Section 3.1.5.3.

Figure B-3. Scaled and offset SAXS plots for six materials after thermal treatment. PT and PE show the best order, with three diffraction peaks clearly visible (namely, (100), (110), and (200)), while the remaining samples show only the first diffraction peak.
Figure C-1. Fourier transform infrared spectra before (dashed lines) and after (solid lines) thermal imprint removal treatment for a) B, b) T, c) R, and d) PL. The C=O mode at 1720 cm$^{-1}$ is clearly visible in all before samples, and eliminated in the after samples, providing evidence of the successful incorporation of the carbamate bond into the original materials, and its successful cleavage after thermal treatment.
Figure C-2. Solid-state $^{13}$C (a) and $^{29}$Si (b) CP MAS spectra for all materials, where ' denotes the sample before thermal imprint removal treatment. $^{13}$C signals are as follows: BPA aromatic signals at 120, 126, 148, and 154 ppm, and CH$_3$ signal at 29.5 ppm; propyl CH$_2$ signals at 9, 23, and 41 ppm; residual ethoxy groups at 15 and 58 ppm. $^{29}$Si signals are as follows: Q signals from silica species (Q$^4$, Si(OSi)$_4$, -114 ppm; Q$^3$, Si(OSi)$_3$(OH), -105 ppm; Q$^2$, Si(OSi)$_2$(OH)$_2$, -96 ppm) and T signals from BPAP (T$^3$, (SiO)$_3$SiR, -70 ppm; T$^2$, (SiO)$_2$SiR(OH), -60 ppm).
Figure C-3. Small-angle X-ray scattering plots for a) B, b) T, c) R, and d) PL. All templated mesoporous samples show the first diffraction peak corresponding to the (100) diffraction plane, but no higher order reflections are evident. This is consistent with our previous SAXS results for MIMO materials prepared with TEOS as the crosslinker, and occurs as a result of BPAP disrupting the TEOS-mediated long-range cooperative self-assembly of a micelle liquid crystalline phase in the material synthesis solution.
Figure C-4. Pseudo-second order plots for BPA only (circles) and competitive (triangles) binding trials for a) B, b) T, c) R, and d) PL, with linear regression results shown.
Appendix D
Supporting Information – Chapter 7

Figure D-1. Total amount (μmol) of BPA bound per gram of MIMO in deionized water before (blue) and after (magenta) the addition of 0.5 mL NaCl(aq) (~0.9 M), using the remaining suspensions from the BPA only kinetic study in Chapter 6, Figure 6-9. The approximate final concentration of NaCl is 0.08 M.