Polymeric Monolithic Stationary Phases for Capillary Reversed-phase Liquid Chromatography of Small Molecules

Kun Liu  
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Polymeric Monolithic Stationary Phases for Capillary Reversed-Phase Liquid Chromatography of Small Molecules

Kun Liu

A dissertation submitted to the faculty of Brigham Young University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Milton L. Lee, Chair
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ABSTRACT

Polymeric Monolithic Stationary Phases for Capillary Reversed-Phase Liquid Chromatography of Small Molecules

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Doctor of Philosophy

Highly cross-linked monoliths prepared from single cross-linking monomers were found to increase surface area and stability. Therefore, seven cross-linking monomers, i.e., 1,3-butanediol dimethacrylate (1,3-BDDMA), 1,4-butanediol dimethacrylate (1,4-BDDMA), neopentyl glycol dimethacrylate (NPGDMA), 1,5-pentanediol dimethacrylate (1,5-PDDMA), 1,6-hexanediol dimethacrylate (1,6-HDDMA), 1,10-decanediol dimethacrylate (1,10-DDDMA), and 1,12-dodecanediol dimethacrylate (1,12-DoDDMA), were used to synthesize highly cross-linked monolithic columns in 75-μm i.d. capillaries by one-step UV-initiated polymerization using dodecanol and methanol as porogens for reversed-phase liquid chromatography (RPLC) of small molecules. Selection of porogen type and concentration was investigated in detail. Isocratic elution of alkylbenzenes at a flow rate of 300 nL/min was conducted for all of the monoliths. Gradient elution of alkylbenzenes and alkylparabens provided high resolution separations. Several of the monoliths demonstrated column efficiencies in excess of 50,000 plates/m. Monoliths with longer alkyl-bridging chains showed very little shrinking or swelling in solvents of different polarities. In addition, highly cross-linked monolithic capillary columns poly(1,6-HDDMA), poly(cyclohexanediol dimethacrylate) [poly(CHDDMA)] and poly(1,4-phenylene diacrylate) [poly(PHDA)], were synthesized and compared for RPLC of small molecules. Isocratic elution of alkylbenzenes was performed using 1,6-HDDMA and CHDDMA monolithic columns. Gradient elution of alkylbenzenes using all three monolithic columns showed good separations. Monolithic columns formed from 1,6-HDDMA, which had a linear alkyl-bridging chain structure, exhibited the highest column efficiencies (86,000 plates/m). Optimized columns showed high permeability and high run-to-run and column-to-column reproducibilities.

Monoliths prepared from controlled/living polymerization was demonstrated exhibiting narrower molecular weight distribution and more homogeneous cross-linked structures due to the reversible character of this polymerization method. Thus, monolithic columns were developed from three cross-linking monomers, i.e., 1, 12-DoDDMA, trimethylolpropane trimethacrylate (TMPTMA) and pentaerythritol tetraacrylate (PETA) using organotellurium-mediated living radical polymerization (TERP) in 150-μm i.d. capillaries for RPLC of small molecules. Selection of the polymerization conditions for the 1,12-DoDDMA monolith was investigated in detail. Isocratic elution of alkylbenzenes was achieved with good efficiency (47,700 to 64,200 plates/m for uracil) using all monolithic columns prepared using TERP.

Keywords: monolith, reversed-phase liquid chromatography, small molecule separation
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Kun Liu
# TABLE OF CONTENTS

ABSTRACT ...................................................................................................................... ii

ACKNOWLEDGMENTS ................................................................................................... iii

TABLE OF CONTENTS ................................................................................................. v

LIST OF ABBREVIATIONS ............................................................................................ ix

LIST OF TABLES ............................................................................................................ xiii

LIST OF FIGURES ......................................................................................................... xv

CHAPTER 1 BACKGROUND AND SIGNIFICANCE ......................................................... 1

1.1 Introduction ............................................................................................................. 1

1.2 Large-molecule separations .................................................................................. 5

1.2.1 Styrene-based monoliths .................................................................................. 9

1.2.2 Acrylate/methacrylate-based monoliths ............................................................ 12

1.3 Small-molecule separations ................................................................................... 17

1.3.1 Styrene-based monoliths ................................................................................ 17

1.3.2 Acrylate/methacrylate-based monoliths ............................................................ 21

1.3.3 Acrylamide monoliths ................................................................................... 25

1.3.4 Amine monoliths ............................................................................................ 25

1.4 Dissertation Overview .......................................................................................... 26

1.5 References ............................................................................................................ 27
CHAPTER 2 HIGHLY CROSS-LINKED POLYMERIC MONOLITHS FOR REVERSED-PHASE CAPILLARY LIQUID CHROMATOGRAPHY OF SMALL MOLECULES

2.1 Introduction ........................................................................................................................................ 35

2.2 Experimental ...................................................................................................................................... 36
  2.2.1 Chemicals and reagents .................................................................................................................. 36
  2.2.2 Fused silica capillary pretreatment ............................................................................................... 37
  2.2.3 Polymeric monolith preparation .................................................................................................... 39
  2.2.4 Capillary liquid chromatography .................................................................................................. 39

2.3 Results and Discussion ...................................................................................................................... 40
  2.3.1 Selection of porogens .................................................................................................................... 40
  2.3.2 Separation of small molecules ..................................................................................................... 40
  2.3.3 Chromatographic efficiency measurements .................................................................................. 47
  2.3.4 Monolith morphologies ................................................................................................................ 51
  2.3.5 Column permeability and stability ............................................................................................... 51
  2.3.6 Reproducibility of poly(1,6-HDDMA) .......................................................................................... 56

2.4 Conclusions ....................................................................................................................................... 56

2.5 References ......................................................................................................................................... 57

CHAPTER 3 HIGHLY CROSS-LINKED POLYMERIC MONOLITHS WITH VARIOUS C6 FUNCTIONAL GROUPS FOR REVERSED-PHASE CAPILLARY LIQUID CHROMATOGRAPHY OF SMALL MOLECULES ....................................................................................................................................................... 59
3.1 Introduction ................................................................. 59
3.2 Experimental ............................................................... 60
  3.2.1 Chemicals and reagents ............................................ 60
  3.2.2 Fused silica capillary pretreatment ............................... 60
  3.2.3 Polymeric monolith preparation .................................. 62
  3.2.4 Capillary liquid chromatography ................................. 63
3.3 Results and Discussion .................................................. 63
  3.3.1 Selection of porogens ............................................... 63
  3.3.2 Monolith morphologies ............................................. 66
  3.3.3 Separation of alkylbenzenes ..................................... 66
  3.3.4 Chromatographic efficiency measurements ................... 67
  3.3.5 Column permeability and rigidity ............................... 73
  3.3.6 Reproducibility of poly(1,6-HDDMA) ............................ 76
3.4 Conclusions ............................................................... 76
3.5 References ............................................................... 80

CHAPTER 4 FABRICATION OF HIGHLY CROSS-LINKED REVERSED-PHASE
MONOLITHIC COLUMNS VIA LIVING RADICAL POLYMERIZATION ......... 82
4.1 Introduction ............................................................... 82
4.2 Experimental ............................................................. 85
  4.2.1 Chemicals and reagents ............................................ 85
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2.2 Fused silica capillary pretreatment</td>
<td>85</td>
</tr>
<tr>
<td>4.2.3 Polymeric monolith preparation</td>
<td>87</td>
</tr>
<tr>
<td>4.2.4 Capillary liquid chromatography</td>
<td>87</td>
</tr>
<tr>
<td>4.3 Results and Discussion</td>
<td>87</td>
</tr>
<tr>
<td>4.3.1 Selection of porogens</td>
<td>87</td>
</tr>
<tr>
<td>4.3.2 Selection of polymerization conditions</td>
<td>88</td>
</tr>
<tr>
<td>4.3.3 Monolith morphologies</td>
<td>93</td>
</tr>
<tr>
<td>4.3.4 Chromatographic efficiency measurements</td>
<td>93</td>
</tr>
<tr>
<td>4.3.5 Column permeability and stability</td>
<td>95</td>
</tr>
<tr>
<td>4.4 Conclusions</td>
<td>98</td>
</tr>
<tr>
<td>4.5 References</td>
<td>101</td>
</tr>
<tr>
<td>CHAPTER 5 FUTURE DIRECTIONS</td>
<td>103</td>
</tr>
<tr>
<td>5.1 Introduction</td>
<td>103</td>
</tr>
<tr>
<td>5.2 Selection of porogens for preparation of monolithic columns</td>
<td>103</td>
</tr>
<tr>
<td>5.3 Investigation of polymerization method selection for synthesis of monolithic columns</td>
<td>107</td>
</tr>
<tr>
<td>5.4 Synthesis of new bi-functional cross-linking monomers for RPLC</td>
<td>108</td>
</tr>
<tr>
<td>5.5 References</td>
<td>113</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

1,3-BDDMA  1,3-Butanediol dimethacrylate
1,4-BDDMA  1,4-Butanediol dimethacrylate
1,5-PDDMA  1,5-Pentanediol dimethacrylate
1,6-HDDMA  1,6-Hexanediol dimethacrylate
1,10-DDDMA 1,10-Decanediol dimethacrylate
1,12-DoDDMA 1,12-Dodecanediol dimethacrylate
2-Me-1,8-ODDMA  2-Methyl-1,8-octanediol dimethacrylate
ACN   Acetonitrile
AIBN   2,2'-Azobis(2-methylpropionitrile)
ATRP   Atom transfer radical polymerization
BACM   4-[(4-Aminocyclohexyl)methyl]cyclohexylamine
BADMA  Bisphenol A dimethacrylate
BAEDA  Bisphenol A ethoxylate diacrylate
BAP    1,4-Bis(acryloyl)piperazine
BMA    Butylmethacrylate
BSA    Bovine serum albumin
BTEE   Ethyl-2-methyl-2- butyltellanyl propionate
BVPE   1,2-Bis(p-vinylphenyl)ethane
COC    Cycloolefin copolymer
CHDDMA Cyclohexanediol dimethacrylate
CQ     (+)-(S)-Camphorquinone
CRP    Controlled/living radical polymerization
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Name</th>
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<tbody>
<tr>
<td>CTA</td>
<td>Chain transfer agent</td>
</tr>
<tr>
<td>DBTTC</td>
<td>Dibenzyltrithiocarbonate</td>
</tr>
<tr>
<td>DMF</td>
<td>$N, N$-Dimethylformamide</td>
</tr>
<tr>
<td>DMPA</td>
<td>2,2-Dimethoxy-2-phenylacetophenone</td>
</tr>
<tr>
<td>DVB</td>
<td>Divinylbenzene</td>
</tr>
<tr>
<td>EDAB</td>
<td>1-Ethyl-4-dimethylaminobenzoate</td>
</tr>
<tr>
<td>EDMA</td>
<td>Ethylene dimethacrylate / ethylene glycol dimethacrylate</td>
</tr>
<tr>
<td>EGDA</td>
<td>Ethylene glycol diacrylate</td>
</tr>
<tr>
<td>EGMEMA</td>
<td>Ethylene glycol methyl ether methacrylate</td>
</tr>
<tr>
<td>EHMA</td>
<td>Ethylhexyl methacrylate</td>
</tr>
<tr>
<td>EVB</td>
<td>Ethylvinylbenzene</td>
</tr>
<tr>
<td>GDMA</td>
<td>Glycerol dimethacrylate</td>
</tr>
<tr>
<td>GMA</td>
<td>Glycidyl methacrylate</td>
</tr>
<tr>
<td>GMMA</td>
<td>Glycerol monomethacrylate</td>
</tr>
<tr>
<td>GNP</td>
<td>Gold nanoparticles</td>
</tr>
<tr>
<td>HEMA</td>
<td>2-Hydroxyethyl methacrylate</td>
</tr>
<tr>
<td>HILIC</td>
<td>Hydrophilic interaction chromatography</td>
</tr>
<tr>
<td>IPA</td>
<td>$N$-Isopropylacrylamide</td>
</tr>
<tr>
<td>LMA</td>
<td>Lauryl methacrylate</td>
</tr>
<tr>
<td>MA</td>
<td>Methacrylamide</td>
</tr>
<tr>
<td>MAA</td>
<td>Methacrylic acid</td>
</tr>
<tr>
<td>MPPB</td>
<td>$N$-Methoxy-4-phenylpyridinium tetrafluoroborate</td>
</tr>
<tr>
<td>MST</td>
<td>Methylstyrene</td>
</tr>
<tr>
<td>Abbr.</td>
<td>Full Name</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------</td>
</tr>
<tr>
<td>MW</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>MWNT</td>
<td>Multi-walled carbon nanotubes</td>
</tr>
<tr>
<td>NMP</td>
<td>Nitroxide-mediated living radical polymerization</td>
</tr>
<tr>
<td>NPGDMA</td>
<td>Neopentyl glycol dimethacrylate</td>
</tr>
<tr>
<td>OD</td>
<td>1-Octadecene</td>
</tr>
<tr>
<td>PCB-HEM</td>
<td>[6,6]-Phenyl-C61-butyric acid 2-hydroxyethyl methacrylate ester</td>
</tr>
<tr>
<td>PA</td>
<td>Phenyl acrylate</td>
</tr>
<tr>
<td>PDA</td>
<td>1,4-Phenyl diacrylate</td>
</tr>
<tr>
<td>PDAM</td>
<td>Pentaerythritol diacrylate monostearate</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>PEEK</td>
<td>Polyetheretherketone</td>
</tr>
<tr>
<td>PETA</td>
<td>Pentaerythritol tetraacrylate</td>
</tr>
<tr>
<td>PHDA</td>
<td>1,4-Phenylene diacrylate</td>
</tr>
<tr>
<td>PS-DVB</td>
<td>Poly(strene-co-divinylbenzene)</td>
</tr>
<tr>
<td>RAFT</td>
<td>Reversible addition-fragmentation chain transfer</td>
</tr>
<tr>
<td>RPLC</td>
<td>Reversed-phase liquid chromatography</td>
</tr>
<tr>
<td>S</td>
<td>Styrene</td>
</tr>
<tr>
<td>SMA</td>
<td>Stearyl methacrylate</td>
</tr>
<tr>
<td>TEPIC</td>
<td>Tris-(2,3-epoxypropyl) isocyanurate</td>
</tr>
<tr>
<td>TERP</td>
<td>Organotellurium-mediated living radical polymerization</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TMB</td>
<td>1,3,5-Trimethylbenzene</td>
</tr>
<tr>
<td>TMPTMA</td>
<td>Trimethylolpropane trimethacrylate</td>
</tr>
</tbody>
</table>
TPM  3-(Trimethoxysilyl)propyl methacrylate
VBC  Vinylbenzyl chloride
LIST OF TABLES

Table 1.1. Column efficiencies for separations of small molecules using organic monolithic columns. ................................................................................................................................. 18

Table 2.1. Compositions of selected monomers. ......................................................................................................................... 41

Table 2.2. Effect of methanol percentage in methanol / dodecanol solutions on column back pressure for a poly(1,12-DoDDMA) monolith. ........................................................................ 42

Table 2.3. Permeabilities of poly(alkanediol dimethacrylate) monolithic columns using different liquids. .......................... 54

Table 2.4. Retention times of uracil and alkylbenzenes showing column-to-column reproducibility of three independently prepared 1,6-HDDMA columns. .................. 55

Table 3.1. Compositions of reagent solutions. .................................................................................................................. 64

Table 3.2. Effect of dodecanol percentage of the total porogen solutions on column back pressure for a poly(CHDDMA) monolith. ........................................................................ 65

Table 3.3. Permeabilities of dimethacrylate/diacrylate monolithic columns for different liquids. ........................................................................................................ 75

Table 3.4. Retention times of uracil and alkylbenzenes showing column-to-column reproducibility of three independently prepared poly(1,6-HDDMA) columns. .......... 79

Table 4.1. Effect of different reagent compositions and capillary i.d. on column efficiency for poly(1,12-DoDDMA) monoliths. ........................................................................................................ 89

Table 4.2. Effect of different reagent compositions on column efficiency for poly(TMPTMA) monoliths. ............................................................................................................... 90

Table 4.3. Effect of different reagent compositions on column efficiency for poly(PETA) monoliths. ............................................................................................................... 91
Table 4.4. Permeabilities of poly(alkanediol multi-methacrylate/multi-acrylate) monolithic columns using different liquids. ................................................................. 100

Table 5.1. Hansen parameters for some solvents at 25 °C......................................................... 105

Table 5.2. Dispersion, polar and hydrogen bonding group contributions................................. 106
LIST OF FIGURES

Figure 1.1. Structures of monomers used to prepare RPLC monoliths included in Chapter 1. 6
Figure 2.1. Chemical structures of n-alkanediol dimethacrylate monomers. 38
Figure 2.2. SEM images of poly(1,12-DoDDMA) monoliths prepared with different percentages of methanol in methanol/dodecanol solution. 43
Figure 2.3. RPLC separations of alkylbenzenes on monoliths synthesized from 1,5-PDDMA, 1,6-HDDMA, 1,10-DDDMA, and 1,12-DoDDMA, respectively. 45
Figure 2.4. RPLC separations of alkylparabens on monoliths synthesized from 1,5-PDDMA, 1,6-HDDMA, 1,10-DDDMA, and 1,12-DoDDMA, respectively. 46
Figure 2.5. Separations of alkylbenzenes on 1,6-HDDMA monolithic column. 48
Figure 2.6. Plate height versus linear velocity for a 1,6-hexanediol dimethacrylate monolithic column using uracil as a non-retained compound. 49
Figure 2.7. Isocratic separations of alkylbenzenes on monoliths synthesized from (A) 1,3-BDDMA and (B) 1,6-HDDMA. 50
Figure 2.8. SEM images of monoliths. (A) poly(1,4-BDDMA), (B) poly(1,3-BDDMA), (C) poly(NPGDMA), (D) poly(1,5-PDDMA), (E) poly(1,6-HDDMA), (F) poly(1,10-DDDMA), (G) poly(1,12-DoDDMA). 52
Figure 2.9. Effect of mobile phase flow rate on column back pressure (A) poly(1,6-HDDMA) and (B) poly(1,12-DoDDMA). 53
Figure 3.1. Chemical structures of dimethacrylate/diacrylate monomers with different C6 functional groups. 61
Figure 3.2. SEM images of monoliths poly(1,6-HDDMA), poly(CHDDMA), and poly(PHDA). 68
Figure 3.3. RPLC separations of alkylbenzenes on monoliths synthesized from 1,6-HDDMA, CHDDMA, and PHDA, respectively. ......................................................... 69

Figure 3.4. Separations of alkylbenzenes on an 1,6-HDDMA monolithic column. .............. 70

Figure 3.5. Separations of alkylbenzenes using (A) 1,6-HDDMA, (B) CHDDMA, and (C) PHDA monolithic columns under isocratic elution conditions. ....................... 71

Figure 3.6. Plot of plate height (H) versus linear velocity for a 1,6-HDDMA monolithic column using toluene as a retained compound. ........................................... 72

Figure 3.7. Effect of mobile phase flow rate on column back pressure. ............................ 74

Figure 3.8. Chromatograms showing column-to-column reproducibility for 1,6-HDDMA monolithic columns using uracil and alkylbenzenes as analytes. ....................... 78

Figure 4.1. General reactions showing (A) mechanism of CRP, (B) thermal dissociation of TERP and (C) degenerative chain transfer mechanism of TERP. ................. 84

Figure 4.2. Chemical structures of multi-methacrylate/multi-acrylate monomers. ............... 86

Figure 4.3. SEM images of monoliths poly(1,12-DoDDMA) (D8), poly(TMPTMA) (T7), poly(PETA) (P6). .......................................................................................... 94

Figure 4.4. RPLC separations of alkylbenzenes on monoliths synthesized from PETA (P6), 1,12-DoDDMA (D8), and TMPTMA (T7), respectively. ....................... 96

Figure 4.5. Plate height versus linear velocity for a poly(TMPTMA) (T7) monolithic column using uracil as a non-retained compound. ........................................... 97

Figure 4.6. Effect of mobile phase flow rate on column back pressure. ............................ 99

Figure 5.1. Structures of monomer 1 for RPLC. ............................................................. 110

Figure 5.2. Structures of monomers 4 and 5. ............................................................... 112
CHAPTER 1 BACKGROUND AND SIGNIFICANCE

1.1 Introduction

Since the 1960s, liquid chromatography (LC) has shown remarkable and steady improvement in performance with advancements in small-particle-packed columns. Between 1975 and 2000, high performance LC columns were typically packed with 5 µm spherical particles in 4.6 mm i.d. stainless-steel tubes for a variety of chromatographic modes, including reversed-phase (RP), ion-exchange, hydrophilic interaction, hydrophobic interaction, chiral, and normal phase. A recent survey by Majors gave a comparison of the relative percentages of use of the different LC modes for the years between 1997 and 2011, during which the percentage of RPLC slowly decreased from 46 to 35%. While RPLC remains, by far, the most popular LC mode, and its use has actually increased over these years, the perception of a decrease has been caused by a greater relative increase in the use of some of the other modes, most notably hydrophilic interaction chromatography (HILIC).

Packed columns are still the dominant column type used in RPLC, with C₁₈ (octadecyilsilane) being the most common stationary phase. However, C₈, C₄, phenyl and cyano groups are also often used as RPLC functional groups for separations of both polar and nonpolar molecules in a variety of industries. Most packed RPLC columns are applied to separations of small molecules, although they also provide important selectivity for the separation of bio-macromolecules.

As the column efficiency can be greatly improved by decreasing the size of the spherical stationary phase particles, columns packed with sub-2 µm particles have recently become popular with the development of pumping systems that can approach 20,000 psi. Today,

efficiencies of columns packed with sub-2 μm fully porous particles and sub-3 μm core-shell particles can almost reach 300,000 plates/m. In a remarkable new development, Wei et al. recently reported an ultra-efficient colloidal crystal column packed with 470 nm silica particles that can provide efficiencies in the tens of millions of plates/m. This improved performance is attributed to the presence of slip flow in the column, which significantly enhances the volumetric flow rate and narrows the velocity distribution of the mobile phase (because of nonzero velocity at the column walls), thereby leading to extremely sharp peaks.

Packed columns, however, also have shortcomings. High pressures are required for both packing and operation, and retaining frits are needed. The inherent instability of silica particles (and silica monoliths) at extreme pH (> 8) continues to be a problem for the separation of many pharmaceuticals; therefore, new stationary phases with a wider pH stability range are needed. Furthermore, nonspecific adsorption of bio-macromolecules due to surface silanol groups has further decreased enthusiasm for the use of silica materials for their separation. Recently, researchers have improved the chemical stability of silica materials (broadened the pH range from 1 to 12) and reduced the nonspecific adsorption of analytes. However, the required lengthy modification procedures and reservations concerning long-term stability of these modified stationary phases continue to dampen enthusiasm for these columns.

Organic polymeric materials are important alternatives for use as LC stationary phases. Horváth et al. introduced poly(styrene-co-divinylbenzene) (PS-DVB) particles for LC. In 1988, nonporous PS-DVB particles were reported for the separation of proteins. Today, commercially available porous PS-DVB particle-packed columns are widely used in LC and can be exposed to denaturing agents and organic solvents. Their excellent chemical stability between pH 1 and 14 allows their use in many applications. Acrylate/methacrylate-based materials have also been
used to prepare polymeric beads for RPLC columns.

Approximately 20 years ago, polymeric monoliths were introduced by Hjertén,31 and Svec and Fréchet.32-34 Monoliths are continuous bed structures composed of highly interconnected pores through a polymeric skeletal structure. Compared to packed columns, monoliths are easier and faster to fabricate, do not require retaining frits, can exhibit improved analyte mass transfer (depending on the monolith morphology), have a lower pressure drop, and possess richer chemistry for providing broad selectivity. The lower pressure drop is attributed to the higher external porosity, which is between 60 and 90% for monolithic columns.35 In contrast, packed columns only provide external porosities from 40 to 65%.36, 37

It should be mentioned for completeness that silica-based monoliths were discovered at approximately the same time as organic polymer monoliths for LC.38-40 There are significant differences between the morphologies of silica-based and polymeric monoliths,20, 26, 41 which lead to quite different chromatographic properties. Silica monoliths have relatively high surface areas (200–300 m²/g), up to 648 m²/g,42 because of their mesopores. This large surface area leads to greater retention and better selectivity for low-molecular-weight compounds, similar to conventional porous particle packed columns. However, they also possess the same adsorptive properties and pH instabilities as their silica particle counterparts.

Organic monoliths are ideal media for the separation of large molecules, such as proteins,43 peptides,44 nucleic acids,45 and synthetic polymers,46 because of their good convective mass transfer and low surface area (usually 20–30 m²/g), the latter of which is a result of their low mesoporosity. Organic polymer monoliths typically exhibit poor performance for the separation of small molecules under typical chromatographic conditions. This has been attributed to the high gel porosity and low mesopore volume.47, 48 Gel porosity corresponds to micropores
within the solvated monolithic stationary phase. However, the existence of these micropores does not affect the separation of proteins and other large biomolecules, due to the small sizes of the micropores (< 2 nm). Only the mesopores (2–50 nm) and macropores (> 50 nm) contribute to bio-macromolecule separations. Recently, some reports have shown improved performance of organic monoliths for small molecule separations by modifying the synthesis conditions.48

Organic monoliths are generally synthesized by in situ polymerization within a capillary column. A variety of initiation methods has been reported for their polymerization, with thermal-33 or photo-initiation49 being the most popular. In addition, microwave irradiation50 and γ radiation51 have also been used. Recently, controlled/living polymerization methods, such as atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer (RAFT), were introduced to better control monolith synthesis.52, 53 These methods allow in situ control of the polymerization process, making it possible to increase homogeneity of the end result. Compared to traditional polymerization methods, living polymerization should produce monoliths with greater homogeneity, which should provide better chromatographic efficiency, because the polymerization rate and phase separation can be better controlled during synthesis.52, 54-56

The general fabrication process for monoliths involves filling of the capillary column with a homogenous polymerization mixture containing initiator, monomer(s), and porogen(s), followed by sealing both capillary ends and initiating the reaction by one of the methods mentioned in the previous paragraph. On completion of polymerization, the capillary is rinsed with a solvent to remove the unreacted monomers, porogens and any other soluble compounds from the pores. A mixture of monomers (i.e., functional monomer and cross-linking monomer) or a single monomer, serving both the functional and cross-linking roles, can be used to synthesize
a monolith. Single monomer systems have shown advantages over mixed monomer systems, i.e.,
easy optimization (easy porogen selection), higher surface area, better stability and better
reproducibility.\textsuperscript{57} However, single monomer systems might reduce the options for surface
modification, depending on the availability of cross-linking monomers that have functional
groups that can be subsequently derivatized.

Porogens are typically inert liquids used to create pores in the monolithic skeleton
during polymerization and phase separation. Porogens are generally selected based on experience
and experimentation, although certain criteria must be addressed. First, the porogens should
produce a homogenous pre-polymer solution with the monomer(s) and they should be
compatible with the initiation method used. Moreover, the solvent strengths of the porogens
should not be very different from each other, as this would have a detrimental effect on monolith
reproducibility. Recently, many studies have reported the use of solubility parameter values as
selection criteria for the porogens. Regardless of some progress made in the rational selection of
porogens, this aspect of monolith synthesis still requires extensive study.\textsuperscript{57-60}

In this chapter, the development of organic monoliths for RPLC is introduced.
Differences in monolith morphologies for the separations of small molecules \textit{versus}
bio-macromolecules are described, and applications are presented for illustration. Most
monomers used for preparation of organic polymer monoliths are either styrene- or
acrylate-based, although acrylamides and amines have also been reported (see Figure 1.1). Each
of these monolith types will be evaluated in the following sections.

1.2 Large-molecule separations

In the early 1990s, organic macroporous monoliths were introduced for the separation of
biomolecules.\textsuperscript{32, 61, 62} Since then, a number of monomers with different reactive groups, such as
Figure 1.1. Structures of monomers used to prepare RPLC monoliths included in Chapter 1.
Figure 1.1. (Continued)
Figure 1.1. (Continued).
vinyl, styryl, acryloyl, and methacryoyl groups, have been investigated for their fabrication.

1.2.1 Styrene-based monoliths

Since PS-DVB polymer beads had already been used for preparation of packed LC columns for decades, it was the earliest and most common material used for organic monolith LC column preparation. Its excellent chemical stability from pH 1 to 14 allows its use in many applications. Therefore, it is claimed that these columns are better than silica columns for separation of biomolecules.63-65

In 1993, Wang et al.34 reported a macroporous monolith with a globular structure prepared in a stainless-steel tube from a mixture of styrene and DVB. It was one of the earliest studies that showed the potential of monoliths for the separation of proteins and other biomolecules. The plate height for cytochrome c, a non-retained protein, was constant for mobile phase linear velocities between 350 to 2,500 cm/h, presumably because of convective mass transfer in the mobile phase. This monolithic column separated a mixture of three proteins (cytochrome c, myoglobin, and ovalbumin) using a gradient program, providing excellent resolution and sharp, narrow peaks.

Gu et al.66 reported the fabrication of a poly(styrene-octadecene-divinylbenzene) [poly(PS-OD-DVB)] monolithic column for the separation of proteins. A PS-DVB monolithic column with the same monomer composition was also prepared for comparison. The PS-OD-DVB monolith demonstrated a higher loading capacity and slightly greater retention of proteins due to the presence of C18 chains in the stationary phase. Compared to the PS-DVB monolith, the PS-OD-DVB monolith showed better apparent selectivity for α and β chains of human hemoglobin, which indicated its potential for difficult bio-macromolecule separations.

Bonn and co-workers44, 67 reported the preparation of a series of monoliths based on
1,2-bis(p-vinylphenyl)ethane (BVPE) as monomer. They synthesized BVPE from p-vinylbenzyl chloride using Grignard dimerization. Poly[methylstyrene-co-1,2-bis-(p-vinylphenyl)ethane] [poly(MST-BVPE)] monoliths prepared by the Bonn group were shown to be useful for the separation of large molecules such as proteins and peptides, as well as small molecules, because of their bimodal pore size distribution. Tetrahydrofuran (THF), dichloromethane and toluene were tested as microporogens. The resultant monoliths had different overall porosities. THF gave the smallest pore size and lowest permeability, while toluene resulted in monoliths with the highest macroporosity. There were no large differences in retention times of proteins separated on the different monoliths, which indicated that the monolith structure and morphology had little effect on large biomolecule separations. However, peak widths at half height and resolution significantly improved by increasing the monomer percentage from 35 to 39% v/v because the mean pore size decreased. In contrast, improvements in capacity, resolution, retention and efficiency were observed for phosphorylated oligothymidylic acids [d(pT)12-18] with a decrease in pore size.

Photo-initiated polymerization has been widely used in the synthesis of monoliths, since it is much faster than thermal-initiated polymerization, and the reaction can be stopped when the irradiation source is removed and the column is flushed with solvents. However, it has not been typically used for preparation of styrene-based monoliths, since styrene absorbs strongly at 254 nm. By adjusting the reaction conditions, many styrene-based monolithic materials have been polymerized even via photo-initiation. Walsh et al. initiated PS-DVB polymerization using a light-emitting diode array with 470 nm visible light, which was the first report of the synthesis of a PS-DVB monolith by photo-initiation. A mixture of (+)-(S)-camphorquinone (CQ), 1-ethyl-4-dimethylaminobenzoate (EDAB), and
\textit{N}-methoxy-4-phenylpyridinium tetrafluoroborate (MPPB) was used as photo-initiator. The resultant monolith showed good chromatographic performance for protein separations using gradient programming.

Flook et al.\textsuperscript{74} reported monoliths prepared from DVB by optimizing the UV irradiation. The column material and porogens were evaluated in this work. Cycloolefin copolymer (COC) tubing, which is stable at high pH, was chosen as a column material for bioanalytical applications. When monoliths obtained from photo-initiation and thermal-initiation were compared, it was observed that photo-initiation led to columns with a lower backpressure and better chromatographic performance for biomolecules.

Detobel et al.\textsuperscript{75} studied the effects of different morphologies and operating parameters of PS-DVB monoliths on peak capacity for intact proteins in gradient elution RPLC. Three PS-DVB monoliths of different lengths (i.e., 50, 100, and 250 mm) and with different macropore sizes, and a commercial capillary column packed with 5 \( \mu \)m porous silica beads were compared. The results showed that the peak capacities increased for all columns with increasing column temperature from 20 to 80\(^\circ\)C. The gradient window also played an important role in peak capacity. A slower gradient program and longer column length led to higher peak capacity, as expected. A maximum theoretical peak capacity of 330 was obtained with a 50-mm-long monolithic column, which increased to 760 when the column length was increased to 250 mm.

Another important parameter for improving peak capacity considered in this study\textsuperscript{75} was the monolith morphology. Two 50-mm-long monolithic columns with similar porosities but different pore sizes were compared within 120 min total analysis time. The column with the smaller domain size, which exhibited a higher column pressure, gave a higher peak capacity (i.e., approximately 450). In addition, this monolithic column provided a higher peak capacity
compared to a 5-μm-particle-packed silica column (approximately 175) of the same length (50 mm). This investigation provided a practical demonstration of the value of monolithic stationary phases and operating conditions in protein analysis.

LC–MS is widely used in applications involving complex mixtures of large biomolecules. RPLC using PS-DVB monolithic columns has often been employed because of its characteristic high recoveries in biological applications. Eeltink et al. analyzed protein digests using LC–MS/MS with PS-DVB monolithic columns of different column lengths (i.e. 50 mm, 250 mm, and 1 m). The 50-mm-long column gave a peak capacity up to 400. When the gradient duration was longer than 10 h, a peak capacity over 1000 could be obtained using a 1 m column. A study using a PS-DVB monolithic column coupled to TOF-MS to analyze protein isoforms was also reported by the same group. Protein isoforms were identified by differences in their oxidation and biotinylation states.

1.2.2 Acrylate/methacrylate-based monoliths

A variety of acrylate and methacrylate monoliths have been fabricated and employed for RPLC of biomolecules such as proteins and nucleotides. Ease of both polymerization and surface modification, as well as broad selectivity have been major reasons for their popularity. The major advantage of acrylates and methacrylates is their short polymerization time, as they can be easily polymerized using photo-initiation. This section summarizes the development and application of acrylate and methacrylate monoliths for RPLC of biomolecules.

One of the earliest applications of methacrylate monolithic columns for protein separation was reported by Lee et al. in 2004 using n-butyl methacrylate (BMA) as monomer and ethylene dimethacrylate (EDMA) as cross-linker. The resultant poly(BMA-EDMA) capillary columns were used for separating a mixture of four proteins (ribonuclease A, cytochrome c,
myoglobin, and ovalbumin) in less than 40 s using a single water/acetonitrile (ACN) step gradient. The separation was reported to be unaffected by the flow rate, thereby permitting faster separations without any loss of chromatographic performance, clearly showing the advantages of monoliths over particulate columns. The same proteins were separated with similar chromatographic performance on poly(butyl methacrylate-co-glycerol dimethacrylate) [poly(BMA-GDMA)] monoliths, thereby indicating that the hydrophobicity of the monolith was defined mainly by the functional monomer, and not by the chemical nature of the internal bridges.\(^{81}\) However, the composition of the cross-linking monomer was shown to influence small molecule separations, as indicated by Xu et al. (Section 1.3),\(^{82}\) and is expected to influence the separation of bio-macromolecules in certain cases, as is observed in other modes of LC.\(^{83}\) Monoliths fabricated in this study were intended for hydrophobic interaction LC, but were found to be suitable for RPLC because of the high monomer/porogen ratio. This increased the surface density of functional groups, making the monolith surface sufficiently hydrophobic to require RPLC conditions for protein elution.

Prium et al. studied the chromatographic performance of BMA-EDMA monoliths for isocratic and gradient elution of peptides. The peak capacity was found to be lower in comparison to particle-packed columns for isocratic elution because of the wider peak widths with increased retention. However, for gradient elution, the performance of the monoliths was found to be comparable to silica packed columns, since the peptides eluted with lower effective retention factors. The peak capacities for both stationary phases were reported to be 75 with steep gradients (15 min).\(^{84}\) Higher peak capacities could be obtained with BMA-EDMA stationary phases by using longer column lengths, but at the expense of time. Geiser et al. evaluated BMA-EDMA monoliths for stability and reproducibility in LC–MS and found
minimum shifts in retention time and backpressure for 2200 consecutive protein separations. The reproducibility was found to be excellent as evidenced by relative standard deviation values of less than 1.5% for retention times of proteins.

After the initial work with BMA-EDMA monoliths, there have been a number of studies to improve the chromatographic performance of acrylate and methacrylate monoliths. The factors governing performance, such as chemical nature of the functional monomer, cross-linker chain length and porogen composition have been extensively studied.

Umemura et al. fabricated poly(hexylmethacrylate-co-ethylene dimethacrylate) monoliths and used them for the separation of four proteins (ribonuclease A, cytochrome c, transferrin and ovalbumin), with a plate count of 3000 at a flow rate of 50 μL/min. The low pressure drop allowed the separation of the four proteins in 20 s at a flow rate of 1000 μL/min. In another study, Eeltink et al. prepared several alkyl methacrylate monoliths using monomers with different chain lengths, such as n-butyl, lauryl (LMA), and stearyl methacrylate (SMA), and the same cross-linker, EDMA, in order to investigate the effect of monomer polarity on chromatographic performance. Among the three types of monoliths studied, poly(LMA-EDMA) provided the best resolution, retention and peak shapes for peptide fragments. Both the BMA and SMA monoliths failed to separate peptides from non-retained compounds and, in addition, the peaks were broad on SMA monoliths. Prium et al. also reported a peak capacity of 125 for an LMA-EDMA monolith in contrast to 90 for a BMA-EDMA monolith of the same column length and with the same gradient. This better performance can be ascribed to the higher retention of analytes with increased alkyl chain length, as well as optimum monolith morphology (i.e., smaller globule and pore sizes).

Recently, Bisjak et al. reported the development of monoliths based on aromatic acrylate
precursors, phenyl acrylate (PA) and 1,4-phenyl diacrylate (PHDA), using thermally initiated free radical polymerization. They expected homogenous monolith morphology with improved chromatographic performance because the monomer and cross-linker had similar chemical structures. Baseline resolution ($R > 8.7$) was obtained for mixtures of proteins under gradient RPLC conditions at a flow rate of 9.4 µL/min. The low RSD values for retention time (0.7–1.6 %) and resolution (2.6–8.3 %) confirm a high batch-to-batch monolith reproducibility. The authors used the same monolith for ion-pair RPLC of oligodeoxynucleotides. The oligodeoxynucleotides were highly retained and separated with baseline resolution. In contrast, the aliphatic methacrylates reported previously were unable to separate oligonucleotides, which may be ascribed to a difference in morphology (porosity) of the two monolith types. The authors reported a plate count of 66,000 plates/m for oligonucleotide separations.

The effect of cross-linker chain length was investigated by Nordberg et al. for BMA monoliths. Four different cross-linkers, i.e., EDMA, diethyleneglycol dimethacrylate (DEGDMA), triethyleneglycol dimethacrylate (TEGDMA) and petaerythritol tetraacrylate (PETA) were studied. The tetracrylate cross-linker formed the densest skeleton (smallest pore size). The separation performance was found to be similar for all of the monoliths, indicating that the hydrophilic bridges had minimal influence on the retention behavior of the column. In contrast, Xu et al. reported the opposite. They analyzed SMA monoliths with dimethacrylate cross-linkers varying in chain length and chain structure (branching isomers). They reported an increase in the hydrophobicity (defined in terms of methylene selectivity) of the monolith with both branching and an increase in molecular chain length from 0.360 nm for EDMA to 1.241 nm for 1,9-nonanediol dimethacrylate. The skeletal and through pore sizes of the monoliths were unaffected by change in cross-linker chain length, however, monoliths with
2-methyl-1,8-octanediol dimethacrylate (2-Me-1,8-ODDMA) as cross-linker were found to have intrinsic porosities twice those for monoliths with EDMA cross-linker. The poly(SMA-2-Me-1,8-ODDMA) monolith showed the best chromatographic performance for a mixture of six model proteins in the RP mode, as well as good long term stability and reproducibility, with retention time RSDs from 0.3 to 0.7% for over 1500 injections. The discrepancy in observations may be because different monomers and porogens were used.

The studies described in Sections 1.2.1 and 1.2.2 represent some of the early attempts in monolith fabrication. Some of their limitations could be a result of the functional monomer used, the large fraction of functional groups buried inside the polymer, and the need for further optimization of each new monomer.\(^9^1\) In an effort to overcome such limitations, new strategies, such as postmodification,\(^9^2\) grafting\(^9^3,^9^4\) and attachment of nanoparticles,\(^9^5\) have been studied. Interestingly, in the 1970s, polymeric nanoparticles were used to modify the surfaces of particle-packed columns for ion-exchange chromatography. Recently, monoliths have been modified with a variety of nanoparticles such as latex,\(^9^6,^9^7\) titanium dioxide and zirconium dioxide powders,\(^9^8\) hydroxyapatite\(^9^9\) and gold.\(^1^0^0\)

Svec and co-workers recently described monoliths coated with gold nanoparticles (GNP) for RPLC of proteins.\(^1^0^0,^1^0^1\) In one of their most recent publications, monoliths containing thiol groups were prepared by reaction of a poly(glycidyl methacrylate-co-ethylene dimethacrylate) [poly(GMA-EDMA)] monolith with cystamine followed by reduction of the disulfide bond, which led to the dramatically enhanced immobilization of GNP and good separations of alkanethiols.\(^9^1\) They compared the chromatographic performance of GMA-EDMA monoliths with and without nanoparticles, and found that the nanoparticles provided significantly better separation of proteins. Moreover, the labile nature of thiol–gold chemistry allows these bound
nanoparticles to be “universal” intermediate ligands that enable changes in monolith surface chemistry via the attachment of thiol-containing compounds with the desired functionality.101

1.3 Small-molecule separations

Organic monoliths have been shown to be useful media for the separation of large molecules; however, their performance for small-molecule separations to date has been much worse than that obtained by using packed and silica monolithic columns. Therefore, much effort has been spent in trying to improve the performance of organic monoliths for the separation of small molecules (see Table 1.1 for representative examples).

1.3.1 Styrene-based monoliths

In 1994, Wang et al. reported the synthesis of PS-DVB monoliths and studied their performance using alkylbenzenes as analytes.102 The resultant columns produced a plate count of 13,500 plates/m for benzene, which was much worse than could be realized using particle-packed and silica monolithic columns. Adding other co-monomers to the polymerization system was suggested to improve their chromatographic performance. Monoliths obtained from DVB, ethylvinylbenzene (EVB) and hydroxyethyl methacrylate (HEMA),103 and styrene, DVB and methacrylic acid (MAA)104 were shown to separate small molecules with chromatographic efficiencies between 20,000 and 28,000 plates/m. This improvement in efficiency was believed to result from changes in monolith structure. However, the resultant efficiency was still far inferior to the performance of silica monoliths.

Recently, Hasegawa et al.105 reported the preparation of PS-DVB and PDVB monoliths using organotellurium-mediated living radical polymerization. The monomers (DVB with and without styrene) and template porogen, polydimethylsiloxane (PDMS), were dissolved in 1,3,5-trimethylbenzene (TMB). Ethyl-2-methyl-2-butyltellanyl propionate (BTEE) was added as
Table 1.1. Column efficiencies for separations of small molecules using organic monolithic columns.

<table>
<thead>
<tr>
<th>Monolith</th>
<th>Porogen(s)</th>
<th>Efficiency (plates/m)</th>
<th>Analyte(^a)</th>
<th>Polymerization method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS-DVB</td>
<td>dodecyl alcohol, toluene</td>
<td>13,500</td>
<td>benzene</td>
<td>Thermal-initiated free radical</td>
<td>102</td>
</tr>
<tr>
<td>PVB-EVB-HEMA</td>
<td>dodecanol</td>
<td>20,000</td>
<td>uracil</td>
<td>Thermal-initiated free radical</td>
<td>103</td>
</tr>
<tr>
<td>PS-DVB-MAA</td>
<td>toluene, isooctane</td>
<td>28,000</td>
<td>benzene</td>
<td>Thermal-initiated free radical</td>
<td>104</td>
</tr>
<tr>
<td>PDVB</td>
<td>PDMS, TMB</td>
<td>34,000</td>
<td>benzene</td>
<td>TERP</td>
<td>105</td>
</tr>
<tr>
<td>PS-DVB</td>
<td>dodecanol, toluene</td>
<td>66,000</td>
<td>n-buty/benzene</td>
<td>Thermal-initiated free radical</td>
<td>48</td>
</tr>
<tr>
<td>MS-BVPE</td>
<td>decanol, toluene</td>
<td>72,000</td>
<td>butyrophenone</td>
<td>Thermal-initiated free radical</td>
<td>108</td>
</tr>
<tr>
<td>PS-DVB-VBC</td>
<td>dodecanol, toluene</td>
<td>73,000</td>
<td>uracil</td>
<td>Thermal-initiated free radical</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>benzene</td>
<td>followed with hypercross-linking</td>
<td></td>
</tr>
<tr>
<td>BMA-EDMA</td>
<td>1-propanol, 1,4-butanediol, water</td>
<td>37,000</td>
<td>benzene</td>
<td>Thermal-initiated free radical</td>
<td>112</td>
</tr>
<tr>
<td>BMA-EDMA</td>
<td>1-propanol, 1,4-butanediol, water</td>
<td>34,000</td>
<td>benzene</td>
<td>Thermal-initiated free radical</td>
<td>113</td>
</tr>
<tr>
<td>BMA-EDMA</td>
<td>1-propanol, 1,4-butanediol, water</td>
<td>47,000</td>
<td>benzene</td>
<td>Photo-initiated free radical</td>
<td>114</td>
</tr>
<tr>
<td>BMA-EDMA</td>
<td>decanol, cyclohexanol</td>
<td>45,000</td>
<td>toluene</td>
<td>Photo-initiated free radical</td>
<td>115</td>
</tr>
<tr>
<td>BMA-EDMA</td>
<td>decanol</td>
<td>59,000</td>
<td>naphthalene</td>
<td>Photo-initiated free radical</td>
<td>116</td>
</tr>
<tr>
<td>BMA-EDMA</td>
<td>1-propanol, 1,4-butanediol</td>
<td>67,000</td>
<td>uracil</td>
<td>Thermal-initiated free radical</td>
<td>117</td>
</tr>
<tr>
<td>LMA-EDMA</td>
<td>1-propanol, 1,4-butanediol</td>
<td>53,000(^b)</td>
<td>amylbenzene</td>
<td>Thermal-initiated free radical</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20,000(^c)</td>
<td>amylbenzene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EHMA-EDMA</td>
<td>1-propanol, 1,4-butanediol</td>
<td>33,000</td>
<td>benzene</td>
<td>RAFT</td>
<td>53</td>
</tr>
<tr>
<td>SMA-2-Me-1,8-ODDMA</td>
<td>tert.-butanol, 1,4-butanediol</td>
<td>52,000</td>
<td>n-buty/benzene</td>
<td>Thermal-initiated free radical</td>
<td>82</td>
</tr>
<tr>
<td>BADMA</td>
<td>DMF, dodecanol</td>
<td>61,000</td>
<td>n-buty/benzene</td>
<td>Photo-initiated free radical</td>
<td>57</td>
</tr>
<tr>
<td>1,10-DDDMA</td>
<td>methanol, dodecanol</td>
<td>53,000</td>
<td>toluene</td>
<td>Photo-initiated free radical</td>
<td>123</td>
</tr>
<tr>
<td>GDMA</td>
<td>monodisperse PS, chlorobenzene</td>
<td>34,000</td>
<td>benzene</td>
<td>Thermal-initiated free radical</td>
<td>125</td>
</tr>
<tr>
<td>GMA-EDMA-MWNT</td>
<td>cyclohexanol, 1-dodecanol</td>
<td>44,000</td>
<td>benzene</td>
<td>Thermal-initiated free radical</td>
<td>126</td>
</tr>
<tr>
<td>GMA-EDMA-PCB-HEM</td>
<td>1-propanol, 1,4-butanediol</td>
<td>120,000</td>
<td>benzene</td>
<td>Thermal-initiated free radical</td>
<td>127</td>
</tr>
<tr>
<td>IPA-MA-BAP</td>
<td>50 mM sodium phosphate buffer (pH=7)</td>
<td>75,000</td>
<td>benzene</td>
<td>Thermal-initiated free radical</td>
<td>129</td>
</tr>
<tr>
<td>BACM-TEPIC</td>
<td>PEG200</td>
<td>180,000</td>
<td>benzene</td>
<td>Thermal-initiated free radical</td>
<td>130</td>
</tr>
</tbody>
</table>

\(^a\)Uracil was considered to be a non-retained; benzene, toluene, n-buty/benzene, amylbenzene, n-hexylbenzene, and naphthalene were retained compounds.

\(^b\)In Silcosteel tubing.

\(^c\)In PEEK tubing.
a polymerization promoter, which provided better control over the monolith morphology. The effects of monomer and PDMS concentrations were investigated to optimize the monolith structures. The results showed that higher efficiencies could be obtained by decreasing the amounts of PDMS and styrene. The amount of BTEE was also adjusted to obtain a well-defined continuous structure. Theoretical plate numbers of 34,000 plates/m for the least retained compound (benzene) and 27,000 plates/m for the most retained compound (hexylbenzene) were obtained using a PDVB monolithic column. Although these efficiencies were not as good as those of PS-DVB monoliths obtained in recent years from other polymerization methods, this was the first time that PS-DVB monoliths prepared from controlled/living radical polymerization (CRP) were shown to separate small molecules.

Nischang et al.\textsuperscript{48} reported the effect of polymerization time (3 and 48 h) on the performance of PS-DVB monoliths. The DVB cross-linker polymerized faster than the monovinyl monomer (i.e., styrene). SEM images demonstrated a fine porous structure with 90% porosity for monoliths polymerized for only 3 h. The total volume of pores between 2 and 100 nm diameter was approximately 0.2 mL/g, which supported the existence of nanopores in the globules. The surface area for this pore volume was 100 m\textsuperscript{2}/g. In comparison, 48 h of polymerization time resulted in a macroporous monolith, for which the surface area was only 5–6 m\textsuperscript{2}/g. Monoliths obtained after 3 h polymerization showed significantly better separations of alkylbenzenes (i.e., 66,000 plates/m for butylbenzene).

Bonn and co-workers\textsuperscript{106-108} reported the preparation of a series of monoliths based on BVPE as a single monomer or copolymerized with MST. An investigation of the effect of polymerization time indicated that the pore size distribution switched from a monomodal distribution to a bimodal distribution, and the monolith increased in porosity with decreasing
polymerization time from 300 to 60 min. The bimodal pore size distribution allowed these monoliths to separate both small and large molecules.

A similar result was also observed for an MST-BVPE monolith. Changes in morphology and pore size distribution of these monoliths resulted in a significant improvement in their chromatographic performance for small molecule separations, whereas there was no significant difference observed in the efficiency for biomolecules. An efficiency of 65,000 plates/m was obtained for butylbenzene for an MST-BVPE monolith polymerized for 45 min. BVPE monoliths polymerized for 60 min, which had a surface area of 102 m²/g and a plate count of 72,100 plates/m for butyrophenone, were successfully applied to the separation of a variety of small molecules (i.e., phenols, phenones, parabens, and β-blockers).

Urban et al. recently reported a hypercross-linked monolith prepared from styrene, vinylbenzyl chloride (VBC), and DVB via Friedel–Crafts alkylation. After hypercross-linking, the monolith possessed more than one order of magnitude increase in surface area compared to a similar monolith without hypercross-linking. A chromatographic efficiency of 73,000 plates/m for uracil (non-retained compound) was demonstrated using the hypercross-linked monolith. This monolith was not particularly useful for the separation of large molecules due to the presence of small mesopores. The effects of different reaction conditions, including composition of reagents (i.e., DVB, VBC and styrene), polymerization time and temperature were investigated in detail. After optimization, the surface areas of the resultant hypercross-linked monoliths reached 631 m²/g, and efficiencies reached 83,200 plates/m for benzene when operated at 80°C with low sample loading. In addition, the hypercross-linked column was coupled with NMR spectroscopy to demonstrate the fast separation of small molecules.
1.3.2 Acrylate/methacrylate-based monoliths

A wide variety of acrylate- and methacrylate-based monomers have been used to produce monoliths for RPLC of both small and large molecules. Coufal et al.\textsuperscript{112} and Moravcová et al.\textsuperscript{113} reported monolithic columns copolymerized from BMA and EDMA in 320 μm i.d. polyimide-clad fused-silica capillaries. The measured column efficiencies were between 30,000 and 40,000 plates/m for alkylbenzenes.

Polymerization at different temperatures was investigated by Szumski et al.\textsuperscript{114} in which monolithic columns were prepared from BMA and EDMA over the temperature range of −15 to 75°C using UV initiation. This study showed that both column efficiency and permeability decreased with increasing polymerization temperature. The best efficiency (approximately 47,000 plates/m) was obtained from a column prepared at −15°C. The alkylbenzenes were baseline separated under isocratic conditions using columns prepared at subzero temperatures. A similar study of the effects of both irradiation intensity (0.4 to 2.0 mW/cm\textsuperscript{2}) and temperature (0 to 20°C) was conducted by Hirano et al.\textsuperscript{115} using BMA and EDMA. Higher UV intensity and decreasing polymerization temperature led to columns with better efficiencies. The best performance (45,000 plates/m) was achieved with a column prepared using 2.0 mW/cm\textsuperscript{2} for 8 min at 0°C, which provided baseline separation of a mixture of alkylbenzenes in 1 min.

Another interesting study was carried out by synthesizing monoliths from BMA and EDMA in an oxidized titanium tube under a gradient temperature program from 20 to 55°C.\textsuperscript{116} Since polymerization could not start below 50°C, the columns were heated gradually at very slow heating rates (0.5°C/min from 20 to 50°C and 0.02°C/min from 50 to 55°C) to provide consistent equilibration and resulting uniform morphology. The best efficiency was 59,000 plates/m for naphthalene. Nischang et al.\textsuperscript{117} reported that the performance of monolithic columns
made from BMA and EDMA was improved by reducing the polymerization time from 48 h to 30 min with higher methylene selectivity. According to their results, the surface areas of the monoliths decreased with increasing polymerization time. A plate count of 67,000 plates/m for a non-retained compound (uracil) was reported.

Monoliths prepared from alkylmethacrylates other than BMA have also been reported. Methacrylates with different functional groups (i.e., C₂, C₆, C₈, C₁₀, C₁₂ and C₁₈) were copolymerized with EDMA. Theoretical plate numbers of these columns varied between 30,000 to 67,000 plates/m. Shu et al. synthesized monolithic columns from LMA and EDMA in polyetheretherketone (PEEK) and silcosteel tubes with similar methacryloylated inner walls. The monolith prepared in silcosteel gave a better chromatographic efficiency (approximately 53,000 plates/m) for amylbenzene. Although monoliths formed in PEEK tubing gave only 20,000 plates/m, the larger diameter PEEK tubing (1 mm i.d., 1/16” o.d.) facilitated connection of the column to conventional LC systems.

Turson et al. prepared monoliths from ethylhexyl methacrylate (EHMA) and EDMA via RAFT polymerization. Dibenzyltrithiocarbonate (DBTTC) was used as a chain transfer agent (CTA) in the living polymerization reaction. The effects of monomer/initiator/CTA ratio and polymerization temperature were investigated. Carefully adjusting the amount of CTA and initiator concentration within a certain range resulted in a slower reaction rate, which improved the column efficiency. In addition, a higher polymerization temperature also led to better column efficiency. This is in contrast to traditional polymerization methods in which worse efficiency is usually obtained by increasing the polymerization temperature. An efficiency of 33,000 plates/m was obtained with a monomer/initiator/CTA ratio of 300:1:6 at 65°C. After polymerization, a hydrophilic layer of poly(glycerol monomethacrylate) [poly(GMMA)] was grafted onto the
monolith surface through post-RAFT reaction. The chromatographic performances of columns with and without the poly(GMMA) surface were compared using BSA and benzene as test analytes. BSA eluted in the void volume with reduced peak tailing, while benzene was retained, demonstrating that the hydrophilic poly(GMMA) layer provided protein exclusion while still providing retention and separation of small hydrophobic compounds. This property is desirable for drug analysis in biological samples.

So far in this chapter, monoliths obtained from monomers containing different alkyl groups have been considered. The work by Xu et al., described in the section on separation of large molecules, also included an evaluation of the effects of different cross-linkers on the separation of small molecules. Compared to monoliths synthesized with EDMA as cross-linker, monoliths containing 2-Me-1,8-ODDMA gave higher chromatographic efficiency (i.e., 52,000 plates/m) for butylbenzene and higher overall selectivity for small molecules.

Recently, we have been investigating monoliths prepared using single monomers, which have traditionally been used as cross-linkers. Good separations of alkylbenzenes and alkylparabens were obtained due to the high surface areas produced from the highly cross-linked structures. Bisphenol A dimethacrylate (BADMA), bisphenol A ethoxylate diacrylate (BAEDA) with an EO/phenol ratio of 2 or 4, and pentaerythritol diacrylate monostearate (PDAM) were used to synthesize monoliths for RPLC of small molecules. BADMA columns gave chromatographic efficiencies as high as 61,000 plates/m for butylbenzene.

Monoliths based on 1,3-butandiol dimethacrylate (1,3-BDDMA), 1,4-butandiol dimethacrylate (1,4-BDDMA), 1,5-pentanediol dimethacrylate (1,5-PDDMA), neopentyl glycol dimethacrylate (NPGDMA), 1,6-hexanediol dimethacrylate (1,6-HDDMA), 1,10-decanediol dimethacrylate (1,10-DDDMA), and 1,12-dodecanediol dimethacrylate as single monomers
demonstrated that better selectivity could be obtained by increasing the chain length between the two dimethacrylate end groups. Monoliths prepared from 1,10-DDDMA exhibited chromatographic efficiencies up to 53,000 plates/m for toluene. Two monomer pairs (i.e., 1,3-BDDMA and 1,4-BDDMA, and NPGDMA and 1,5-PDDMA) were used to compare monoliths containing branching and nonbranching alkyl groups of the same carbon number. Monoliths with branching group(s) in the alkyl bridge between the two dimethacrylate groups gave higher efficiencies than their corresponding linear isomeric polymers. Recently, we improved the efficiency of 1,6-HDDMA monoliths to 86,000 plates/m for toluene by improving the homogeneity of the monolithic structure.

Glycerol dimethacrylate has been a relatively popular monomer for the preparation of monoliths for RPLC. Aoki et al. used GDMA as a single monomer and monodisperse polystyrene (PS) of different molecular weights (MW) as template porogens for preparation of monoliths. The results indicated that the morphologies of monoliths prepared with ultrahigh MW PS became more fused (and less globular) compared to those prepared with low MW PS. A bimodal pore size distribution was observed. The measured column efficiency was 34,000 plates/m.

Chambers et al. used multi-walled carbon nanotubes (MWNT) in the synthesis of GMA-EDMA monoliths to modify the monolith surface area; the pore size of the monolith was reduced to 0.47 μm. A plate count of 44,000 plates/m was achieved for benzene when THF was added to the mobile phase to improve the chromatographic performance. In another study, they improved the performance of GMA-EDMA monoliths (i.e., 85,000 plates/m for benzene) by adding 1 wt% C60-fullerene containing [6,6]-phenyl-C61-butyric acid 2-hydroxyethyl methacrylate ester (PCB-HEM) monomer. A high efficiency for organic polymer-based
monolithic columns (i.e., 120,000 plates/m) was achieved for benzene at the optimum mobile phase linear velocity of 0.33 mm/s.\textsuperscript{127}

1.3.3 Acrylamide monoliths

Acrylamides are typically used for preparing monoliths for HILIC,\textsuperscript{128} however, they can also be used for RPLC. Maruska et al.\textsuperscript{129} reported monoliths prepared from \textit{N}-isopropylacrylamide (IPA), methacrylamide (MA), and 1,4-bis(acryloyl)piperazine (BAP), with ammonium sulfate dissolved in 50 mM sodium phosphate buffer for capillary LC and CEC. After filling part of the capillary column with a mixture of polymerization reagents, a buffer solution containing initiator and salt was introduced into the capillary to form a gradient monolith. Various gradient monoliths prepared in this manner were evaluated with methanol/water mobile phase and alkylbenzenes as test compounds. Those with a smoother gradient provided the best performance. The chromatographic efficiency reached 75,000 plates/m for benzene under pressure-driven LC conditions.

1.3.4 Amine monoliths

Monoliths prepared from 4-[(4-aminocyclohexyl)methyl]cyclohexylamine (BACM) and tris(2,3-epoxypropyl) isocyanurate (TEPIC) were reported by Hosoya et al.\textsuperscript{130} Alkylbenzenes were used as test compounds under isocratic conditions. The morphologies and separation performances of two columns of different lengths were compared. The SEM images illustrated that the shorter column (TEPIC-BACM-S), which was 18.2 cm long, had a small average domain size, while the longer column (TEPIC-BACM-L, 150.5 cm) had a rather large domain size. The TEPIC-BACM-L and TEPIC-BACM-S columns gave 180,000 (an exceptionally high efficiency for organic polymer monoliths) and 40,000 plates/m for benzene, respectively; however, there was no clear explanation of the reason for the different domain sizes and
efficiencies. The difference in efficiency could be due to the better homogeneity of the longer monolithic column morphology, which was observed in the SEM images.

1.4 Dissertation Overview

My research focused on the preparation of polymeric monolithic capillaries for RP of small molecules. Chapter 2 reports a group of highly cross-linked monolithic capillary columns synthesized from 1,3-BDDMA, 1,4-BDDMA, NPGDMA, 1,5-PDDMA, 1,6-HDDMA, 1,10-DDDMA, and 1,12-DoDDMA, in 75-µm i.d. capillaries for RPLC of small molecules. Gradient elution of alkylbenzenes and alkylparabens provided high resolution separations. Optimized monoliths synthesized from all seven cross-linking monomers showed high permeability and reproducibility. Chapter 3 describes three monolithic capillary columns for RPLC of small molecules prepared from single monomers, (i.e., 1,6-HDDMA, CHDDMA and PHDA). Selection of porogen type and concentration was investigated in detail. Isocratic elution of alkylbenzenes was performed using 1,6-HDDMA and CHDDMA monolithic columns. Gradient elution of alkylbenzenes using all three monolithic columns showed good separations. Monolithic columns formed from HDDMA, which had a linear alkyl-bridging chain structure, exhibited the highest column efficiencies (86,000 plates/m). In Chapter 4, monolithic columns were developed from three cross-linking monomers [i.e., 1,12-DoDDMA], trimethylolpropane trimethacrylate (TMPTMA) and pentaerythritol tetraacrylate (PETA)] using organotellurium-mediated living radical polymerization (TERP) in 150-µm i.d. capillaries for RPLC of small molecules. Selection of polymerization conditions, such as monomer ratio, porogen ratio, initiator ratio and capillary inner diameter, for the 1,12-DoDDMA monolith was investigated in detail. Isocratic elution of alkylbenzenes was achieved with good efficiency using all monolithic columns. Chapter 5 presents some proposed future directions in selection of
porogens and polymerization methods for polymer monolith preparation, and synthesis procedures for potential single cross-linking monomers, which could be used for producing polymeric monoliths for RPLC.

1.5 References


42. Ma, X.; Sun, H.; Yu, P. *J. Mater. Sci.* **2008**, *43*, 887-891.


CHAPTER 2 HIGHLY CROSS-LINKED POLYMERIC MONOLITHS FOR REVERSED-PHASE CAPILLARY LIQUID CHROMATOGRAPHY OF SMALL MOLECULES*

2.1 Introduction

Monolithic stationary phases for liquid chromatography (LC) were introduced in the late 1980s and early 1990s\(^1\)-\(^4\) with promise of overcoming some limitations of conventional packed columns.\(^5\) Monoliths are often called continuous porous beds, continuous polymer rods or continuous column supports.\(^1\) Compared to packed columns, monoliths are easy and fast to fabricate, and do not require frits, have low back pressure and can operate at high flow rates. Rapid separations can be realized due to their high permeability. In addition, their skeletal structures can be optimized and controlled during the preparation process,\(^6,7\) because the through-pores are not strictly dependent on spherical particle packing geometries, as is the case for packed columns. The attractive advantages of monolithic columns have been described in many excellent reviews.\(^5,10\)

Inorganic (silica) monolithic columns were introduced in 1996 using a sol-gel process,\(^11,12\) and are characterized by a bimodal pore size distribution. Large through-pores allow them to be used with high flow rates and low back pressure. The smaller pores provide high surface area. This helps to improve the resolution of small molecules. However, separations of high molecular weight compounds, such as proteins, are limited by the low number of small macropores (50-100 nm).\(^10,13\)

Monolithic stationary phases can also be synthesized from organic monomers. Most monoliths prepared from these monomers have been used for the separation of peptides and proteins.\(^14,15\) However, such polymeric materials also exhibit several disadvantages. Compared

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to inorganic monoliths, organic polymeric monoliths generally suffer from significantly lower chromatographic efficiencies for low-molecular weight compounds. Organic monoliths are usually suitable for the separation of high molecular weight compounds due to their monomodal macropore-distribution. In addition, organic polymeric monoliths can swell or shrink with organic solvents in the mobile phase, leading to reduced chromatographic performance and poor mechanical stability. Recently, several publications have reported the separation of small molecules with organic monoliths. However, most applications still focus on high-molecular-weight compounds.

A conventional polymerization system for monolith preparation includes initiator, functional monomer, cross-linking monomer and porogen or porogen mixture. It has been reported that higher cross-linker concentration can provide higher mechanical stability and higher surface area. In this chapter, a group of highly cross-linked polymeric monolithic stationary phases prepared from single alkanediol methacrylate based monomers are introduced. The structures of these monomers are shown in Figure 2.1. The morphologies and separation performances of these monoliths were studied. These monoliths were successfully used for separation of low-molecular-weight compounds, such as alkylbenzenes and alkylparabens.

2.2 Experimental

2.2.1 Chemicals and reagents

2,2-Dimethoxy-2-phenylacetophenone (DMPA, 99%) and 3-(trimethoxysilyl)propyl methacrylate (TPM, 98%) were purchased from Sigma–Aldrich (St Louis, MO, USA); 1,5-pentanediol dimethacrylate (1,5-PDDMA) and 1,10-decanediol dimethacrylate (1,10-DDDMA) (see Figure 2.1) were purchased from Polysciences (Warrington, PA, USA); and 1,3-butanediol dimethacrylate (1,3-BDDMA), 1,4-butanediol dimethacrylate (1,4-BDDMA),
neopentyl glycol dimethacrylate (NPGDMA), 1,6-hexanediol dimethacrylate (1,6-HDDMA) and 1,12-dodecanediol dimethacrylate (1,12-DoDDMA) (see Figure 2.1) were gifts from Sartomer (Exton, PA, USA). Water, methanol, decanol, dodecanol, propylbenzene, butylbenzene, amylbenzene and uracil were also obtained from Sigma–Aldrich; acetonitrile (ACN), iso-butanol, and ethylbenzene were purchased from Fisher Scientific (Pittsburgh, PA, USA); toluene was purchased from Mallinckrodt (Phillipsburg, NJ, USA); tetrahydrofuran (THF) was purchased from Curtin Matheson Scientific (Houston, TX, USA); and methyl paraben, ethyl paraben, propyl paraben and butyl paraben were purchased from Fluka (Buchs, Switzerland). All porogenic solvents and chemicals for monolith and mobile phase buffer preparations were HPLC or analytical reagent grade, and were used as received. Buffer solutions were prepared with HPLC water and filtered through a 0.22-µm membrane filter.

2.2.2 Fused silica capillary pretreatment

First, UV-transparent fused silica capillary tubing (75-µm i.d., 375-µm o.d., Polymicro Technologies, Phoenix, AZ, USA) was treated with TPM in order to anchor the polymer to the capillary wall. The treatment procedures were reported by Vidič et al.\textsuperscript{28} and Coutios et al.\textsuperscript{29} The capillary was connected to a syringe pump for washing with ethanol and water for 30 min, respectively. The inner surface of the capillary tubing was treated with 2 M HCl solution, and placed in a GC oven at 110 °C for 3 h. The tubing was rinsed with water and ethanol and dried at 110 °C with a flow of nitrogen gas overnight. Then the capillary tubing was filled with 15% TPM/toluene (wt/wt) solution and placed in a GC oven at 35 °C overnight. Finally, the tubing was washed with toluene and acetone, successively, and dried with nitrogen gas at room temperature.
Figure 2.1. Chemical structures of n-alkanediol dimethacrylate monomers.
2.2.3 Polymeric monolith preparation

Monomer solutions were prepared in 1-dram (4 mL) glass vials by admixing initiator, monomer, and porogen solvents (see Table 2.1 for reagent compositions). Each solution was vortexed and then degassed by sonication for a few seconds to avoid excessive evaporation of methanol. Then, the reaction mixture was introduced into one end of the silanized capillary by capillary action. The other end of the capillary was left empty for UV detection. After filling with solution, the capillary was sealed with rubber septa at both ends and placed directly under a PRX 1000-20 exposure unit UV lamp (390±15 nm, 1000 W, TAMARACK Scientific, Corona, CA, USA). Monoliths obtained after exposing with UV light from 1 to 6 min were flushed with methanol and then water until stable pressure readings were obtained. Similar back pressures (per unit column length) and morphology (based on microscope images) were observed when the polymerization time was longer than 3 min. Therefore, a polymerization time of 3.5 min was selected for all monoliths. After a monolithic column was prepared, it was then flushed with methanol and water sequentially using an HPLC pump to remove porogens and possible unreacted residual monomers. The monolithic columns were characterized by scanning electron microscopy using an FEI Philips XL30 ESEM FEG (Hillsboro, OR, USA) without coating with a conductive gold layer.

2.2.4 Capillary liquid chromatography

An Eksigent Nano 2D LC system (Dublin, CA, USA) was used for the chromatographic experiments. The injection volumes were 20 nL for alkylbenzenes and 30 nL for alkylparabens. The two mobile phase components for elution of alkylbenzenes in RPLC were water (mobile phase A) and acetonitrile (mobile phase B). On-column detection was performed using a Crystal 100 variable wavelength UV–Vis absorbance detector (Thermo Separation Products, MA, USA).
Chrom Perfect software (Mountain View, CA, USA) was used for data collection and treatment. UV absorbance was monitored at 214 nm.

2.3 Results and Discussion

2.3.1 Selection of porogens

The selection of porogenic solvent or solvent combination is an important step in the preparation of monoliths. One of the monomers, 1,12-DoDDMA, was chosen for detailed study of porogen selection. Several solvents with different polarities were used to synthesize the monoliths. It was found that 1,12-DoDDMA formed a monolith when dissolved in methanol and iso-butanol after UV light initiation. A soft or hard transparent gel was obtained with polymerization using toluene, THF, or ACN, indicating that these were potentially “good” solvents for 1,12-DoDDMA. Rigid macroporous monoliths were found when methanol and iso-butanol were combined with decanol or dodecanol. Toluene, THF, and ACN still resulted in gels when combined with decanol and dodecanol. Although 1,12-DoDDMA could form monoliths with decanol, the monoliths gave very poor chromatographic performance. When iso-butanol was combined with dodecanol, the final monolith gave very high back pressure (over 3000 psi at a mobile phase flow rate of 100 nL/min). Therefore, a combination of methanol and dodecanol appeared to be the best porogen system for the 1,12-DoDDMA monolith. The ratio of monomer to total porogens was investigated and the final ratio was set at 31.3:68.7. Table 2.2 shows the effect of methanol (poor solvent) to dodecanol (good solvent) ratio on back pressure in forming rigid monoliths from 1,12-DoDDMA. Figure 2.2 shows SEM images of these monoliths, which indicate that the pore size becomes larger with an increase in methanol to dodecanol ratio.

2.3.2 Separation of small molecules

I obtained rigid structural monoliths using all of the monomers. All could be used to
**Table 2.1.** Compositions of selected monomers.

<table>
<thead>
<tr>
<th>Monolith</th>
<th>Monomer</th>
<th>Methanol (g/wt %)</th>
<th>Dodecanol (g/wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,3-BDDMA</td>
<td>0.36/31.86</td>
<td>0.46/40.71</td>
<td>0.31/27.43</td>
</tr>
<tr>
<td>1,4-BDDMA</td>
<td>0.36/32.14</td>
<td>0.38/33.93</td>
<td>0.38/33.93</td>
</tr>
<tr>
<td>NPGDMA</td>
<td>0.36/31.86</td>
<td>0.31/27.43</td>
<td>0.46/40.71</td>
</tr>
<tr>
<td>1,5-PDDMA</td>
<td>0.36/32.43</td>
<td>0.34/30.63</td>
<td>0.41/36.94</td>
</tr>
<tr>
<td>1,6-HDDMA</td>
<td>0.36/31.86</td>
<td>0.52/46.02</td>
<td>0.25/22.12</td>
</tr>
<tr>
<td>1,10-DDDMA</td>
<td>0.36/31.58</td>
<td>0.51/44.74</td>
<td>0.27/23.68</td>
</tr>
<tr>
<td>1,12-DoDDMA</td>
<td>0.36/31.30</td>
<td>0.50/43.48</td>
<td>0.29/25.22</td>
</tr>
</tbody>
</table>

*a* All monoliths contained 1 wt% DMPA to monomer.

*b* wt % related to total polymerization mixture.
Table 2.2. Effect of methanol percentage in methanol / dodecanol solutions on column back pressure for a poly(1,12-DoDDMA) monolith.

<table>
<thead>
<tr>
<th>Methanol (wt%)</th>
<th>Column back pressure (MPa)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>59.3</td>
<td>22.36 ± 0.49</td>
</tr>
<tr>
<td>61.5</td>
<td>12.76 ± 0.16</td>
</tr>
<tr>
<td>62.5</td>
<td>4.81 ± 0.10</td>
</tr>
<tr>
<td>64.3</td>
<td>3.20 ± 0.09</td>
</tr>
<tr>
<td>66.5</td>
<td>0.24 ± 0.01</td>
</tr>
</tbody>
</table>

\(^a\) Conditions: 10 cm × 75 μm i.d. monolithic column, methanol, 300 nL/min flow rate.

\(^b\) Average of three trials ± standard deviation.
**Figure 2.2.** SEM images of poly(1,12-DoDDMA) monoliths prepared with different percentages of methanol in methanol / dodecanol solution: (A) 59.3%, (B) 61.5%, (C) 63.3%, (D) 64.3%, and (E) 66.5%. 
separate alkylbenzenes and alkylparabens. Figure 2.3 shows gradient elution chromatograms of uracil, toluene, ethylbenzene, propylbenzene, butylbenzene, and amylbenzene with the monoliths listed in Table 2.1. The flow rate was 300 nL/min and the gradient was 40–100% ACN in 10 min. A mixture of ACN and water (70%/30% v/v) was used as the solvent for the alkylbenzene sample (0.25% v/v each alkylbenzene standard). As can be seen in Figure 2.3, all peaks had good symmetries and narrow peak widths at half peak height, ranging between 8.7 and 5.2 s for the alkylbenzenes. Figure 2.4 shows gradient elution chromatograms of alkylparabens. The flow rate was 300 nL/min and the gradient was 20–100% B in 10 min. A mixture of ACN and water (30%/70% v/v) was used as solvent for the alkylparaben sample (0.7 mg/mL each alkylparaben standard). Columns prepared from 1,4-BDDMA, 1,3-BDDMA, and NPGDMA can also separate alkylbenzenes and alkylparabens using the same conditions as in Figures 2.3 and 2.4 (chromatograms not included). The resolution obtained using these columns was not as good as for poly(1,5-PDDMA), poly(1,6-HDDMA), poly(1,10-DDDMA), and poly(1,12-DoDDMA). Monoliths with longer alkyl-bridging chain length showed greater retention of both alkylbenzenes and alkylparabens, which was due to an increase in hydrophobicity of the monolith with longer alkyl-bridging chain.

Figure 2.5 shows the elution of alkylbenzenes using a 1,6-HDDMA monolithic column with different gradients and flow rates. The six compounds were eluted within 8 min with better resolution using a 10 min gradient from 40% to 100% B and a flow rate of 600 nL/min in Figure 2.5B compared to Figure 2.5A. As expected, a shallower gradient led to longer elution time, and provided better resolution. For example, resolution values for toluene and ethylbenzene were 3.79 and 5.20 in Figures 5A and 5C, respectively. The same trend was observed when a shallower gradient was used to separate alkylparabens with this column.
Figure 2.3. (A), (B), (C), and (D) are RPLC separations of alkylbenzenes on monoliths synthesized from 1,5-PDDMA, 1,6-HDDMA, 1,10-DDDMA, and 1,12-DoDDMA, respectively. Conditions: 16 cm × 75 μm i.d. monolithic column; mobile phase component A was water, and B was acetonitrile; linear A-B gradient from 40% to 100% B in 10 min, and then isocratic elution with 100% B; 300 nL/min flow rate; on-column UV detection at 214 nm. Peak identifications: uracil, toluene, ethylbenzene, propylbenzene, butylbenzene and amylbenzene in order of elution.
Figure 2.4. (A), (B), (C), and (D) are RPLC separations of alkylparabens on monoliths synthesized from 1,5-PDDMA, 1,6-HDDMA, 1,10-DDDMA, and 1,12-DoDDMA, respectively. Conditions: 16 cm × 75 μm i.d. monolithic column; linear A-B gradient from 20% to 100% B in 10 min, and then isocratic elution with 100% B; 300 nL/min flow rate; on-column UV detection at 214 nm, other conditions are the same as in Figure 2.3. Peak identifications: uracil, methylparaben, ethylparaben, propylparaben and butylparaben, in order of elution.
2.3.3 Chromatographic efficiency measurements

Column efficiencies were measured for all of the alkanediol dimethacrylate monoliths. The theoretical plate numbers varied between 30,000 to 35,500 plates/m for uracil as a non-retained compound at 120 nL/min (0.45 mm/s) flow rate, which was the optimized flow rate for the 1,6-HDDMA monolithic column based on its van Deemter curve (Figure 2.6). The isocratic conditions used were 30% water/70% acetonitrile (v/v), 300 nL/min flow rate, and on-column UV detection at 214 nm.

The plate numbers for all of the monolithic columns were between 14,000 and 35,000 plates/m measured using uracil at 300 nL/min (i.e., 1.13 mm/s). Other conditions were the same as above. The column efficiencies (N/m)/retention factors (k) for toluene as a retained compound for all of the monolithic columns were 14,879/0.384 (1,4-BDDMA), 19,593/0.320 (1,3-BDDMA), 53,779/0.707 (1,10-DDDMA), and 49,323/1.113 (1,12-DoDDMA). Figure 2.7 shows chromatograms of alkylbenzenes using two different monolithic columns under isocratic condition. The efficiencies of the alkanediol dimethacrylate-based monoliths with alkyl chains greater than C5 were comparable to the performance of polymeric monoliths reported previously.17, 34, 35

Two monomer pairs (i.e., 1,3-BDDMA and 1,4-BDDMA, and NPGDMA and 1,5-PDDMA) were used to compare monoliths from branching and non-branching alkyl groups of the same carbon number. The monoliths, especially NPGDMA, with two branching groups in the alkyl bridge between the two dimethacrylate groups gave higher efficiencies (plates/m) when compared to their corresponding linear isomeric polymers (19,593/0.320 and 14,879/0.384 for 1,3-BDDMA and 1,4-BDDMA, respectively, and 48,877/0.453 and 35,147/ 0.497 for NPGDMA
Figure 2.5. Separations of alkylbenzenes on 1,6-HDDMA monolithic column. Conditions: linear A-B gradient from 40% to 100% B in (A) 5 min, 300 nL/min flow rate, (B) 10 min, 600 nL/min flow rate, and (C) 10 min, 300 nL/min flow rate; other conditions are the same as in Figure 2.3.
Figure 2.6. Plate height versus linear velocity for a 1,6-hexanediol dimethacrylate monolithic column using uracil as an non-retained compound. Conditions: 16 cm × 75 μm i.d. column; mobile phase component A was water, and B was acetonitrile; 30% A/70% B mobile phase.
Figure 2.7. Isocratic separations of alkylbenzenes on monoliths synthesized from (A) 1,3-BDDMA and (B) 1,6-HDDMA. Conditions: 16 cm × 75 μm i.d. monolithic column; mobile phase component A was water, and B was acetonitrile; 30% A/70% B mobile phase; 300 nL/min flow rate; on-column UV detection at 214 nm. Peak identifications: uracil, toluene, ethylbenzene, propylbenzene, butylbenzene and amylbenzene in order of elution.
and 1,5-PDDMA, respectively). This is most likely due to differences in monolith morphology and pore size distribution of the monoliths prepared from the different monomers.

### 2.3.4 Monolith morphologies

Figure 2.8 shows SEM images of monoliths synthesized from 1,4-BDDMA, 1,3-BDDMA, NPGDMA, 1,5-PDDMA, 1,6-HDDMA, 1,10-DDDMA, and 1,12-DoDDMA. From the SEM images, we see that all seven monoliths formed with small globules. However, poly(1,10-DDDMA) and poly(1,12-DoDDMA) have much smaller globules than the other five dimethacrylate-based monoliths, which resulted in higher back pressures and sharper chromatographic peaks than obtained using the other three monoliths formed from linear alkanediol dimethacrylates.

### 2.3.5 Column permeability and stability

Column permeability was used to evaluate the stability of the monoliths. To obtain plots of back pressure versus flow rate, acetonitrile, methanol and water were pumped through a 16-cm long monolithic column at six different flow rates from 0.05 to 0.5 μL/min. Linear relationships between back pressure and flow rate (R^2>0.999 for all monoliths) clearly indicated that the monoliths were mechanically stable [Figure 2.9 shows poly(1,6-HDDMA and poly(1,12-DoDDMA) as examples]. The permeabilities calculated based on Darcy’s law are listed in Table 2.3. For 1,5-PDDMA, 1,6-HDDMA, 1,10-DDDMA, and 1,12-DoDDMA monolithic columns, the results were similar for all three solvents, indicating that these monoliths shrank or swelled very little in solvents of different polarities. Monoliths with shorter alkyl-bridging chains, especially poly(1,4-BDDMA), have greater permeabilities. This may be due to the fact that monoliths with shorter alkyl-bridging chains have less hydrophobicities.
Figure 2.8. SEM images of monoliths. (A) poly(1,4-BDDMA), (B) poly(1,3-BDDMA), (C) poly(NPGDMA), (D) poly(1,5-PDDMA), (E) poly(1,6-HDDMA), (F) poly(1,10-DDDMA), (G) poly(1,12-DoDDMA); see structures in Figure 2.1.
Figure 2.9. Effect of mobile phase flow rate on column back pressure (A) poly(1,6-HDDMA) and (B) poly(1,12-DoDDMA) (average of three repetitions). Conditions: 16 cm × 75 μm i.d. monolithic columns.
Table 2.3. Permeabilities of poly(alkanediol dimethacrylate) monolithic columns using different liquids.

<table>
<thead>
<tr>
<th>Liquid</th>
<th>Relative polarity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Viscosity (mPa s)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>1,4-BDDMA</th>
<th>1,3-BDDMA</th>
<th>NPGDMA</th>
<th>1,5-PDDMA</th>
<th>1,6-HDDMA</th>
<th>1,10-DDDMA</th>
<th>1,12-DoDDMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1.00</td>
<td>0.89</td>
<td>22.57±3.88</td>
<td>4.48±0.39</td>
<td>0.77±0.07</td>
<td>7.12±1.11</td>
<td>5.52±1.14</td>
<td>0.96±0.01</td>
<td>1.07±0.10</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>0.46</td>
<td>0.37</td>
<td>39.44±5.08</td>
<td>6.10±0.24</td>
<td>2.99±0.64</td>
<td>8.16±0.20</td>
<td>5.80±0.23</td>
<td>0.77±0.02</td>
<td>1.08±0.04</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.76</td>
<td>0.54</td>
<td>16.33±3.90</td>
<td>6.44±0.22</td>
<td>0.72±0.10</td>
<td>6.42±0.08</td>
<td>4.81±0.28</td>
<td>0.74±0.01</td>
<td>0.99±0.02</td>
</tr>
</tbody>
</table>

<sup>a</sup> Relative polarity data are from ref 32.

<sup>b</sup> Viscosity, η, data are from online CRC Handbook of Chemistry and Physics, 89<sup>th</sup> ed.; CRC: Boca Raton, FL, 2008-2009.

<sup>c</sup> Permeability $k = \eta L u / \Delta P$, where η is the viscosity, L is the column length (16 cm in this case), u is the solvent linear velocity, and ΔP is the column back-pressure.

<sup>d</sup> Average of six trials at different flow rates ± standard deviation.
Table 2.4. Retention times of uracil and alkylbenzenes showing column-to-column reproducibility of three independently prepared 1,6-HDDMA columns.a

<table>
<thead>
<tr>
<th></th>
<th>Retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uracil</td>
</tr>
<tr>
<td>Column 1</td>
<td>2.118</td>
</tr>
<tr>
<td>Column 2</td>
<td>2.112</td>
</tr>
<tr>
<td>Column 3</td>
<td>2.072</td>
</tr>
<tr>
<td>Mean value</td>
<td>2.101</td>
</tr>
<tr>
<td>RSDb</td>
<td>1.20%</td>
</tr>
</tbody>
</table>

a Conditions are the same as in Figure 2.3.
b Relative standard deviation
2.3.6 Reproducibility of poly(1,6-HDDMA)

In addition to good chromatographic performance, reproducibility and stability are basic requirements for a monolithic column, especially when the column is to be used for routine analysis. Run-to-run and column-to-column reproducibilities (see Table 2.4) were measured for the poly(1,6-HDDMA) monolithic column. The run-to-run and column-to-column RSD values based on retention times (n = 3) were 0.25% and 1.20%, respectively. More than 60 runs were conducted to test the stability of the poly(1,6-HDDMA) monolithic column. There was no noticeable change observed in column performance. Due to the highly cross-linked network, monoliths synthesized from single cross-linking monomers typically exhibited excellent stability, as demonstrated here and in our previous work.19, 33

2.4 Conclusions

New monolithic RPLC stationary phases based on single monomers were synthesized using UV-initiated free radical polymerization. These new monolithic columns were successfully used for the separation of low-molecular weight compounds under RP conditions. SEM images were taken which showed different globule sizes for monoliths made from different dimethacrylates. Smaller globules resulted in higher back pressures and sharper chromatographic peaks. Among the monoliths prepared from linear alkanediol dimethacrylates, poly(1,10-DDDMA) provided the highest efficiency (plates/m) overall. Investigation of two pairs of isomer monomers showed that monoliths with branching groups in the alkyl bridge between the two dimethacrylate groups gave higher efficiencies compared to their linear counterparts. Gradient elution of alkylbenzenes and alkylparabens was achieved with high resolution using all seven columns. The test analytes were completely separated in 15 min using 300 nL/min (1.13 mm/s) flow rate. Good run-to-run and column-to-column (n = 3)
reproducibilities were observed, which are mainly attributed to the use of single monomers in their preparation.

2.5 References

CHAPTER 3 HIGHLY CROSS-LINKED POLYMERIC MONOLITHS WITH VARIOUS C6 FUNCTIONAL GROUPS FOR REVERSED-PHASE CAPILLARY LIQUID CHROMATOGRAPHY OF SMALL MOLECULES†

3.1 Introduction

Polymeric monolithic stationary phases characterized by continuous porous beds† were introduced in the late 1980s and early 1990s by Hjertén,1, 2 and Svec and Fréchet.3-6 They are potentially good alternatives to packed columns for high performance liquid chromatography (LC). Compared to packed columns, monolithic columns have several attractive advantages, which have been described by many excellent reviews.7-13 The most common monomers used to synthesize organic polymeric monoliths include styrene,14 acrylates,15 and acrylamides.16 Polymeric monoliths are particularly suitable stationary phases for separations of peptides,17 proteins,18 nucleic acids,19 and synthetic polymers.20 However, their low mesoporosities and surface areas lead to low chromatographic resolution for small molecules. Recently, most attention should be focused on improving chromatographic efficiency and mechanical stability of monolithic columns. Some researchers have reported improvements in monoliths for small molecule separations by modifying the preparation conditions.14, 21-24

A typical polymerization system for monolith preparation includes two or more monomers (functional and cross-linking monomers). It has been reported that high cross-linker concentration can provide high mechanical stability and high surface area.25, 26 Actually, cross-linkers not only provide monolith rigidity, but they can also influence their surface properties by serving as functional monomers. Most acrylic monolith studies report the use of ethylene glycol diacrylate (EGDA) or ethylene glycol dimethacrylate (EGDMA) as

† This chapter was largely reproduced from: Liu, K.; Tolley, H. D.; Lawson, J. S.; Lee, M. L. J. Chromatogr. A 2013, 1321, 80-87.
Cross-linkers. Only a couple of these studies focused on the effect of the cross-linker. Recent work has suggested that use of a single-monomer/cross-linker in the synthesis provides simpler optimization of the polymerization system, improved column-to-column reproducibility, better mechanical stability and higher surface area due to the highly cross-linked network.

In this chapter, a group of highly cross-linked polymeric monolithic stationary phases prepared from single methacrylate/acylate based monomers were prepared. The structures of these monomers are shown in Figure 3.1. The separation performances of these monoliths were studied using a standard mixture of low-molecular-weight alkylbenzenes.

3.2 Experimental

3.2.1 Chemicals and reagents

2,2-Dimethoxy-2-phenylacetophenone (DMPA, 99%) and 3-(trimethoxysilyl)propyl methacrylate (TPM, 98%) were purchased from Sigma–Aldrich (St Louis, MO, USA); 1,6-hexanediol dimethacrylate (1,6-HDDMA) (see Figure. 3.1) was a gift from Sartomer (Exton, PA, USA); 1,4-cyclohexanediol dimethacrylate (CHDDMA) and 1,4-phenylene diacrylate (PHDA) (see Figure. 3.1) were purchased from Polysciences (Warrington, PA, USA). Water, methanol, decanol, dodecanol, propylbenzene, butylbenzene, amylbenzene and uracil were also obtained from Sigma–Aldrich; ACN, iso-butanol, DMF and ethylbenzene were purchased from Fisher Scientific (Pittsburgh, PA, USA); toluene was purchased from Mallinckrodt (Phillipsburg, NJ, USA); THF was purchased from Curtin Matheson Scientific (Houston, TX, USA). All porogenic solvents and chemicals for monolith and mobile phase buffer preparations were HPLC or analytical reagent grade, and were used as received.

3.2.2 Fused silica capillary pretreatment

UV-transparent fused silica capillary tubing (75-µm i.d., 375-µm o.d., Polymicro
Figure 3.1. Chemical structures of dimethacrylate/diacrylate monomers with different C6 functional groups.
Technologies, Phoenix, AZ, USA) was treated with TPM following the procedure described in Section 2.2.2. Before using HCl, the inner surface of the capillary tubing was treated with 1 M NaOH solution for 1 h. Both ends were sealed with GC septa and the capillary was heated in a GC oven at 120 °C for 3 h. Then, the tubing was washed with water to remove NaOH.

### 3.2.3 Polymeric monolith preparation

All monomer solutions were prepared in 1-dram (4 mL) glass vials by admixing initiator, monomer, and porogen solvents (see Table 3.1 for reagent compositions). All solutions were vortexed and then degassed by sonication for a few seconds to avoid excessive evaporation of porogenic solvents. The prepolymer mixture containing PHDA was heated in a GC oven at 70 °C for 30 s to promote dissolution of PHDA in the porogens, and the resultant solution was vortexed for 30 s to ensure that it was well-mixed. Then, the reaction mixture was introduced into one end of the silanized capillary by helium gas pressure. A 5-cm long empty section was left at the exit end of the capillary to provide for a detection window at the end of the monolith bed. The capillary was sealed with rubber septa at both ends and placed directly under a PRX 1000-20 exposure unit UV lamp (390±15 nm, 1000 W, TAMARACK Scientific, Corona, CA, USA). Polymerization times of 1 to 6 min were evaluated. The resultant monolith was flushed with methanol and then water to remove any porogens and unreacted residual monomers until a stable pressure reading was obtained. Similar back pressures (per unit column length) were observed when the polymerization time was longer than 3 min for HDDMA and CHDDMA, and 3.5 min for PHDA. Therefore, 3.5 min for HDDMA and CHDDMA monoliths and 4 min for PHDA monoliths were selected as polymerization times to ensure complete conversion of the monomers. The monoliths were characterized using an FEI Helios Nanolab 600 (Hillsboro, OR, USA) scanning electron microscope after coating with a thin (∼10 nm) conducting layer of gold.
3.2.4 Capillary liquid chromatography

The LC instrument and detector used for all chromatographic experiments in this chapter were as the same as described in Section 2.2.4. The injection volume was 30 nL.

3.3 Results and Discussion

3.3.1 Selection of porogens

The selection of porogenic solvent(s) is an important step in the preparation of monoliths. One of the monomers, CHDDMA, was chosen for detailed study of porogen selection. Several solvents with different polarities were used in the synthesis of the monoliths. It was found that a transparent gel was obtained after polymerization using decanol or dodecanol, indicating that these were potentially “good” solvents for CHDDMA. The monomer would not polymerize using toluene or ACN. CHDDMA formed a monolith when dissolved in methanol or isobutanol after UV light initiation. However, the structures of these monoliths were not rigid enough to be used as stationary phases. Rigid macroporous monoliths were obtained when methanol or iso-butanol were combined with decanol or dodecanol. Although CHDDMA could form monoliths with decanol, the monoliths gave very poor chromatographic efficiency. When iso-butanol was combined with dodecanol, the final monolith gave much higher back pressure than monoliths prepared from the mixture of methanol and dodecanol. In addition, it was much easier to fill the capillary using monomer solutions that contained methanol, and to flush the columns after polymerization, as the viscosity of methanol is much lower than that of iso-butanol. Therefore, a combination of methanol and dodecanol appeared to be the best porogen system for the CHDDMA monolith. The data in Table 3.2 show how the column back pressure increases with an increase in the amount of dodecanol. In forming rigid monoliths from CHDDMA, dodecanol behaves as a “good” solvent and methanol as a “poor” solvent. The ratio of monomer
Table 3.1. Compositions of reagent solutions.

<table>
<thead>
<tr>
<th>Monolith</th>
<th>Composition (g/wt%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monomer</td>
</tr>
<tr>
<td>1,6-HDDMA</td>
<td>0.36/33.03</td>
</tr>
<tr>
<td>CHDDMA</td>
<td>0.36/40.00</td>
</tr>
<tr>
<td>PHDA</td>
<td>0.36/22.93</td>
</tr>
</tbody>
</table>

<sup>a</sup> All monoliths contained 1 wt% DMPA to monomer.
<sup>b</sup> wt % related to total polymerization mixture.
Table 3.2. Effect of dodecanol percentage of the total porogen solutions on column back pressure for a poly(CHDDMA) monolith.a

<table>
<thead>
<tr>
<th>Dodecanol (wt%)</th>
<th>Column back pressure (MPa)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.99</td>
<td>4.27 ± 0.02</td>
</tr>
<tr>
<td>37.05</td>
<td>5.07 ± 0.02</td>
</tr>
<tr>
<td>39.07</td>
<td>5.33 ± 0.02</td>
</tr>
<tr>
<td>41.01</td>
<td>7.49 ± 0.02</td>
</tr>
<tr>
<td>43.39</td>
<td>9.31 ± 0.03</td>
</tr>
</tbody>
</table>

*a Conditions: 10 cm × 75 μm i.d. monolithic column, ACN, 300 nL/min flow rate.

b Average of three trials ± standard deviation.
to total porogens was investigated, and the final ratio was set at 40.0:60.0 (wt/wt).

At room temperature, PHDA is a solid and is very difficult to dissolve in mixtures of methanol and dodecanol. Although its solubility improves with increasing temperature, the boiling point of methanol is only 67.4 °C, which means that methanol evaporates during heating, which changes the composition of the monomer solutions. Therefore, methanol could not be used for synthesis of poly(PHDA) monoliths. Iso-butanol (boiling point 107.89 °C) was, therefore, tested as an alternative to methanol. Unfortunately, PHDA did not totally dissolve until the temperature was increased to 100 °C, which indicated that iso-butanol was not a good choice either. By using a mixture of dodecanol and DMF (individual boiling points of 259 and 153 °C, respectively), PHDA could be totally dissolved in 15 s at 70 °C. Consequently, dodecanol and DMF were used as porogens for preparation of poly(PHDA) monoliths.

3.3.2 Monolith morphologies

Figure 3.2 shows SEM images of monoliths synthesized from HDDMA, CHDDMA, and PHDA. From the SEM images, it is clear that all three monoliths had skeletal structures composed of small globules. Poly(PHDA) had smaller throughpores than the other two monoliths, which resulted in higher back pressure (17.24 MPa at 300 nL/min flow rate). Poly(1,6-HDDMA) contained some large pores (Figure 3.2A), which led to low back pressure (2.24 MPa at 300 nL/min flow rate).

3.3.3 Separation of alkylbenzenes

I obtained rigid structural monoliths using all three of the monomers, and all of the monolithic columns could be used to separate alkylbenzenes. Figure 3.3 shows gradient elution chromatograms of uracil, toluene, ethylbenzene, propylbenzene, butylbenzene, and amylbenzene with the monoliths formed from the reagents listed in Table 3.1. The flow rate was 300 nL/min
and the gradient was 40–100% ACN in 10 min. As can be seen in Figure 3.3, all peaks had good symmetries and narrow peak widths, ranging between 3.4 and 5.6 s at half height.

Figure 3.4 illustrates the elution of alkylbenzenes using an 1,6-HDDMA monolithic column with different gradients and flow rates. The six compounds were eluted within 4 min using a 5 min gradient from 40% to 100% ACN and a flow rate of 600 nL/min (Figure 3.4A). As expected, a shallower gradient led to longer elution time, but provided better resolution. For example, resolution values for ethylbenzene and propylbenzene were 3.75 and 4.03 in Figure 3.4A and 3.4B, respectively.

### 3.3.4 Chromatographic efficiency measurements

Figure 3.5 shows isocratic elution of alkylbenzenes using 1,6-HDDMA, CHDDMA, and PHDA monoliths at 300 nL/min (i.e., 1.13 mm/s), which was the optimized flow rate for CHDDMA monolithic columns. 1,6-HDDMA and CHDDMA monoliths showed good separation performance for alkylbenzenes under isocratic conditions. However, the alkylbenzenes could not be baseline separated using the PHDA monolith. The plate numbers for all of the monolithic columns were between 20,000 and 47,000 plates/m measured using uracil as a non-retained compound and between 40,000 to 65,000 plates/m for 1,6-HDDMA and CHDDMA columns using alkylbenzenes as retained compounds at 300 nL/min.

The maximum theoretical plate numbers were 86,000 (k = 0.364), 62,000 (k = 0.334), 54,000 (k = 0.216) plates/m for toluene as a retained compound using 1,6-HDDMA, CHDDMA, and PHDA, respectively. A van Deemter curve for the 1,6-HDDMA column is shown in Figure 3.6. The performance of the 1,6-HDDMA monolith was comparable to the performance of polymeric monoliths reported previously.22, 30
Figure 3.2. SEM images of monoliths. (A) and (B) poly(1,6-HDDMA), (C) and (D) poly(CHDDMA), (E) and (F) poly(PHDA); see structures in Figure 3.1.
Figure 3.3. (A) - (C) are RPLC separations of alkylbenzenes on monoliths synthesized from 1,6-HDDMA, CHDDMA, and PHDA, respectively. Conditions: 16 cm × 75 μm i.d. monolithic column; mobile phase component A was water, and B was acetonitrile; linear A-B gradient from 40% to 100% B in 10 min, and then isocratic elution with 100% B; 300 nL/min flow rate; on-column UV detection at 214 nm. Peak identifications: uracil, toluene, ethylbenzene, propylbenzene, butylbenzene and amylbenzene in order of elution.
Figure 3.4. Separations of alkylbenzenes on an 1,6-HDDMA monolithic column. Conditions: linear A-B gradient from 40% to 100% B in (A) 5 min, 600 nL/min flow rate, (B) 10 min, 600 nL/min flow rate, and (C) 10 min, 300 nL/min flow rate; other conditions and peak identifications are the same as in Figure 3.3.
Figure 3.5. Separations of alkylbenzenes using (A) 1,6-HDDMA, (B) CHDDMA, and (C) PHDA monolithic columns (Table 3.1) under isocratic elution conditions. Conditions: 16 cm × 75 μm i.d. monolithic column; 30% A/70% B mobile phase; 300 nL/min flow rate; other conditions and peak identifications are the same as in Figure 3.3.
**Figure 3.6.** Plot of plate height ($H$) versus linear velocity for a 1,6-HDDMA monolithic column using toluene as a retained compound (average of three repetitions). Conditions: 16 cm × 75 μm i.d. column; mobile phase component A was water, and B was ACN; 30% A/70% B mobile phase; on-column UV detection at 214 nm.
All of the monomers used to prepare monoliths in this work have C6 groups, albeit with different structures. The monolith with a linear C6 structure demonstrated the best efficiency, followed by those with cyclohexyl and then phenyl groups. It appears that the flexibility of the linear C6 groups allows better analyte diffusion into the stationary phase (i.e., better mass transfer). In contrast, the structures of the monoliths became more rigid when the functional groups in the monoliths changed from linear C6 to cyclohexyl and phenyl groups, which made it more difficult for analyte diffusion into the polymer and interaction with the functional groups. The PHDA monolith had quite different properties compared to the other monoliths. It was formed from a diacrylate monomer, which does not contain the two methyl groups that are characteristic of the dimethacrylate monomers (i.e., 1,6-HDDMA and CHDDMA). In addition, due to the conjugated effect of the phenyl moiety, the phenoxy group is a weaker electron donor than the alkoxy group. These properties cause the PHDA molecule to have the least hydrophobicity among the three monomers, which leads to a monolithic column that has the lowest selectivity for alkylbenzenes (Figure 3.5C).

3.3.5 Column permeability and rigidity

Back pressure measurements and column permeability calculations for monoliths exposed to different solvents were used to evaluate the rigidities of the monoliths. To obtain plots of back pressure versus flow rate, acetonitrile, methanol and water were pumped through 16-cm lengths of monolithic columns at six different flow rates from 50 to 500 nL/min. Linear relationships (Figure 3.7) between back pressure and flow rate ($R^2>0.999$ for all monoliths) clearly indicated that the monoliths were mechanically stable. The permeabilities calculated based on Darcy’s law are listed in Table 3.3. All monolithic columns were found to slightly shrink in polar solvents, with the highest permeability in water and only slight swelling in ACN.
Figure 3.7. Effect of mobile phase flow rate on column back pressure (average of three repetitions).

Conditions: 16 cm × 75 μm i.d. monolithic columns.
Table 3.3. Permeabilities of dimethacrylate/diacrylate monolithic columns for different liquids.

<table>
<thead>
<tr>
<th>Liquid</th>
<th>Relative polarity(^a)</th>
<th>Viscosity (mPa s)(^b)</th>
<th>Permeability (×10(^{-14}) m(^2))(^c,d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1,6-HDDMA</td>
<td>CHDDMA</td>
</tr>
<tr>
<td>Water</td>
<td>1.00</td>
<td>0.89</td>
<td>5.54±1.07</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>0.46</td>
<td>0.37</td>
<td>2.15±0.39</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.76</td>
<td>0.54</td>
<td>5.43±1.30</td>
</tr>
</tbody>
</table>

\(^a\) Relative polarity data are from ref 36.

\(^b\) Viscosity, \(\eta\), data are from online CRC Handbook of Chemistry and Physics, 89\(^{th}\) ed.; CRC: Boca Raton, FL, 2008-2009.

\(^c\) Permeability \(k = \eta Lu/\Delta P\), where \(\eta\) is the viscosity, \(L\) is the column length (16 cm in this case), \(u\) is the solvent linear velocity, and \(\Delta P\) is the column back-pressure.

\(^d\) Average of six trials at different flow rates ± standard deviation.
3.3.6 Reproducibility of poly(1,6-HDDMA)

In addition to good chromatographic performance, reproducibility is a very important characteristic of any LC column. Run-to-run and column-to-column reproducibilities were measured for the poly(1,6-HDDMA) monolithic column. Chromatograms illustrating column-to-column reproducibilities for three independent poly(1,6-HDDMA) monolithic columns are shown in Figure 3.8. The run-to-run and column-to-column RSD values based on retention times (n = 3) were less than 0.26% and 0.70%, respectively (see Table 3.4). The theoretical plate number RSD values for a poly(1,6-HDDMA) column (3 replicate measurements at 10 different velocities) ranged between 0.778% and 7.16%. Measurements (n = 3) of the maximum theoretical plate number (86,000 plates/m) gave and RSD value of 1.58 %. More than 60 runs were conducted to test the robustness of the poly(1,6-HDDMA) monolithic column. There was no noticeable change observed in column performance. Due to the highly cross-linked network, monoliths synthesized from single cross-linking monomers typically exhibited excellent robustness, as demonstrated here and in our previous work.22, 34

3.4 Conclusions

New monolithic RPLC stationary phases based on single monomers were synthesized using UV-initiated free radical polymerization. The performances of these new monolithic columns were demonstrated using alkylbenzenes under RPLC conditions. SEM images were taken which showed different globular morphologies for monoliths made from different dimethacrylates/diacrylates. Among the monoliths, those prepared from the linear 1,6-hexanediol dimethacrylate monomer provided the highest efficiency (86,000 plates/m) and lowest backpressure. Gradient elution of alkylbenzenes was achieved with high resolution using all three columns. The test analytes were completely separated in 10 min using 300 nL/min (1.13
mm/s) flow rate. Good run-to-run and column-to-column (n=3) reproducibilities were observed, which are mainly attributed to the use of single monomers in the preparation of the monoliths.
Figure 3.8. Chromatograms showing column-to-column reproducibility for 1,6-HDDMA monolithic columns using uracil and alkylbenzenes as analytes. Conditions and peak identifications are the same as in Figure 3.3.
Table 3.4. Retention times of uracil and alkylbenzenes showing column-to-column reproducibility of three independently prepared poly(1,6-HDDMA) columns.\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Uracil (min)</th>
<th>Toluene</th>
<th>Ethylbenzene (min)</th>
<th>Propylbenzene (min)</th>
<th>Butylbenzene (min)</th>
<th>Amylbenzene (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Column 1</strong></td>
<td>1.920</td>
<td>4.189</td>
<td>4.730</td>
<td>5.333</td>
<td>5.966</td>
<td>6.561</td>
</tr>
<tr>
<td><strong>Column 2</strong></td>
<td>1.914</td>
<td>4.131</td>
<td>4.666</td>
<td>5.265</td>
<td>5.908</td>
<td>6.505</td>
</tr>
<tr>
<td><strong>Column 3</strong></td>
<td>1.923</td>
<td>4.159</td>
<td>4.692</td>
<td>5.293</td>
<td>5.932</td>
<td>6.521</td>
</tr>
<tr>
<td><strong>Mean value</strong></td>
<td>1.919</td>
<td>4.160</td>
<td>4.696</td>
<td>5.297</td>
<td>5.935</td>
<td>6.529</td>
</tr>
<tr>
<td><strong>RSD</strong></td>
<td>0.24%</td>
<td>0.70%</td>
<td>0.69%</td>
<td>0.65%</td>
<td>0.49%</td>
<td>0.44%</td>
</tr>
</tbody>
</table>

\(^a\) Conditions are the same as in Figure 3.3.
\(^b\) Relative standard deviation
3.5 References


CHAPTER 4 FABRICATION OF HIGHLY CROSS-LINKED REVERSED-PHASE MONOLITHIC COLUMNS VIA LIVING RADICAL POLYMERIZATION

4.1 Introduction

Polymeric monolithic stationary phases have played an important role as stationary phases for liquid chromatography (LC) since they were introduced in the late 1980s and early 1990s by Hjertén,1, 2 and Svec and Fréchet.3-6 Up to now, most monoliths are prepared through conventional free radical polymerization. During the process of free radical polymerization, cross-linkers with or without functional monomers are mixed with initiator and porogen(s). It is very difficult to control the polymerization process and the resultant monolith morphology, because it takes only seconds for an individual chain to propagate from initiation to termination.7 This characteristic of free radical polymerization usually results in the formation of massive structures (microgels) and random polymers,8, 9 and finally a heterogeneous polymer network structure with mediocre separation performance. Therefore, more homogeneous structures with well-defined skeletal and pore sizes are desirable for obtaining better separation performance.

Recently, controlled/living radical polymerization (CRP) was introduced and investigated for the synthesis of organic monoliths. The controlled/living radical polymerization system is a reversible activation/deactivation process. The growing rate of an individual chain is controlled by the balance between the growing radical and dormant species because of its reversible character. Therefore, the period of chain propagation in CRP is much longer than in free radical polymerization, which gives these chains sufficient time for relaxation so that the reaction species distribute uniformly.10, 11 Figure 4.1A shows the general mechanism of CRP.12 All controlled/living radical polymerization includes three steps: activation, propagation and deactivation (see Figure 4.1A).13 The most important aspect of CRP is the formation of a
reversibly generated active P• radical. When a P• radical is formed, it can react with the monomers, and the polymer chains propagate before deactivation to the dormant species, P-X. Due to reaction reversibility, the resultant polymers exhibit narrower molecular weight distributions and more homogeneous cross-linked structures compared to polymers obtained from conventional free radical polymerization. Yu et al. reported that poly(ethylene glycol dimethacrylate) (PEGDMA) and poly(ethylene glycol dimethacrylate-ethylene glycol methyl ether methacrylate) (PEGDMA-PEGMEMA) monoliths were synthesized via atom transfer radical polymerization (ATRP). A poly(1,3-glycerol dimethacrylate) (PGDMA) monolith was obtained by ATRP as well. In addition, poly(styrene-co-divinylbenzene) (PS-DVB) monoliths were successfully prepared from nitroxide-mediated living radical polymerization (NMP). Organotellurium-mediated living radical polymerization (TERP) is a new branch of CRP. Yamago’s group investigated a series of organotellurium compounds which could produce carbon-centered radicals by thermolysis to initiate the polymerization reactions. Figure 4.1B illustrates the mechanism of TERP without the presence of azo initiator (i.e., AIBN), which is a thermal dissociation (TD) mechanism. High initiation temperature and long polymerization time are required because the generation of carbon-centered radicals is very slow in this mechanism. When azo initiators are introduced into the system, the initiating radicals are created from these initiators first under mild thermal conditions, and the polymerization follows the degenerative chain transfer (DT) mechanism (see Figure 4.1C). Therefore, relatively gentle polymerization conditions could be used for monolith preparation. Recently, PS-DVB, PDVB, and poly(N,N-methylenebisacrylamide) monoliths have been successfully prepared using TERP.
Figure 4.1. General reactions showing (A) mechanism of CRP, (B) thermal dissociation of TERP and (C) degenerative chain transfer mechanism of TERP.\textsuperscript{13}
In this study, I successfully synthesized organic monolithic capillary columns by TERP using three monomers 1,12-dodecanediol dimethacrylate (1,12-DoDDMA), trimethylolpropane trimethacrylate (TMPTMA) and pentaerythritol tetraacrylate (PETA). All columns could separate alkylbenzenes under isocratic conditions. This is the first time that monoliths prepared from methacrylates/acrylates via TERP were used for small molecule separations.

4.2 Experimental

4.2.1 Chemicals and reagents

The reagents 2,2'-azobis(2-methylpropionitrile) (AIBN, 98%) and 3-(trimethoxysilyl)propyl methacrylate (TPM, 98%) were purchased from Sigma–Aldrich (St. Louis, MO, USA); 1,12-dodecanediol dimethacrylate (1,12-DoDDMA), trimethylolpropane trimethacrylate (TMPTMA) and pentaerythritol tetraacrylate (PETA) were gifts from Sartomer (Exton, PA, USA) (see Figure 4.2). Ethyl-2-methyl-2-butylltellanyl propionate (BTEE) was kindly supplied by Dr. Takashi Kameshima, Otsuka Chemical Co. (Osaka, Japan). Water, 1,4-butanediol, propylbenzene, butylbenzene, amylbenzene and uracil were also obtained from Sigma–Aldrich; acetonitrile (ACN), DMF and ethylbenzene were purchased from Fisher Scientific (Pittsburgh, PA, USA); toluene cyclohexanol and ethylene glycol were purchased from Mallinckrodt (Phillipsburg, NJ, USA). All porogenic solvents and chemicals for monolith and mobile phase buffer preparations were HPLC or analytical reagent grade, and were used as received.

4.2.2 Fused silica capillary pretreatment

UV-transparent fused silica capillary tubing (75-, 100-, and 150-µm i.d., 375-µm o.d., Polymicro Technologies, Phoenix, AZ, USA) were treated with TPM following the procedures described in Section 3.2.2.
Figure 4.2. Chemical structures of multi-methacrylate/multi-acrylate monomers.
4.2.3 Polymeric monolith preparation

All monomer solutions were prepared in 1-dram (4 mL) glass vials by admixing initiator, monomer, and porogen solvents (see Tables 4.1, 4.2 and 4.3 for reagent compositions). All solutions were vortexed and then degassed by sonication for a few seconds to avoid increasing the temperature of mixture solutions to initiate the polymerizations. The solutions were purged with nitrogen gas for 5 min. The reaction promoter BTEE was added into the reaction solution with a 10 μL syringe. Then the reaction mixture was introduced into one end of the silanized capillary by helium gas pressure. A 5 cm long empty section was left at the other end of the capillary to provide a detection window immediately following the monolith. After filling with polymerization solution, the capillary was sealed with rubber septa at both ends and placed in a 60 °C oil bath for 24 h. The obtained monoliths were flushed with methanol and then water to remove porogens and possible unreacted residual monomers until stable pressure readings were obtained. The monolithic columns were characterized using an FEI Helios Nanolab 600 (Hillsboro, OR, USA) scanning electron microscope after coating a thin (~10 nm) conducting layer of gold on each capillary column end.

4.2.4 Capillary liquid chromatography

The LC instrument and detector used for all chromatographic experiments in this chapter were as the same as described in Section 2.2.4. The injection volume was 30 nL.

4.3 Results and Discussion

4.3.1 Selection of porogens

The selection of porogenic solvent(s) is an important step in the preparation of monoliths. One of the monomers, 1,12-DoDDMA, was chosen for detailed study of porogen selection. Several solvents with different polarities were used to synthesize the monoliths. It was
found that a transparent gel or monolith was obtained with polymerizations using toluene, cyclohexanol or DMF, individually, indicating that these were potentially “good” solvents for 1,12-DoDDMA. The monomer could not dissolve in pure ethylene glycol, 1,4-butanediol or mixtures of ethylene glycol, 1,4-butanediol and toluene. However, rigid macroporous monoliths were obtained when ethylene glycol or 1,4-butanediol were combined with cyclohexanol or DMF using thermal polymerization at 60 °C for 24 h. Although 1,12-DoDDMA could form monoliths using combinations of cyclohexanol and ethylene glycol, DMF and ethylene glycol, and DMF and 1,4-butanediol, the final monolithic columns gave higher back pressures and poorer chromatographic efficiencies than monoliths prepared from the mixture of cyclohexanol and 1,4-butanediol. Consequently, cyclohexanol and 1,4-butanediol were used for preparation of poly(DoDDMA) monoliths.

4.3.2 Selection of polymerization conditions

To obtain a homogeneous monolithic column, both gelation and phase separation must take place at the appropriate time. If gelation occurs first, the resultant structure will have fine features; however, the heterogeneity of the skeleton may increase, which will result in poor chromatographic performance, since chain diffusion is frozen by early gelation. On the other hand, early precipitation of the monolith leads to large globules and large pores, which leads to a reduction in monolith surface area. Therefore, it is desirable for gelation and phase separation to take place at the same time to produce uniform skeletal and pore structures with small pores. The effects of different polymerization reagent amounts and capillary inner diameters for poly(1,12-DoDDMA) monoliths were studied (listed in Table 4.1) to find the combination that gave the highest column efficiency. Uracil was used as a non-retained analyte, and toluene was used as a retained compound in these efficiency tests.
Table 4.1. Effect of different reagent compositions and capillary i.d. on column efficiency for poly(1,12-DoDDMA) monoliths.\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Column</th>
<th>Reagent Composition</th>
<th>Efficiency$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cyclohexanol/1,4-butadieniol</td>
</tr>
<tr>
<td>Cyclohexanol to 1,4-butadieniol ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>0.10</td>
<td>24.97</td>
</tr>
<tr>
<td>D2</td>
<td>0.10</td>
<td>24.97</td>
</tr>
<tr>
<td>D3</td>
<td>0.10</td>
<td>24.90</td>
</tr>
<tr>
<td>D4</td>
<td>0.10</td>
<td>25.00</td>
</tr>
<tr>
<td>D5</td>
<td>0.10</td>
<td>25.02</td>
</tr>
<tr>
<td>D6</td>
<td>0.10</td>
<td>25.02</td>
</tr>
<tr>
<td>Total porogen to monomer ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D7</td>
<td>0.10</td>
<td>28.50</td>
</tr>
<tr>
<td>D8</td>
<td>0.10</td>
<td>23.21</td>
</tr>
<tr>
<td>D9</td>
<td>0.10</td>
<td>21.75</td>
</tr>
<tr>
<td>D10</td>
<td>0.10</td>
<td>19.95</td>
</tr>
<tr>
<td>Initiator percentage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D11</td>
<td>0.05</td>
<td>25.02</td>
</tr>
<tr>
<td>D12</td>
<td>0.10</td>
<td>24.97</td>
</tr>
<tr>
<td>Total porogen to monomer ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D13</td>
<td>0.10</td>
<td>23.15</td>
</tr>
<tr>
<td>D14</td>
<td>0.10</td>
<td>23.22</td>
</tr>
<tr>
<td>D15</td>
<td>0.10</td>
<td>23.22</td>
</tr>
<tr>
<td>Effect capillary i.d.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D16</td>
<td>0.10</td>
<td>25.00</td>
</tr>
<tr>
<td>D17</td>
<td>0.10</td>
<td>24.97</td>
</tr>
</tbody>
</table>

\textsuperscript{a} All monoliths were polymerized at 60 °C for 24 h.
\textsuperscript{b} Conditions: 300 nL/min flow rate.
\textsuperscript{c} Column efficiency (plates/m).
\textsuperscript{d} Percentage by weight.
\textsuperscript{e} Could not flush the column with over 400 bar (5800 psi).
Table 4.2. Effect of different reagent compositions on column efficiency for poly(TMPTMA) monoliths.\textsuperscript{a, b}

<table>
<thead>
<tr>
<th>Column</th>
<th>Reagent Composition</th>
<th>Efficiency\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initiator\textsuperscript{d}</td>
<td>Monomer\textsuperscript{d}</td>
</tr>
<tr>
<td>Cyclohexanol to 1,4-butanediol ratio</td>
<td>T1</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>T5</td>
<td>0.10</td>
</tr>
<tr>
<td>Total porogen to monomer ratio</td>
<td>T6</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>T7</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>T8</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>T9</td>
<td>0.10</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Percentage of mass. All monoliths were polymerized at 60 °C for 24 h.
\textsuperscript{b}Conditions: 150 μm i.d. monolithic column, 300 nL/min flow rate.
\textsuperscript{c}Column efficiency (plates/m).
\textsuperscript{d}Percentage by weight.
\textsuperscript{e}Could not be measured because of large gaps in the monolith structure.
Table 4.3. Effect of different reagent compositions on column efficiency for poly(PETA) monoliths.a, b

<table>
<thead>
<tr>
<th>Column Reagent Composition</th>
<th>Efficiency&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Efficiency&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclohexanol to 1,4-butanol ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1 0.10 25.02 55.20 19.78 0.6 3.0 2.8</td>
<td>31,400/35,100</td>
<td></td>
</tr>
<tr>
<td>P2 0.10 24.97 56.23 18.80 0.6 3.0 3.0</td>
<td>39,800/58,100</td>
<td></td>
</tr>
<tr>
<td>P3 0.10 24.97 57.70 17.33 0.6 3.0 3.3</td>
<td>39,500/46,100</td>
<td></td>
</tr>
<tr>
<td>P4 0.10 24.96 58.35 16.68 0.6 3.0 3.5</td>
<td>39,300/42,100</td>
<td></td>
</tr>
<tr>
<td>P5 0.10 24.91 59.75 15.34 0.6 3.0 3.9</td>
<td>e</td>
<td></td>
</tr>
<tr>
<td>Total porogen to monomer ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2 0.10 24.97 56.25 18.78 0.6 3.0 3.0</td>
<td>39,800/58,100</td>
<td></td>
</tr>
<tr>
<td>P6 0.10 23.21 57.47 19.32 0.6 3.3 3.0</td>
<td>43,300/60,200</td>
<td></td>
</tr>
<tr>
<td>P7 0.10 22.73 57.95 19.32 0.6 3.4 3.0</td>
<td>40,700/42,000</td>
<td></td>
</tr>
<tr>
<td>P8 0.10 21.71 58.69 19.60 0.6 3.6 3.0</td>
<td>24,600/29,300</td>
<td></td>
</tr>
<tr>
<td>P9 0.10 19.98 59.95 20.07 0.6 4.0 3.0</td>
<td>f</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Percentage of mass. All monoliths were polymerized at 60 °C for 24 h.
<sup>b</sup> Conditions: 150 μm i.d. monolithic column, 300 nL/min flow rate.
<sup>c</sup> Column efficiency (plates/m).
<sup>d</sup> Percentage by weight.
<sup>e</sup> Could not flush the column with over 400 bar (5800 psi).
<sup>f</sup> Could not be measured because of large gaps in the monolith structure.
First, the porogen ratio (i.e., cyclohexanol to 1,4-butanediol) was investigated. Other amounts were constant for all columns [i.e., total porogen/monomer was 3:1 (wt/wt), initiator was 0.1% (wt) of monomer, promoter (BTEE) volume was 0.6 μL, and capillary i.d. was 150 μm]. The data in Table 4.1 show that column efficiency increased to a point with an increase in amount of cyclohexanol. The best efficiencies were obtained for uracil and toluene when the cyclohexanol to 1,4-butanediol ratio was 3 to 1. Column efficiency became worse when the percentage of cyclohexanol increased further. Along with this increase in cyclohexanol composition, gelation occurred earlier than phase separation, and heterogeneity was, therefore, introduced into the monolith structure, which resulted in a decrease in column efficiency.

Second, the total porogen to monomer ratio was studied for 3:1 cyclohexanol/1,4-butanediol. When the total porogen to monomer ratio was raised from 2.5:1 to 3.3:1, the column efficiency improved from approximately 7,700 and 11,000 plates/m to 43,800 and 48,000 plates/m for uracil and toluene, respectively. However, with a further decrease in monomer, the monolith skeleton became thinner and the pore size became larger, which led to a decrease in column efficiency.

Third, the added amount of initiator (AIBN) and added amount of promoter (BTEE) were considered. With an increase in the amount of AIBN or BTEE, the population of growing chains increased, which resulted in lower average molecular weight of each growing chain and reduction in tendency for phase separation. If the amount of AIBN or BTEE added was too small, phase separation occurred earlier than gelation. Large globules and pores were formed, and the column efficiency was poor. On the other hand, if the amount of AIBN or BTEE was too large, gelation took place earlier, and the resultant monolith skeleton was not homogeneous. From the experimental results, the column with initiator percentage of 0.10% and promoter amount of 0.6
μL BTEE gave the best performance.

Finally, monolithic columns prepared in capillaries with different inner diameters (i.d.) were compared. With increasing capillary i.d. from 75 to 150 μm, the pore volume and column efficiency both increased. Therefore, 3:1 (wt/wt) total porogen to monomer ratio, 3.3:1 cyclohexanol to 1,4-butanediol porogen ratio, 0.1% initiator (i.e., 0.1% of the amount of monomer), and 0.6 μL of promoter (BTEE) in a 150 μm capillary were selected for preparation of poly(1,12-DoDDMA) monoliths.

The polymerization conditions for TMPTMA and PETA were also investigated, as listed in Tables 4.2 and 4.3. The combination of 3:1 (wt/wt) total porogen to monomer ratio, and 3.3:1 cyclohexanol to 1,4-butanediol resulted in the best efficiencies for both poly(TMPTMA) and poly(PETA) monoliths. The effects of initiator ratio, capillary i.d. and promoter volume were not examined for either TMPTMA or PETA.

4.3.3 Monolith morphologies

Figure 4.3 shows SEM images of monoliths synthesized from 1,12-DoDDMA (D8), TMPTMA (T7) and PETA (P6). From the SEM images, it appears that all three monoliths formed small globules. Poly(TMPTMA) had smaller throughpores than the other two monoliths, which resulted in the highest back pressure (2.25 Mpa at 300 nL/min flow rate) (Figures 4.3C and D). Poly(PETA) contained some large globules (Figures 4.3E and F), which led to very low back pressure (0.39 MPa at 300 nL/min flow rate).

4.3.4 Chromatographic efficiency measurements

Rigid structural monoliths were obtained using all of the monomers, and all monolithic columns could be used to separate alkylbenzenes. Figure 4.4 shows isocratic chromatograms of uracil, toluene, ethylbenzene, propylbenzene, butylbenzene, and amylbenzene at 300 nL/min.
Figure 4.3. SEM images of monoliths. (A) and (B) poly(1,12-DoDDMA) (D8), (C) and (D) poly(TMPTMA) (T7), (E) and (F) poly(PETA) (P6); see structures in Figure 4.2.
(i.e., 0.283 mm/s) with D8, P6 and T7 monolithic columns listed in Tables 4.1, 4.2 and 4.3. As poly(PETA) has a C5 functional group, which is weaker than those in poly(1,12-DoDDMA) (i.e., C12) and poly(TMPTMA) (i.e., C6), it shows the lowest hydrophobicity. Poly(PETA) monoliths exhibit the weakest selectivity for alkylbenzenes. Therefore, an isocratic program with less ACN (40% H2O and 60% ACN) was used for poly(PETA) columns. As can be seen in Figure 4.4, all peaks had good symmetries and narrow peak widths at half peak height for the alkylbenzenes. The plate numbers for all of the monolithic columns were between 43,300 and 53,300 plates/m measured using uracil as a non-retained compound and between 47,700 to 64,200 plates/m for, 1,12-DoDDMA, TMPTMA and PETA columns using alkylbenzenes as retained compounds at 300 nL/min.

Poly(1,12-DoDDMA) demonstrated the best selectivity among these three monolithic columns because its functional group (i.e., C12) has the highest hydrophobicity compared to the other two monoliths. However, poly(PETA) and poly(TMPTMA) showed better column efficiencies than poly(1,12-DoDDMA), as the shorter chain lengths of the functional groups and greater number of double bonds in TMPTMA and PETA lead to more homogeneous structures than 1,12-DoDDMA.

A van Deemter curve for a T7 column was determined as shown in Figure 4.5. The maximum theoretical plate number was 58,800 plates/m for uracil as a non-retained compound. Compared to the performance of good polymeric monoliths reported previously, the performance of these monoliths was comparable.

4.3.5 Column permeability and stability

Column permeability was used to evaluate the stability of the monoliths. To obtain plots of back pressure versus flow rate, acetonitrile, methanol and water were pumped through each
Figure 4.4. RPLC separations of alkylbenzenes on monoliths synthesized from PETA (P6), 1,12-DoDDMA (D8), and TMPTMA (T7), respectively. (A) P6 monolithic column: 12.5 cm × 150 μm i.d.; 40% A/60% B mobile phase; (B) D8 monolithic column: 15 cm × 150 μm i.d.; 30% A/70% B mobile phase; (C) T7 monolithic column: 13 cm × 150 μm i.d.; 30% A/70% B mobile phase; 300 nL/min flow rate; on-column UV detection at 214 nm. Peak identifications: uracil, toluene, ethylbenzene, propylbenzene, butylbenzene and amylbenzene in order of elution.
Figure 4.5. Plate height versus linear velocity for a poly(TMPTMA) (T7) monolithic column using uracil as a non-retained compound (average of three repetitions). Conditions: 13 cm×150 μm i.d. column; 30% water/70% acetonitrile mobile phase; on-column UV detection at 214 nm.
10-cm long monolithic column at six different flow rates from 100 to 1000 nL/min. Linear relationships (Figure 4.6) between back pressure and flow rate ($R^2>0.999$ for all monoliths) clearly indicated that the monoliths were mechanically stable. The permeabilities calculated based on Darcy’s law are listed in Table 4.4. All monolithic columns were found to slightly shrink in polar solvents, which led to higher permeability in water. They swelled slightly in acetonitrile as well, which resulted in the sharp peaks observed in Figure 4.4.

4.4 Conclusions

New monolithic RPLC stationary phases based on single monomers were synthesized using organotellurium-mediated living radical polymerization. These new monolithic columns were successfully used for the separation of alkylbenzenes under RP conditions. SEM images were taken which showed different globule sizes for monoliths made from different dimethacrylates/diacrylates. Permeability tests indicated that the monoliths were mechanically stable. A van deemter curve for a poly(trimethylolpropane trimethacrylate) monolith was determined for uracil, which demonstrated the highest efficiency of 58,800 plates/m.
Figure 4.6. Effect of mobile phase flow rate on column back pressure (average of three repetitions).

Conditions: 10 cm×150 μm i.d. monolithic columns.
Table 4.4. Permeabilities of poly(alkanediol multi-methacrylate/multi-acrylate) monolithic columns using different liquids.

<table>
<thead>
<tr>
<th>Liquid</th>
<th>Relative polarity$^a$</th>
<th>Viscosity (mPa s)$^b$</th>
<th>Permeability ($\times 10^{-14}$ m$^2$)$^c,d$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1,12-DoDDMA</td>
</tr>
<tr>
<td>Water</td>
<td>1.00</td>
<td>0.89</td>
<td>3.33±0.24</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.76</td>
<td>0.54</td>
<td>2.14±0.22</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>0.46</td>
<td>0.37</td>
<td>1.71±0.02</td>
</tr>
</tbody>
</table>

$^a$ Relative polarity data are from ref 25.

$^b$ Viscosity, $\eta$, data are from online CRC Handbook of Chemistry and Physics, 89th ed.; CRC: Boca Raton, FL, 2008-2009.

$^c$ Permeability $k = \eta L u / \Delta P$, where $\eta$ is the viscosity, L is the column length (10 cm in this case), u is the solvent linear velocity, and $\Delta P$ is the column back-pressure.

$^d$ Average of six trials at different flow rates ± standard deviation.
4.5 References


CHAPTER 5 FUTURE DIRECTIONS

5.1 Introduction

The properties of organic monoliths, such as broad chromatographic selectivity, high porosity, and independent optimization of through-pore and skeleton size, make them attractive as LC stationary phases. On the other hand, organic monoliths have not generally demonstrated acceptable performance for small-molecule RPLC; their chromatographic efficiencies fall far short of efficiencies obtained today using modern silica-based small-particle and monolithic RPLC. Considerable effort is now being focused on improving the performance of organic monoliths for small-molecule applications. Adjusting and controlling the reaction conditions, utilizing new monomers and porogens, and introducing new additives and particles into the monolith structure are some of the current efforts. Since the bed structures of organic monolithic columns, characterized by surface area, monolith skeletal structure, and pore size and distribution, affect monolith performance, accurate measurements of these properties are essential for future advancements. I believe that improvements in chromatographic performance will be accomplished most efficiently by systematically tailoring the monolith morphology in response to measurements of quantitative descriptors. Identifying and controlling the factors governing morphology and the ability to correlate these quantitative descriptors with chromatographic performance measurements would greatly aid in structure-directed optimization of synthetic methods.

5.2 Selection of porogens for preparation of monolithic columns

The morphologies and homogeneities of monolithic columns are very important for separations. The pore structure of a monolith is influenced by several variables, including initiator nature and concentration, monomer to cross-linker ratio, and porogen nature and ratio,
which includes monomer to porogen ratio and ratio of different porogens if more than one porogen is used. The most important factors that affect synthesis of the desired porous structure of a polymer monolith are the selection of porogen(s) and porogen ratio. Up to now, there is still no general theory proposed for porogen selection. Selection of appropriate porogens still must primarily depend on experiments and experience. According to experiments in our lab, several basic factors are important. First, the porogen or porogen combination must be miscible with all reagent components. A homogeneous polymerization mixture solution is a prerequisite for developing a good monolith. Second, both poor and good solvents are required in the polymerization mixture, so that the morphology can be adjusted by varying the ratio between the different types of porogens. Third, the porogen must be compatible with the initiation technique.1

According to some literature reports, polarity and solubility of the solvents affect the formation of monoliths.2,3 The work of Courtois et al. predicted that porogens that exhibited high dipole moment values were likely to produce monoliths with small pore diameter for the monomer system containing glycidyl methacrylate, triethylene glycol dimethacrylate and trimethylol-propane trimethacrylate.3 Similar results were found in previous work in our lab. When we prepared poly(hydroxyethyl acrylate-co-triethylene glycol diacrylate) monoliths, the replacement of methanol with water resulted in monoliths with lower permeability.4

The solubility parameter (δ) is another guide for selection of porogens.5 Various articles have mentioned that if the solvent has a similar δ value as the monomer, the solvent can be considered to be a “good” solvent, while a solvent is a “poor” solvent for the monomer if there is a large difference between the two δ values of the monomer and solvent.

Hansen parameters, which include four, are potential references for porogen selection. The parameters δd, δp, δh and δt represent dispersion, polarity, hydrogen-bonding and total
Table 5.1. Hansen parameters for some solvents at 25 °C.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>( \delta_d )</th>
<th>( \delta_p )</th>
<th>( \delta_h )</th>
<th>( \delta_t )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>15.1</td>
<td>12.3</td>
<td>22.3</td>
<td>29.6</td>
</tr>
<tr>
<td>Iso-butanol</td>
<td>15.1</td>
<td>5.7</td>
<td>16.0</td>
<td>22.7</td>
</tr>
<tr>
<td>Decanol</td>
<td>17.6</td>
<td>2.7</td>
<td>10.0</td>
<td>20.4</td>
</tr>
<tr>
<td>Butanediol</td>
<td>16.6</td>
<td>10</td>
<td>21.5</td>
<td>28.9</td>
</tr>
<tr>
<td>Cyclohexanol</td>
<td>17.4</td>
<td>4.1</td>
<td>13.5</td>
<td>22.4</td>
</tr>
<tr>
<td>Toluene</td>
<td>18.0</td>
<td>1.4</td>
<td>2.0</td>
<td>18.2</td>
</tr>
<tr>
<td>DMF</td>
<td>17.4</td>
<td>13.7</td>
<td>11.3</td>
<td>24.8</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>17.0</td>
<td>11.0</td>
<td>26.0</td>
<td>32.9</td>
</tr>
</tbody>
</table>
Table 5.2. Dispersion, polar and hydrogen bonding group contributions.

<table>
<thead>
<tr>
<th>Groups</th>
<th>z</th>
<th>$\Sigma_z F_d / J \text{ cm}^3 \text{ mol}^{-1}$</th>
<th>$\Sigma_z F_p / J \text{ cm}^3 \text{ mol}^{-1}$</th>
<th>$\Sigma_z U_h / J \text{ mol}^{-1}$</th>
<th>$V / \text{ cm}^3 \text{ mol}^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>-CH$_2$-</td>
<td>270</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16.6</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>420</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>31.7</td>
</tr>
<tr>
<td>=CH$_2$</td>
<td>400</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>32.1</td>
</tr>
<tr>
<td>=CH-</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12.4</td>
</tr>
<tr>
<td>-O</td>
<td>80</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-1.0</td>
</tr>
<tr>
<td>COO</td>
<td>-70</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-19.2</td>
</tr>
<tr>
<td>-COO</td>
<td>390</td>
<td>490</td>
<td>7</td>
<td>20000</td>
<td>8.2</td>
</tr>
<tr>
<td>-OH</td>
<td>210</td>
<td>500</td>
<td>20000</td>
<td>10.5</td>
<td></td>
</tr>
</tbody>
</table>

$^a F_d =$ Dispersion group molar attraction constant.

$^b F_p =$ Polar group molar attraction constant.

$^c U_h =$ Hydrogen bonding parameter group molar attraction constant.

$^d V =$ Molar volume group constant.
cohesion, respectively. Based on many experimental results, the relation among these parameters should be:

\[
\delta_t^2 = \delta_d^2 + \delta_p^2 + \delta_h^2 \tag{5.1}
\]

The combination of these parameters describes the miscibility behavior of all polymer-liquid systems.

\[
\delta_d = \left( \sum z^2 F_d \right) / V \tag{5.2}
\]

\[
\delta_p = \left( \sum z^2 F_p \right)^{1/2} / V \tag{5.3}
\]

\[
\delta_h = \left( \sum z^{-2} U_h / V \right)^{1/2} \tag{5.4}
\]

Table 5.1 lists some examples of Hansen parameters for solubility for some organic solvents. They would be useful as guidelines for initial selection of porogen(s). In the process of forming monoliths, the type and ratio of porogen(s) could be adjusted in the system in order to obtain good monolithic stationary phases. Based on Equations 5.1 to 5.4, and the constants in Table 5.2, Hansen solubility parameters of some monomers could be estimated. From the Hansen parameters, solvents could be selected as potential porogens, which could lead to monoliths with the desired structures, if the Hansen parameters for the porogens were close to those of the monomer system.

### 5.3 Investigation of polymerization method selection for synthesis of monolithic columns

Various polymerization methods have been reported for the synthesis of organic monoliths. Conventional free radical polymerization, which includes thermal-, photo-, microwave, and γ radiation, is the most popular method. In addition, controlled/living polymerization methods, such as atom transfer radical polymerization (ATRP), reversible addition-fragmentation chain transfer (RAFT), nitroxide-mediated living radical
polymerization (NMP)\textsuperscript{13} and organotellurium-mediated living radical polymerization (TERP)\textsuperscript{14} have also been introduced, and are receiving increasing attention in this field. Due to their different properties, and the different reaction conditions and reagents that are needed for each polymerization method, I suggest that it is necessary to compare the polymerization conditions, such as polymerization temperature, polymerization time, accessibility of equipment and reagents, environmental conditions (e.g., anhydrous and anaerobic environments), morphology, efficiency and applications of resultant monoliths from these methods. Their characteristics should be studied and compared to determine if new polymerization methods could be invented. If guidelines for choosing polymerization methods could be established, it would be much more convenient and faster to find the optimum conditions for preparation of monoliths.

5.4 Synthesis of new bi-functional cross-linking monomers for RPLC

It has been demonstrated that single cross-linking monomers have advantages compared to monoliths prepared from a combination of vinyl and divinyl monomers, such as improved column-to-column reproducibility, increased surface area, higher rigidity and better mechanical stability.\textsuperscript{1} However, single bi-functional cross-linking monomers are not as widely available as traditional functional monomers. Therefore, design and investigation of new single monomers (i.e., structures shown in Figure 5.1) are necessary.

Monomer 1, a typical diacrylate or dimethacrylate, can be used as a monomer for preparation of monoliths. Monomer 1 has a brush-like long alkyl chain, which is a good functional group for reversed-phase conditions, and it can be synthesized straightforwardly (Scheme 1) from esterification of 2-alkyl-1,2-propanediol (2) and acryloyl chloride (or methacryloyl chloride). Compound 2 can be prepared by reduction of the corresponding
dimethyl malonate 3, which can be synthesized from the reaction of a commercial 1-bromo aliphatic chain and dimethyl malonate under basic conditions with EtONa.\textsuperscript{15}

If $R_1$ is a phenyl group, monomer 4 can be synthesized simply as shown in Scheme 5.1, since it can be straightforwardly prepared from esterification using commercial 2-phenyl-1,2-propanediol (2, $R_1 = \text{Ph}$). Monomers 4 and 5 could also be used to prepare monoliths (see Figure 5.2) for RPLC.
Figure 5.1. Structures of monomer 1 for RPLC.
**Scheme 5.1.** Synthesis route for monomer 1.

\[
\begin{align*}
\text{H}_2\text{CO} & \quad \xrightarrow{\text{EtONa, R}_1\text{Br}} \quad \text{EtOH} \\
\text{H}_2\text{CO} & \quad \xrightarrow{\text{R}_1\text{OH}} \\
\text{HO} & \quad \xrightarrow{\text{R}_1\text{OH}} \\
\end{align*}
\]

(1) NaBH₄, LiCl
THF, EtOH
20-30°C, 1h
(2) HCl < 20°C, 1h

**R**₁ = \( n\text{C}_\text{H}_{2n+1} \) (n > 6), Phenyl, and Benzy1
**R**₂ = H, CH₃
Figure 5.2. Structures of monomers 4 and 5.
5.5 References


