FUNDAMENTAL STUDIES WITH FUNCTIONALIZED LOW TEMPERATURE GLASSY CARBON IN LIQUID CHROMATOGRAPHY, SOLID-LIQUID EXTRACTION, AND CAPILLARY ELECTROPHORESIS

DISSERTATION

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By

Justin W. Shearer, B.S.

* * * * *

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Dissertation Committee:

Professor Susan V. Olesik, Adviser
Professor Terry L. Gustafson
Professor Derek J. Hansford

Approved by

________________
Adviser
Chemistry Graduate Program
ABSTRACT

The application of functionalized low temperature glassy carbon (LTGC) in three areas of separation science is examined. Liquid chromatography was performed with two fluorine-containing LTGC stationary phases, thermally processed to 200 °C and 400 °C, in an effort to determine the interactions that are important in obtaining separation by employing the solvation parameter model by Abraham. The interactions that are important for describing retention on the 200 °C processed F-LTGC stationary phase are hydrogen bond basicity > dispersion > hydrogen bond acidity > dipolarity/polarizability. The interactions that describe the retention on the 400 °C processed F-LTGC are hydrogen bond basicity > dispersion > excess molar refraction > hydrogen bond acidity. The solvation parameter model for the 200 °C processed F-LTGC showed similar trends in the relative importance of intermolecular interactions as previously found for octadecyl-polysiloxane stationary phases, while the 400 °C processed F-LTGC had similar intermolecular interactions with solutes as found with porous glassy carbon in that π–π interactions with the carbon surface contribute more so to the retention.

High surface area media are desirable substrates for solid-phase extraction. The high surface area promotes extraction of small quantities of solute, which permits lower detection limits of extracted analytes. Solid-phase extraction was performed with
resorcinol-formaldehyde carbon cryogels and fluorine-containing LTGC (F-LTGC) coated cryogels to determine differences in extraction abilities of the F-LTGC as a function of thermal processing. The extraction ability was compared to that observed for bare cryogels, and it was determined that the extraction abilities of the F-LTGC coated gels appeared different for F-LTGC processed between 200-600 °C, with respect to the bare cryogels. The extraction behavior of F-LTGC thermally processed at >800 °C was determined to be similar to the bare cryogel. Insight into solute characteristics that favor extraction was obtained, and it was determined that dispersion is important, as planar molecules are better extracted by F-LTGC coated carbon aerogels than uncoated carbon cryogels.

Capillary electrophoresis and chip electrophoresis were performed using F-LTGC and a silicon-containing LTGC (Si-LTGC). A precursor to the study of the electrophoretic behavior of LTGC chips and channels involved studying the ability to fabricate micrometer and nanometer features of LTGC. Micrometer and nanometer features were successfully fabricated by employing replica molding. The electrophoretic performance for fused silica, F-LTGC, and Si-LTGC (both LTGCs processed at 200 °C) is examined and compared. A comparison of electrophoretic peak areas shows that there is a difference between the performance between the LTGCs and fused silica column in capillary electrophoresis. Both LTGCs used produced peaks with smaller peak areas than on fused silica, which is attributed to analyte sorption on the LTGC surfaces. Shorter
retention times were also observed for LTGC columns with respect to fused silica. The pressure-assisted electrophoretic behavior of Si-LTGC and F-LTGC chips was compared using a fluorescent dye, rhodamine B. F-LTGC chips produced electropherograms with longer retention times, smaller peak areas, and larger peak widths than Si-LTGC chips. The longer retention times, broader peaks, and smaller peak areas is indicative of more interaction/sorption by F-LTGC than Si-LTGC.
To my loving wife and family

My biggest supporters
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VITAE

September 13, 1979 . . . . . . . Born, Connellsville, Pennsylvania, USA

May, 2002 . . . . . . . . . . . . . B.S., Chemistry (ACS Certified), Westminster
College, New Wilmington, Pennsylvania

2002-2007 . . . . . . . . . . . . . Graduate Teaching Associate and Research
Assistant, the Ohio State University, Department of
Chemistry, Columbus, Ohio

2007-present . . . . . . . . . . . . Instructor in Chemistry, University of North,
Alabama, Department of Chemistry and Industrial
Hygiene, Florence, Alabama

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1.1 Overview

Carbon based separation media are increasingly being used in many areas of separation science. Hypercarb, a porous-graphitic carbon, is very attractive for use in liquid chromatography, as it displays excellent robustness over the entire spectrum of pH [1, 2]. Carbon separation media have also been investigated in gas chromatography (GC), solid phase microextraction (SPME), and as an electrode in capillary and microchip capillary electrophoresis applications. The following dissertation discusses the application of carbonaceous media in three types of separations: liquid chromatography, solid-liquid extraction, and microchip capillary electrophoresis. Each of the following chapters provides an introduction that pertains specifically to the material and techniques employed to perform the study, while this introductory chapter should serve to inform the reader of some basics of the techniques employed and serve to calibrate the reader with what has been done in the field prior to this work.

1.2 Thermodynamics and Separations

In order for an analyte to be retained in a chromatographic application, there must
be partitioning between two phases. In liquid chromatography these phases are the mobile phase and the stationary phase and in solid-phase extraction (SPE) these phases are the liquid and the solid sorbent in SPE. This partitioning is governed by the equilibrium expression, as seen in equation 1.1:

\[ K = \frac{[\text{solute}]_s}{[\text{solute}]_m} \]  

(1.1)

This equation relates the concentration of solute in the stationary phase in liquid chromatography or solid sorbent in solid-phase extraction to the concentration of analyte in the mobile phase in liquid chromatography and the solution in solid-phase extraction.

In liquid chromatography, the general resolution equation emphasizes the importance of thermodynamics, especially phase equilibrium, which occur during partitioning:

\[ R = \frac{\sqrt{N}}{4} \frac{(\alpha - 1)}{\alpha} \frac{k}{1 + k} \]  

(1.2)

where \( N \) represents efficiency, \( k \) is the retention factor, and \( \alpha \) is the selectivity factor, the ratio of the retention factors for the two species being separated. The retention factor is described by Equation 1.2:

\[ k = \frac{t_r - t_m}{t_m} = \frac{V_r - V_m}{V_m} = K \frac{V_s}{V_m} \]  

(1.3)

and is dependent on the amount of time a species spends in the column (\( t_r \)) with respect to a species that has no interaction with the stationary phase (\( t_m \)). The time can be easily converted to volume (\( V \)) by multiplying by the volumetric flow rate used during analysis. The \( K \) in the third expression is the equilibrium constant of phase transfer, and is
described in equation 1.1. The retention factor is, therefore, a measure of the thermodynamics of phase transfer equilibria. This relation between retention factor and equilibrium constant demonstrates the link between partition coefficient and free energy.

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

(1.4)

Where \( \Delta G \) is the free energy of partitioning, \( R \) is the ideal gas constant, \( T \) is temperature in Kelvin, and \( K \) is the partition coefficient. The free energy of transfer between two phases, partitioning, is dependent on how a solute interacts with the components of the two phases.

1.3 Linear Free Energy Relationships

Linear free energy relationships are a useful tool that can describe retention mechanisms on stationary phases in liquid and gas chromatography [3]. Employment of this statistical model takes advantage of the thermodynamic and kinetic principles that govern liquid chromatography. Initially, solvatochromism was employed to investigate the relative strength of chemical and physical interactions a solute undergoes with its surroundings [4, 5, 6, 7]. Solvatochromism refers to effect of solvent on some spectroscopic property of a molecule. Solvatochromic scales of solvent strength were developed by the physical organic chemists; Kamlet, Taft, Abboud, and coworkers [4, 5, 6]. They developed scales for the dipolarity/polarizability, hydrogen bond donor, and hydrogen bond acceptor strength of solutes. These scales were initially developed to systematically probe the solvent effects on spectroscopic properties of solutes, reaction rates, and equilibrium constants of chemical reactions [4, 5, 6]. The \( \beta \)-scale is essentially
a measure of solvent’s ability to accept a hydrogen bond, and was obtained for many solvents by examining the $^{19}$F-NMR shift by exposing 4-fluorophenol to different solvents [4]. The $\alpha$-scale of solvent hydrogen bond donations abilities was derived by monitoring the shifts of an ultraviolet-visible (UV-vis) band of a pyridine molecule as it is dissolved in different solvents [5]. Kamlet et al. derived the $\pi^*$-scale of solvent polarities by observing the spectral shifts of electronic transitions [6]. The careful determination of these scales of solvent intermolecular forces and scaling them from 0-1 permits correlations to be drawn from free energy related properties.

Linear free energy relationships or linear solvation energy relationships (LSERs) are employed to relate a free energy related property to various chemical or physical interactions. LSERs have been used to relate spectroscopic properties and chromatographic properties [8, 3, 9]. The general equation for LSERs as they pertain to liquid chromatography is shown in Equation 1.5:

$$XYZ = XYZ_0 + \text{intermolecular interactions} + \text{cavity formation}$$

(1.5)

where XYZ represents a free energy related property that is solvent dependent and measurable, such as spectroscopic shift, rate constant of a reaction, partition coefficient or retention factor [3]. LSERs are used to describe the relative importance of various intermolecular forces involved in the retention of various analytes in liquid chromatography. The thermodynamic relationship between retention factor, k, and partition coefficient, K, permits one to use retention factor as a free energy related property. This relationship justifies the validity of LSER models based on retention factor, a chromatographic figure of merit, to liquid and gas chromatographies.
Equation 1.6 is an LSER model that has been used to study a large range of analyte sorption in gas chromatography and liquid chromatography [3, 8, 9]. The logarithm of retention factor is used to ensure that a linear relationship between retention factor and free energy is maintained [3]. This LSER model correlates the observed retention with various specific intermolecular interactions. $\Sigma \alpha_2^H$, $\Sigma \beta_2^H$ and $\pi_2^*$ are Abraham solute parameters that describe the solute’s overall hydrogen bond acidity, hydrogen basicity and dipolarity/polarizability, respectively [10, 11, 12]. $V_x$ is the McGowan’s characteristic volume, which describes dispersive interactions and can be calculated from the structure of the molecule [11, 12]. $R_2$ is the excess molar refraction; this term is added to compensate for lumping solute dipolarity and polarizability into one $\pi^*$ descriptor. $R_2$ can be estimated from refractive index measurements [13].

$$\log k = \log k_0 + vV_x + s\pi_2^* + rR_2 + a\Sigma \alpha_2^H + b\Sigma \beta_2^H \quad (1.6)$$

The coefficients’, $\log k_0$, $v$, $s$, $r$, $a$, and $b$, relative magnitude represents the relative importance of specific intermolecular interaction to retention. The LSER model of chromatographic retention has undergone many iterations and is now most commonly employed as the solvation parameter model by Abraham [14]. The LSER equation simplifies to the solvation parameter model as seen in Equation 1.7:

$$\log k = c + vV + sS + eE + aA + bB \quad (1.7)$$

The terms in the solvation parameter are very similar to those used in the LSER model, except the greek characters have been reduced to arabic letters. The $V$ is McGowan’s volume, $S$ is the solute dipolarity/polarizability, $E$ is the excess molar refraction, $A$ is the hydrogen bond acidity (donation), and $B$ is the hydrogen bond basicity. The solvation
parameter model by Abraham is employed in the following work as a tool to understand the role interactions play in the retention behavior of two carbon-based stationary phases in reversed-phase liquid chromatography.

1.4 Low Temperature Glassy Carbon

Low temperature glassy carbon (LTGC) is a polymer that was first developed by Callstrom et al., and is unique in that temperatures between 200 °C and 600 °C are sufficient to produce a sp$^2$-hybridized network of carbon [15]. Olesik and colleagues have performed the bulk of the work in which LTGCs have been applied to separations. Specifically two functionalized LTGCs have been employed for these studies, a silicon-containing LTGC (Si-LTGC) and a fluorinated LTGC (F-LTGC). The chemical structure of the diethynl aromatic oligomers that form LTGC are shown in Figure 1.1. The LTGCs used in the following studies are soluble in common organic solvents, and the high levels of unsaturation permits polymerization at relatively low temperatures. Upon polymerization, the LTGC forms a type of glassy carbon. Glassy carbon can be thought of as having two main areas where interactions can occur. The first of these areas are flat basal planes, which permit interactions similar to sheets of graphite. The second area is the edge planes of the glassy ribbons. One accepted structure of glassy carbon is that amorphous ribbons of graphite sheets in close proximity to one another. Some of the applications in which functionalized low temperature glassy carbon has been used are high performance liquid chromatography (HPLC) [16, 17, 18], supercritical fluid chromatography (SFC) [19, 20], and solid phase microextraction (SPME) [21, 22].
Figure 1.1: (A) Chemical structure of Si-containing low temperature glassy carbon (Si-LTGC): (B) Chemical structure of fluorine-containing low temperature glassy carbon (F-LTGC).
1.5 Carbon Phases in Liquid Chromatographic Separations

Glassy carbon/porous graphitic carbon (PGC) stationary phases are receiving increased attention in liquid chromatographic separations. Glassy carbon surfaces exhibit excellent mechanical strength, are resistant to chemical attack, and have selective retention characteristics [1]. Selective separations of highly polar analytes, such as nucleosides [23] and anions [2, 24] are possible with a glassy carbon/PGC surface, while the selective separation of structural isomers of nonpolar analytes is also readily achieved [2]. Low temperature glassy carbons (LTGCs) convert to glassy carbon at relatively low temperatures (200-400 °C) compared to commonly used precursor polymers that require high temperatures (2000-3000 °C) to produce PGC [15, 25].

1.6 Organic Aerogels

Carbon aerogels are highly porous materials that possess unique nano- and macroscopic properties. Various synthetic conditions and processing parameters have been investigated for the production of carbon aerogels [26, 27, 28]. Resorcinol: formaldehyde (R:F) aerogels have been described as possessing porosities greater than 80%, surface areas between 400 - 1200 m² g⁻¹, and pore volumes that vary with the synthetic and processing conditions [27].

Various synthetic conditions; ratios of starting materials, pH of the initial sol, and drying conditions; have been studied to determine their effects on the pore size in the resulting aerogels. It has been determined that R: F aerogels synthesized with a low resorcinol to catalyst ratio (R:C) (50:1) produced gels that exhibited small particles (3-5
nm) with large diameter connections between particles [29, 30]. R:F aerogels synthesized through low R:C ratios were determined to have higher densities than gels produced with large R:C ratios. The particles in the R:F aerogels synthesized using high R:C ratios were determined to be between 16-200 nm [29, 30, 31]. These gels appeared as a pearl necklace.

1.7 Extraction Media in Separation Science

Extractions of analytes are performed by exploiting the preferential partitioning of a substance between two different phases. If a substance exists in only one form, the partitioning of that substance is described using the partition coefficient (K). The partition coefficient is dependent on the concentration of the substance in each phase at equilibrium, as shown in Equation (1.1). In solid-phase extraction, the sorbent is represented by s and the analyte being extracted is in a mixture, by m. Solid phase extraction (SPE) encompasses extracting a substance from a liquid phase into a solid phase, and can be performed in one of two ways: batch and packed. In batch solid phase extractions, the solid is mixed with the solution containing the analyte of interest, while in packed solid phase extraction, the solution is often passed through a tube packed with the solid material.

Since the extraction of the solute being extracted is dependent upon equilibrium between the solution and solid adsorbent, a solid with a high surface area will shift equilibrium toward the solid. A sorbent with a high surface area increases the amount of solution in contact with the sorbent, and promotes extraction of analytes from solutions.
with relatively low concentrations. Many solids used in SPE have surface areas between 200 and 800 m$^2$g$^{-1}$ [32]. Two other properties that make SPE successful are that a reproducible percentage of solute be sorbed to the solid and that the solutes are easily desorbed from the solid medium [32]. It is imperative that SPE sorbents exhibit good chemical stability, as acidic, alkaline, or organic solvents are used to desorb the solutes [32]. Thermal desorption is also sometimes employed to remove solids from the SPE sorbent [32]. It is also important that the sample solution is able to have good contact with the solute solution [32]. This means that a hydrophobic SPE sorbent will not have good surface contact with aqueous solutions.

Quantification in solid phase extraction is attainable by employing auxiliary tests post SPE. The amount of solute extracted from solution can be obtained by exploiting a quantifiable property of the concentrated solution and solution after exposure to the sorbent and comparing the response to a calibration curve. This method would permit examination of the solution before and after extraction, and simple subtraction of the determined concentrations would result in the amount of solute extracted by the sorbent. The percent extracted would then be calculated by dividing the amount of solute extracted by the amount of solute in the concentrate. The percent recovered can also be calculated after the adsorbed solute is desorbed from the sorbent and monitored by some other technique. This concentration, as determined from a calibration curve, could be compared using similar steps as in determining the amount and percent of solute extracted. Several techniques used to determine the amount extracted, percent extracted, amount recovered and percent recovered include ultraviolet-visible spectroscopy, high
performance liquid chromatography, and gas chromatography [32].

1.8 Microfabrication Techniques

The fabrication of micrometer features has been accomplished using photolithography for several years in the semiconductor industry [33, 34, 35]. Photolithographic processes employ chemical and energetic processes to create micrometer features. Electromagnetic radiation is used to modify the chemistry of a photosensitive polymer, photoresist, in such a way as to change the solubility of the photoresist. Alkaline and acidic solutions can also be used to etch solid surfaces in order to produce micrometer features [33, 35]. Electron beam lithography has been used for the fabrication of nanometer features [34, 36]. Soft lithographic processes, which permit the fabrication of micrometer features in elastomeric polymers, have been gaining increased exposure [37]. Soft lithography also includes the use of the microfabricated elastomeric material as a template by which other polymeric micrometer and nanometer features can be fabricated [38].

1.9 Capillary Electrophoresis and Microchip Capillary Electrophoresis

Analyte separations may be attainable by applying an electric potential across capillaries or channels. The applied electric field provides the driving force with which analytes and a mobile phase are moved through the column. Electrophoresis and electroosmosis are the major fluid movement processes that occur in channels and capillaries as a result of an applied potential. Jorgenson et al. were among the first to
conduct free solution electrophoresis in capillary columns [39]. Microfluidics has been increasingly studied since the seminal paper on the topic was published by Manz et al. [40]. Capillaries and microchips provide a platform that allows low volumes of samples and solvents to be analyzed with high resolution [41].

Capillary electrophoresis and capillary microchip electrophoresis have been demonstrated as effective tools for separating biomolecules and ionic species [39, 42, 43, 44, 45, 46, 47]. The most typical substrate used in capillary electrophoresis is fused silica and modified fused silica [41, 42, 43, 44, 45, 46, 47, 48]. Fused silica (glass) and polydimethylsiloxane (PDMS) are the most typical platforms utilized in microchip capillary electrophoresis [48, 49, 50, 51]. Capillary electrophoresis is not limited to being performed in fused silica capillaries. Coated or packed capillaries can also be used as the column in capillary electrophoresis [41]. Surface coatings have been used to reduce interactions from the silanols present on the surface of fused silica capillaries. The reduced interaction of the surface silanols from fused silica will often result in better separation efficiencies, peak shapes, and recovery of analytes. This means that it is undesirable for an analyte to interact with the wall or the surface of the column material when performing electrically driven separations. Dynamic and chemically bonded coatings are employed to modify the surface of fused silica. Dynamic coatings are obtained by rinsing the capillary with a solution containing the coating reagent, and can require significant maintenance [41]. Bonded coatings are often obtained by performing a series of sequential reactions to prepare the fused silica surface for further modification with a desired polymer [41].
1.10 Research Focus

The chapters in this dissertation present a detailed discussion of the preparation, evaluation, and application of Si-LTGC and F-LTGC media in three areas of separation science. The second chapter discusses the preparation and application of F-LTGC coated porous zirconia in reversed-phase liquid chromatography. The focus of this chapter is to elucidate important contributions in retention using the free energy based solvation parameter models discussed previously. The solvation parameter models provide information about the retention mechanism of the low temperature glassy carbon surface. The third chapter encompasses the synthesis of resorcinol: formaldehyde based carbon aerogels, which have high surface area, and the investigation of the ability to adsorb environmentally important compounds. The third chapter also discusses coating aerogels with F-LTGC and investigating sorption as a function of thermal processing. The adsorption of analytes in solid-phase extraction is performed to discern what control over the selectivity of adsorption is attainable by using coated carbon aerogels. The fourth chapter in this dissertation discusses studies performed in collaboration with the Dr. Nick Ferrell from the OSU Department of Biomedical Engineering, and explores the efficacy of producing LTGC micrometer features through soft lithography. The final chapter discusses the application of low temperature glassy carbon electrically driven separations. This chapter explores the electrophoretic behavior of Si-LTGC modified fused silica and F-LTGC modified fused silica. Also, soft lithographically fabricated microchannels are tested as a potential candidate for microfluidic devices. The tests performed demonstrate
the feasibility of carbon-based fluidic devices. There is great desire in obtaining separations on smaller scales, which permit analyses to be performed more quickly and cheaply. The underlying theme in this dissertation is that functionalized low temperature glassy carbons can be used for a wide range of separation applications.
1.11 References


CHAPTER 2

SOLVATION PARAMETER MODELS FOR RETENTION ON PERFLUORINATED AND FLUORINATED LOW TEMPERATURE GLASSY CARBON STATIONARY PHASES IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

2.1 Introduction

The use of glassy carbon/porous graphitic carbon (PGC) stationary phases are receiving increased attention in liquid chromatographic separations. Glassy carbon surfaces exhibit excellent mechanical strength, are resistant to chemical attack, and have selective retention characteristics [1]. Selective separations of highly polar analytes, such as nucleosides [2] and anions [3, 4] are possible with a glassy carbon/PGC surface, while the selective separation of structural isomers of nonpolar analytes is also readily achieved [5]. Low temperature glassy carbon (LTGC) stationary phases are produced from precursor polymers that have several advantages over PGC. These precursor oligomers are readily soluble in organic solvents, which allow columns or particles to be coated using standard coating procedures. Because the precursor polymers are highly unsaturated, they convert to glassy carbon with minimal mass loss in comparison to the commonly-used resorcinol-formaldehyde copolymers. The oligomers used to make the LTGCs convert to glassy carbon at relatively low temperatures (200-400 °C) compared to
commonly used precursor polymers that require high temperatures (2000-3000 °C) to produce PGC [6, 7].

The incorporation of fluorine heteroatoms into a carbonaceous matrix typically produces a material that is strongly hydrophobic and inert [8, 9]. Fluorine containing stationary phases have been studied in gas chromatography (GC) [8, 9, 10, 11, 12] and liquid chromatography [13, 14, 15, 16, 17, 18, 19, 20]. Vernon et al. illustrated that fluorinated stationary phases exhibit reduced dispersive interactions than similar non-fluorinated stationary phases [10, 11]. The weak dispersive interactions that are possible with fluorinated stationary phases have made them attractive for the separation of pharmaceuticals, proteins, halogenated solutes, and other polar solutes [11]. It has also been noted that fluoroaromatic phases display stronger dispersive interactions than fluoroalkyl phases [12]. Fluorinated stationary phases also exhibit high selectivity for halogenated compounds, as well as alcohols, ketones, and nitro-containing compounds, which all have lone electron pairs [21]. Further, a liquid chromatographic separation of proteins using a fluorinated stationary phase with substantial retention of biological activity was previously demonstrated [22]. Recent studies of HPLC under reversed-phase conditions showed the perfluorinated F-LTGC to be more biocompatible toward proteins than the octadecyl-polysiloxane stationary phases [23].

The perfluorinated and fluorinated low temperature glassy carbon (F-LTGC) stationary phases studied in this paper are unique in that the fluorine heteroatom is covalently bound to the glassy carbon surface (Figure 2.1). The perfluorinated stationary phase has been thermally processed to a final temperature of 200 °C, while 400 °C is the
Figure 2.1: Chemical structure of perfluorinated diethynyl aromatic oligomer.
maximum temperature used to produce the fluorinated LTGC. The retentive behavior of
the resulting F-LTGCs will be compared to the results of previous studies on alkyl-
bonded liquid chromatographic stationary phases, carbon stationary phases, and
fluorinated stationary phases [18, 24, 25, 26, 27, 28]

Perfluorinated and fluorinated LTGC was previously studied as a possible surface
for extraction of halobenzenes from headspace samples. The studied materials displayed
increased selectivity toward halogens; however, the perfluorinated LTGC showed greater
selectivity than the fluorinated phase. The selectivity factor increases relative to the size
of halogen for the particular molecules studied (i.e. \(-F < -Cl < -Br < -I\)) [29]. The
observed selectivity differences warranted further investigation of this F-LTGC.

To study the perfluorinated and fluorinated LTGC selectivity, the solvation
parameter model is used to describe the relative importance of various intermolecular
forces involved in the retention of analytes on these two stationary phases. The solvation
parameter model used to describe distribution between two condensed phases is shown in
Equation 2.1. This equation relates a free energy related property, in this case retention
factor (k), to specified interactions. Respectively, \(S\), \(A\), and \(B\) correspond to the
dipolarity/polarizability, hydrogen bond acidity, and hydrogen bond basicity for solutes
as determined by Abraham [27, 30, 31, 32]. \(V\) is the McGowan’s characteristic volume
which describes the magnitude of dispersive interactions and cavity formation processes
in the stationary phase [27, 30, 31]. \(V\) can be calculated from the structure of a molecule
[27, 30, 31]. \(E\) is the excess molar refractivity of the solute. This term is added into the
model to compensate for \(S\) including both solute dipolarity and polarizability. The excess
molar refraction reflects the tendency of π-electron and lone pair electrons [27]. $E$ can be estimated from refractive index measurements of solute molecules [33].

$$\log k = c + v V + s S + e E + a A + b B$$ (2.1)

The coefficients $c$, $v$, $s$, $e$, $a$, and $b$ can be determined by using multivariate linear least squares analysis. The relative magnitudes of the coefficients represent the importance of each specific interaction relative to retention. Table 2.1 lists the solutes studied in this paper with their Abraham descriptors, McGowan’s characteristic volumes, and excess molar refractions [32, 34]. A comparison of the model coefficients for the two different stationary phases will be made. The chromatographic data were collected with a fixed mobile phase composition. Accordingly, the difference in the coefficients will describe changes in the properties of the stationary phase as a function of processing temperature.

2.2 Experimental

2.2.1 Materials

HPLC-grade acetonitrile, water, and toluene were obtained from Fisher Scientific (Fair Lawn, NJ, USA) and were used as received. Dodecane (99.9%) and all solutes were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Rhinophase porous zirconia particles, (3.1 μm, 300 Å) were obtained from ZirChrom Separations, Inc. (Anoka, MN, USA). Fluorinated low-temperature glassy carbon precursor was synthesized in-house using a previously published method [6]. Polyimide-coated fused silica capillary tubing (250 μm i.d.) was obtained from Polymicro Technologies.
<table>
<thead>
<tr>
<th>Compound</th>
<th>E</th>
<th>S</th>
<th>A</th>
<th>B</th>
<th>V</th>
<th>log k&lt;sub&gt;200 °C&lt;/sub&gt;</th>
<th>log k&lt;sub&gt;400 °C&lt;/sub&gt;</th>
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<td>1,2-dichloroethane&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.416</td>
<td>0.64</td>
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<td>0.02</td>
<td>0.617</td>
<td>0.119</td>
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<td>Methylene chloride&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0.57</td>
<td>0.10</td>
<td>0.05</td>
<td>0.494</td>
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<td>-0.237</td>
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<td>0.09</td>
<td>0.891</td>
<td>0.352</td>
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<td>0.65</td>
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<td>0.839</td>
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<td>0.78</td>
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<td>0.04</td>
<td>0.961</td>
<td>0.521</td>
<td>0.462</td>
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<td>Fluorobenzene</td>
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<td>0.734</td>
<td>0.15</td>
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<td>0.975</td>
<td>0.442</td>
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<td>0.67</td>
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<td>4-Nitrophenol&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.070</td>
<td>1.72</td>
<td>0.82</td>
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<td>0.949</td>
<td>-0.128</td>
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<td>0.00</td>
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<td>1.014</td>
<td>-0.043</td>
<td>-0.109</td>
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<td>0.14</td>
<td>0.716</td>
<td>0.188</td>
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<td>0.33</td>
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<td>0.465</td>
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<td>0.00</td>
<td>0.50</td>
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<td>0.46</td>
<td>1.073</td>
<td>0.081</td>
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<td>m-Xylene&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.623</td>
<td>0.52</td>
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<td>0.16</td>
<td>0.998</td>
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<td>0.28</td>
<td>0.891</td>
<td>0.037</td>
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<td>N-Methyl aniline</td>
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<td>0.90</td>
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<td>0.43</td>
<td>0.957</td>
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<td>o-Xylene&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.56</td>
<td>0.00</td>
<td>0.16</td>
<td>0.998</td>
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<td>0.32</td>
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<td>Phenol</td>
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<td>p-Xylene&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.52</td>
<td>0.00</td>
<td>0.16</td>
<td>0.998</td>
<td>0.507</td>
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<td>Toluene</td>
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<td>0.14</td>
<td>0.857</td>
<td>0.318</td>
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<td>Valerophenone&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.95</td>
<td>0.00</td>
<td>0.50</td>
<td>1.436</td>
<td>0.438</td>
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<tr>
<td>Acetone&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.70</td>
<td>0.04</td>
<td>0.49</td>
<td>0.547</td>
<td>-0.51</td>
<td>-0.329</td>
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<td>0.42</td>
<td>0.37</td>
<td>0.48</td>
<td>0.449</td>
<td>-0.263</td>
<td>-0.287</td>
</tr>
<tr>
<td>Methanol&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.44</td>
<td>0.43</td>
<td>0.47</td>
<td>0.308</td>
<td>-0.248</td>
<td>-0.331</td>
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</table>

Table 2.1: List of compounds used to determine the Solvation Parameter Models with their respective descriptor for interactions. <sup>a</sup>statistical outlier in all cases; <sup>b</sup>solute used in 400 °C study only; <sup>c</sup>solute used in 200 °C study only. Blanks in log k column result from no peak being observed.
(Phoenix, AZ, USA). Whatman syringe-tip filters with 0.2 μm pores were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.2.2 Solute Preparation

The analytes were prepared by diluting the solutes to a concentration of 10 mg/mL with the mobile phase (60:40 (v/v) acetonitrile: water solution) used for analysis. The diluted solutes were then passed through a 0.2 μm Whatman syringe-tip filter obtained through Fisher Scientific.

2.2.3 Chromatography

2.2.3.1 Glassy Carbon Coating Process

The stationary phase was prepared using a previously described method with a few modifications [35]. The porous zirconia particles were encapsulated with the perfluorinated oligomeric precursor (3% w/w) via a solvent slurry process that used toluene as a good solvent for the carbon precursor and dodecane as a non-solvent for the oligomeric precursor. The toluene was slowly evaporated at a constant rate and dodecane was added such that the solvent level remained constant throughout the coating process. Once the zirconia was coated, the resulting particles were thermally processed using a Lynberg/Blue quartz tube furnace (Asheville, NC, USA) [35]. The temperature was increased from ambient temperature (20-25 °C) to the final temperature (200 or 400 °C) at a rate of 1 °C/minute. The final temperature was maintained for a minimum of 10 hours. A reducing gas environment (95% N₂: 5% H₂) was continually purged through the
quartz tube to maintain an inert atmosphere during the temperature processing. The perfluorinated phase was thermally processed to a final temperature of 200 °C, while the fluorinated phase was thermally processed to a final temperature of 400 °C.

2.2.3.2 Column Packing

The thermally processed particles were packed into 30 cm long fused silica capillaries with a 250 μm inner diameter using an acetone slurry at 3.447 x 10^7 Pa (5000 psi). Once the fused silica was completely packed, the pressure was increased to 4.826 x 10^7 Pa (7000 psi) and held constant for a minimum of one hour. The packed column was then allowed to vent to ambient pressure through a 50 μm i.d. fused silica capillary restricting tube.

2.2.3.3 Mobile Phase

A 60:40 (v/v) acetonitrile-water mixture was used as the mobile phase in the chromatography experiments. The chromatography was performed using the constant pressure mode of an ISCO 260D syringe pump (Teledyne ISCO, Inc., Lincoln, NE, USA) in order to maintain a flow rate of 10 μL/min.

2.2.3.4 Chromatographic System

The chromatographic system consisted of an ISCO 260D syringe pump operated in constant pressure mode to reduce pressure fluctuations. The samples were injected with a 4-port Valco Instruments (Valco Instruments Co. Inc., Houston, TX, USA) high-
pressure injection valve that was fitted with a 60 nL rotor. The detector for these studies was either a Thermo Separation Products UV1000 or a Spectrophysics UV2000 operated at a wavelength of 210 nm or 254 nm. A flow cell was made by removing a portion of the polyimide coating of some fused silica tubing with an internal diameter of 250 μm, thus reducing the detector dead volume. The chromatograms were collected by using EZChrom Chromatography Data System V6.0 (Scientific Software Inc, San Ramon, CA, USA) and analyzed by Peak Fit (Jandel Scientific, San Rafael, CA, USA).

2.2.3.5 Particle Characterization

Surface characterization of the particles was performed using a Kratos Axis Ultra XPS and was compared to previous data obtained on a Perkin-Elmer Model 550 ESCA-Auger spectrometer with a magnesium X-ray source. Scanning Electron Microscopy (SEM) was performed on a JEOL JSM-820 SEM with Oxford eXL energy dispersive x-ray analyzer. The images were obtained with an accelerating voltage of 12 keV. The mean diameter of the particles was measured using Scion Image Beta 4.02 (Scion Corporation, Frederick, MD, USA).

2.2.3.6 Statistical Analysis

Multivariate linear least squares analysis was performed using SYSTAT 11 (Systat Software Inc., Point Richmond, CA, USA) to determine the coefficients and the statistical error of the model. The multivariate linear regression analysis was performed stepwise, in order to obtain a relevant model. The models were obtained by determining
the validity of the model one descriptor at a time. This means that each model was comparatively analyzed to others using the multilinear regression statistical goodness of fit (adjusted $R^2$) in SYSTAT. A descriptor (coefficient and parameter) was added and included only when it improved the overall statistics. SYSTAT determines outliers based on residual analysis. If the residual of a data point does not fall within $(1.5 \times \text{interquartile range (IQR)} + \text{IQR})$, then the data point is marked as an outlier. The IQR is determined by first rank ordering the residuals of the fit. The IQR is the absolute value of the difference between the median of the lower half and the median of the upper half of the residuals [36, 37]. Once a data point was identified as an outlier, it was removed from consideration and a new model was calculated. The validity of a model was also determined by calculating an $F_{\text{stat}}$ using equation 2.2, where $k$ is equal to the number of variables included in the model, and $n$ is the number of data points used to determine the model. Systat calculated an $F_{\text{calc}}$ for each model. The $R^2$ is calculated using linear regression mathematics. A desirable $R^2$ is 0.99, but 0.9 has been determined to be acceptable for this study.

2.3 Results and Discussion

SEM analysis of the coated zirconia was performed. Figure 2.2 compares the micrograph of the zirconia particles with and without coating. The figure also shows that the zirconia particles were uniformly coated. The mean particle diameter was determined to be 3.4 $\mu$m, which is approximately 10% larger than the reported value, 3.1 $\mu$m, from
Figure 2.2: SEM images of unmodified ZrO\textsubscript{2} (left) and F-LTGC coated ZrO\textsubscript{2} (right) that has been thermally processed to 200 °C. The scale bar represents 10 μm. The images were collected using 12 keV accelerating voltage.
ZirChrom and the value determined from Figure 2.2. The particles shown on the right in Figure 2.2 were coated and processed to a final temperature of 200 °C. No difference in mean particle size was determined by SEM analysis of the particles processed at 200 °C and 400 °C.

X-ray photoelectron spectroscopy was previously reported for the thermally processed F-LTGC [29]. The initial weight percent of fluorine present in the unprocessed oligomer was determined to be 36.0%. The weight percent of fluorine for F-LTGC processed at 200 °C and 400 °C was shown to be 34.0% and 16.0%, respectively. The amount (weight %) of fluorine remaining at a processing temperature of 200 °C is nearly the same as the unprocessed precursor, while at 400 °C the weight percent of fluorine is approximately half of the unprocessed precursor [29]. Therefore, the 200 °C F-LTGC will be referred to as perfluorinated, and the 400 °C as fluorinated.

The retention factor, k, for the sorption of solutes onto columns containing the two different stationary phases was determined. Values for coefficients; c, v, s, e, a, and b were determined by performing multivariate linear least squares using the chromatographically determined retention factor and descriptor values found in Table 2.1. It is important to understand what the coefficients represent before explaining the importance of the parameters in the retention data. The c term is the intercept of the linear relationship. The sign of the remaining coefficients (v, s, e, a, and b) indicates the direction of the interactions. A positive coefficient indicates that the solute/stationary phase interactions are greater than the solute/mobile phase interactions, and that favors retention. The converse of this statement is also true, that a negative coefficient implies
<table>
<thead>
<tr>
<th>Processing Temperature</th>
<th>n</th>
<th>c</th>
<th>e</th>
<th>s</th>
<th>a</th>
<th>b</th>
<th>v</th>
<th>R</th>
<th>$F_{\text{calc}}$</th>
<th>$F_{\text{stat}}$ (n₁, n₂)</th>
<th>$R^2$</th>
</tr>
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<tbody>
<tr>
<td>200 °C</td>
<td>25</td>
<td>-0.39</td>
<td>-</td>
<td>-0.21</td>
<td>-0.43</td>
<td>-1.28</td>
<td>1.17</td>
<td>0.99</td>
<td>347.61</td>
<td>3.098 (4, 20)</td>
<td>0.986</td>
</tr>
<tr>
<td>400 °C</td>
<td>23</td>
<td>-0.86</td>
<td>0.58</td>
<td>-</td>
<td>-0.45</td>
<td>-0.90</td>
<td>0.73</td>
<td>0.94</td>
<td>36.55</td>
<td>2.928 (4, 18)</td>
<td>0.893</td>
</tr>
</tbody>
</table>

Table 2.2. Solvation Parameter Models determined from retention data of solutes on perfluorinated and fluorinated stationary phases under isocratic reversed-phase conditions with a mobile phase composition of 60:40 MeCN:H₂O
that a specific interaction decreases retention, meaning that the mobile phase/solute interaction is stronger than the stationary phase/solute interaction. Another important point is that the absolute value of the coefficient describes the relative importance of a descriptor in the retention model. A large absolute value suggests a large contribution in the retention of a given stationary phase, while a small absolute value suggests that the contribution of a descriptor is not as significant.

The data for the 200 °C processed F-LTGC show that not all of the interactions contribute significantly to retention. Table 2.2 shows the solvation parameter models that were determined for the different stationary phases tested in this study. The determined solvation parameter model equations for the 200 °C processed F-LTGC is shown in Equation 2.3, while Equation 2.4 shows the solvation parameter model for the 400 °C processed F-LTGC.

\[
\log k = -0.39 - 0.21 S - 0.43 A - 1.64 B + 1.53 V \\
\log k = -0.86 + 0.58 E - 0.45 A - 0.90 B + 0.73 V
\]  

Equation 2.3 (2.4)

The variance-covariance matrices (Tables 2.3 and 2.4) of the parameters that are statistically important were determined to test the validity of the solvation parameter models shown in Equations 2.3 and 2.4. The matrix (Table 2.3) for the parameters of the 29 solutes used to determine the retention characteristics of the 200 °C processed F-LTGC stationary phase shows slight correlation between S and V (0.564). The remaining correlation values are less than 0.49, indicating little covariance among the parameters. The matrix for the solutes used with 400 °C processed F-LTGC shows that the largest correlation is between the E and V parameters (0.646). The remaining correlation values
<table>
<thead>
<tr>
<th></th>
<th>E</th>
<th>A</th>
<th>B</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.037</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>-0.02</td>
<td>0.229</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>0.646</td>
<td>-0.276</td>
<td>0.312</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 2.3:** Correlation matrix for solutes used to determine the solvation parameter model for the 200 °C F-LTGC stationary phase using only statistically relevant descriptors.

<table>
<thead>
<tr>
<th></th>
<th>S</th>
<th>A</th>
<th>B</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>-0.083</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.489</td>
<td>0.363</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>0.564</td>
<td>-0.419</td>
<td>0.287</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 2.4:** Correlation matrix for solutes used to determine the solvation parameter model for the 400 °C F-LTGC stationary phase using only statistically relevant descriptors.

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34
are all less than or equal to 0.32, which indicates minimal correlation between those parameters. These correlation matrices for the solute sets employed here displays similar covariance as matrices previously presented for other solute sets where linear solvation energy relationship (LSER) were employed \([18, 25, 27]\). For example, correlations between E/S (0.597) and E/V (0.565) were reported by Reta et al. \([18]\). The matrices presented in this paper demonstrate that the overall covariance is acceptable. The covariance of the parameters that are determined to be statistically insignificant in the models are irrelevant to this discussion and do not add useful information in determining the validity of solvation parameter models. The overall correlation between the parameters modeled in this study is small, but some consideration must be given to the correlations observed.

Once the model is determined, it can be used to estimate the log k of the solutes used in the study. Figure 2.3 shows plots of the observed log k versus predicted log k using the derived models shown in Equations 2.3 and 2.4. An ideal plot of observed log k versus predicted log k would have a slope of 1 and an intercept of 0, as an ideal plot and the model would produce the same retention factor for any given solute used in the model. There is a high correlation observed for the model achieved with the perfluorinated stationary phase. The perfluorinated carbon shows that there is very good correlation between predicted and observed retention factors \((R^2 = 0.986)\). A similar plot for the 400 °C F-LTGC stationary phase is shown in Figure 2.3b. The \(R^2\) for this plot is 0.893, lower than the plot for the perfluorinated phase. The goodness of fit \((R^2)\) for the plots matches the square multiple R obtained for the models through SYSTAT. This
Figure 2.3: Plots of predicted log $k$ versus observed log $k$. (A) Plot for 200 °C F-LTGC ($m=1.003$, $b=0.003$, $R^2=0.986$). (B) Plot for 400 °C F-LTGC ($m=1.000$, $b=-0.001$, $R^2=0.893$). The numbers on the plots indicate the solute as numbered in Table 2.1.
demonstrates the ability of the model to predict the retention data with good correlation. Equation 2.3 shows the solvation parameter model for the 200 °C processed F-LTGC stationary phase tested. A few solutes were determined to be outliers, ethanol, methanol, methylene chloride, and 1, 2-dichloroethane. Upon deleting these solutes from the model, the statistical parameters increased to obtain a strong model for the perfluorinated retention data. The primary contributors to the model are the dispersion and H-bond basicity, which are positive and negative, respectively. Dipolar interactions are also statistically important in the negative direction, which decreases retention on the 200 °C processed F-LTGC stationary phase. The $E$ interaction is statistically negligible in the linear regression. The $b$ coefficient is the most significant coefficient and it has a negative sign. Therefore solutes that are strong hydrogen bond acceptors will be less retained than those that are not. These data also indicate that the mobile phase is a much stronger hydrogen bond donor than the stationary phase. The surface of the 200 °C processed F-LTGC should not readily participate in hydrogen bonding, as nearly all the possible sites for hydrogen atoms should be occupied by a covalently bound fluorine. The dispersion/cavity formation term, $v$, has the second largest absolute value in the model. The positive sign associated with the $v$ term indicates that the solutes with larger McGowan’s characteristic volumes are better retained on the stationary phase. The positive sign also indicates that the cavity formation energy and dispersive interactions with the perfluorinated stationary phase are stronger than those with the mobile phase. The hydrogen bond acidity term was determined to be statistically significant in the negative direction, which means that solutes with considerable $a$ values would have
stronger interaction with the mobile phase than the stationary phase. The $s$ coefficient is statistically significant but smaller in magnitude than $v$ or $b$ and with a negative sign. The negative sign indicates that the dipolarity/polarizability of the mobile phase is higher than that of the stationary phase.

The model for the 400 °C F-LTGC stationary phase is shown in Equation 2.4. Three aliphatic solutes (acetone, ethanol, and methanol) were statistical outliers in the regression model, and were therefore not used to determine the coefficients in the model. The coefficients with the largest magnitude are $b$ and $v$, with $b$ having a negative sign and $v$ a positive sign. Further, the solute’s excess molar refraction and hydrogen bond acidity are statistically significant in the model for the 400 °C processed F-LTGC with the $e$ term adding to retention and $a$ term decreasing retention. The negative signs for the $a$ and $b$ coefficients indicate that the mobile phase is a stronger hydrogen bond acid and hydrogen bond base than the F-LTGC surface. The $e$ term emerges as a contributor to the model because the solutes are able to undergo $\pi-\pi$ interactions with the condensed ring structure of the glassy carbon. This will be discussed further below.

In comparing the parameters that are important in the two models, there are differences observed between the 200 °C and 400 °C processed F-LTGC stationary phases. The 200 °C model displays interaction importance in the order: H-bond basicity > dispersion > dipolarity, while the 400 °C model shows H-bond basicity > dispersion > excess molar refraction > H-bond acidity. The $b$ and $v$ terms are smaller in the 400 °C model. The $a$ terms are statistically the same for each stationary phase and negative, thus indicating that solutes with strong H-bond acidity prefer to interact with the mobile phase.
for both stationary phases studied. However, it should be noted that of the solutes analyzed in this study, there are few with significant values of $A$. Given this information, the contribution of $a$ to the model is considered with care. The $s$ term was determined to be statistically significant for the perfluorinated LTGC processed to 200 °C (Equation 2.3), but insignificant for the fluorinated LTGC processed to 400 °C (Equation 2.4). The two stationary phases also demonstrate different directionality of the $s$ and $e$ terms. The $s$ term describes the dipolar interactions that a solute has between the stationary phase and mobile phase. While the $e$ term has been used to compensate for including the polarizability of solutes into the $s$ term, it has also been used to describe $\pi-\pi$ interactions and $n$-electron interactions [18]. The emergent importance of the $e$ term and suppression of the $s$ term can be attributed to the differences in the surface chemistries of the two stationary phase surface. A larger percentage of delocalized $\pi$ electrons are available at the surface of the 400 °C F-LTGC as loss of approximately 50% of the fluorine at the surface has occurred (34% weight fluorine for 200 °C processed F-LTGC and 16% weight fluorine for 400 °C processed F-LTGC) [29], and the increased temperature causes further graphitization of the LTGC [6]. The dipolar interactions with the mobile phase, which is apparent with the perfluorinated phase, is overcome by the strong $\pi$-interactions with the carbon surface that has been thermally processed to 400 °C. This emergence of importance of $\pi$-electron interactions is similar to that noted for a LTGC stationary phase processed to ~800 °C, which contains a silicon atom per repeat unit, Si-LTGC [24].
Previous studies of aromatic and aliphatic reversed-phase stationary phases by using linear solvation energy relationships (LSERs) have shown that dispersive interactions ($V$) and the hydrogen bond basicity ($B$) of the solute are the most important interactions central to retention in RPLC [18, 25, 27]. In all these cases, the $V$ coefficient is large and positive, and the $B$ coefficient is large and negative. Nearly all coefficients, except the $V$ coefficient, were determined to be negative in the LSER studies, which means that their interaction is favorable toward the mobile phase, decreasing retention [18, 25, 27]. The $e$ coefficient was determined to add to retention with a few phenyl stationary phases examined [18, 27]. Solute retention on C$_8$ and C$_{18}$ columns have been studied, and also exhibit the same trend, that dispersion and H-bond basicity are the most important interactions described in LSER models [25, 27].

Stationary phases that include fluorine atoms have been studied and compared to C$_{18}$ as well [18, 38, 39]. Marchand et al. [38] showed that perfluoro and fluorinated alkyl phases have dispersive interactions of varying strength due to significant differences in polarizability between a fluorinated phase and an octadecyl-polysiloxane stationary phase. Also, for aromatic solutes $\pi-\pi$ interactions with fluorophenyl phases were very important [38, 39]. Using Principal Component Analysis, Eurby et al. illustrated that a perfluorinated stationary phase has retention attributes that are often orthogonal to that observed with C-18 columns for hydrophilic and lipophilic bases [39]. Reta et al. demonstrated the $e$ and $s$ coefficients to be statistically zero on a perfluorophenyl phase with a methanol modified mobile phase, and the $e$ coefficient was determined statistically important and negative for two fluoroalkyl phases [18]. All other
coefficients, except the $v$ coefficient, were determined to be negative in this study, which means that interactions are favorable toward the mobile phase, decreasing retention [18]. Poole et al. examined retention differences between perfluorhexylpropylsiloxane-bonded and octadecylsiloxane-bonded stationary phases using solvation parameter models [20]. The solvation parameter model presented for the perfluorinated stationary phase on silica exhibited very similar relationships as observed in this work. Poole et al. determined that the dipolarity/polarizability of the solute positively affected retention, where as the dipolarity interaction was determined to be negative for the fluorinated phase used in this study [20]. Giardina studied the same F-LTGC in solid phase microextraction (SPME), and determined the selectivity toward halogented compounds decreased as processing temperature increased, and that selectivity followed the trend: iodobenzene $>$ bromobenzene $>$ chlorobenzene $>$ fluorobenzene [29]. The F-LTGC phases used in this studied also displayed the selectivity as described by Giardina [29].

Other types of carbon-based stationary phases were previously examined using similar multivariate analysis [5, 24, 28]. A LTGC that contains a silicon per repeat unit in the precursor polymer was investigated, and it was found that the major contributors to retention were dispersion and H-bond basicity of the solute [5, 24, 28]. The effect of processing temperature was explored by Engel et al. [24]. At lower processing temperatures (200-500 °C) the retention on the Si-LTGC is governed by dispersion interactions $>$ the hydrogen basicity of the solute $>$ the hydrogen-bond acidity of the solute $>$ the dipolarity of the solute [24]. At higher processing temperatures, (800 °C) the importance of the solutes dipolarity increased and positively influenced retention. Since
this model does not use the excess molar refraction, the polarizability is also lumped into the dipolarity variable [24]. The F-LTGC graphitizes at lower temperatures than the Si-LTGC [29]. Therefore, the retention behavior for the F-LTGC processed at 400 °C should be expected to be similar to that of Si-LTGC processed at 800 °C. One advantage the F-LTGC has for chromatographic separations compared to the Si-LTGC is the F-LTGC is more homogeneous in its surface chemistry and is markedly more resistant to oxidation [29]. Jackson et al. studied other carbon stationary phases, porous glassy carbon and carbon coated zirconia particles, using multivariate analysis to discern the relative importance of specific interactions on retention. The relative importance of interactions for solute retention on these carbons was similar to that of the 800°C processed Si-LTGC (dispersion > solute’s hydrogen bond basicity > dipolarity > solute hydrogen bond acidity) [28]. Both Engel et al. and Jackson et al. noted that one of the significant differences between the interactions of solutes of carbon and on octadecyl polysiloxane (ODS) stationary phase was that solute dipolarity negatively impacted retention with ODS and positively impacted retention on the carbon surfaces. Interestingly, the 200 °C processed F-LTGC has a solvation parameter model similar to that found for ODS, while the 400°C processed F-LTGC has a model similar in trends to that observed for other carbon stationary phases [24, 28].

2.4 Conclusions

This chapter discusses the application of a fluorine-containing precursor polymer in reversed-phase liquid chromatography. The polymer can easily be coated onto
chromatographic supports and converted to graphitic carbon at relatively low temperature. The retention of neutral solutes on two variants of the F-LTGC polymer have been performed and compared to performance of several chromatographic stationary phases. The important interactions determined for the 200 °C F-LTGC are solute hydrogen bond basicity > dispersion > solute hydrogen bond acidity > dipolarity/polarizability. The important interactions for the 400 °C F-LTGC were determined to be solute H-bond basicity > volume/dispersion > molar refractivity. The variations of important interactions with respect to the other stationary phases were observed, as well as variations between the two different F-LTGC materials used. The differences observed among the two phases studied have been linked to the surface chemistry of the material at a specific processing temperature, as the 200 °C F-LTGC was determined to be perfluorinated and the 400 °C F-LTGC was determined to be fluorinated.
2.5 References


3.1 Introduction

Carbon aerogels are a highly porous material that possess unique nanoscopic and macroscopic properties. Various synthetic and processing conditions have been investigated for the production of carbon aerogels [1, 2, 3]. Resorcinol: formaldehyde (R:F) aerogels have been described as possessing porosities greater than 80%, surface areas between 400-1200 m²g⁻¹, and pore volumes that vary with the synthetic and processing conditions [2]. There have also been studies investigating the thermal and electrical conductivity of R:F aerogels [4] and the elastic properties of R:F aerogels [5]. The electrochemical properties have been determined to be dependent on the density of the carbon aerogel [4]. Carbon aerogels have been shown to display electrical conductivities over a range of 0.6 to 20 Ω⁻¹ cm⁻¹ over a density range of 60 to 650 kg m⁻³ [4]. Elastic properties such as shear modulus spanning 0.3 to 70 MegaPascals for cryogels with a density range of 100 to 400 kg m⁻³ have been reported [5]. Aerogels with lower densities have been shown to display smaller shear modulus than aerogels that are
Figure 3.1: The addition and condensation reactions that take place during the formation of gel from a sol mixture of resorcinol, formaldehyde, and an acidic catalyst.
more dense [5].

R:F aerogels can be synthesized via sol-gel polycondensation, using a base-catalyzed addition-condensation of formaldehyde to resorcinol. Resorcinol (Figure 3.1), 1, 3-dihydroxybenzene, is able to add formaldehyde (Figure 3.1) in the 2-, 4-, and/or 6-positions of the aromatic ring. The addition is shown in Figure 3.1, and the condensation reaction is also shown in Figure 3.1. Briefly, the formaldehyde adds to the resorcinol with the basic catalyst assisting in condensation [2, 6]. The hydroxymethyl groups formed in the addition of the formaldehyde to the resorcinol are able to undergo condensation reactions with the aid of sodium carbonate, a basic catalyst, to form methylene and methylene ether linkages, as shown in Figure 3.1.

Various synthetic conditions; ratios of starting materials, pH of the initial sol, and drying conditions; have been studied to determine their effects on the pore size in the resulting aerogels. It has been determined that R:F aerogels synthesized with a low resorcinol to catalyst ratio (R:C) (50:1) produced gels that exhibited small particles (3-5 nm) with large diameter connections between particles [7, 8]. R:F aerogels synthesized through low R:C ratios were determined to have higher densities than gels produced with large R:C ratios. The particles in the R:F aerogels synthesized using high R:C ratios were determined to be between 16-200 nm [7, 8, 9]. These gels appeared as a pearl necklace.

The drying technique employed dictates the name with which a dried gel is referred [10]. Gels that contain aqueous solutions in the pore structure should be referred to as hydrogels. A xerogel is produced by simply evaporating the solvent at ambient pressure [6]. Freeze-drying to remove the solvent from the pores produces a cryogel
An aerogel is produced when supercritical drying is employed to remove the solvent from the pores of the hydrogel [1].

Carbon aerogels have been used for various applications; from catalysis [12, 13], electrochemistry [14, 15, 16] and adsorption [17, 18, 19, 20, 21, 22, 23, 24, 25]. Metal-doped (Ce- and Zr-) and metal-containing (Cr-, Mo-, W-, Fe-, Ru-, Co-, Ni-, Pd-, Pt-, Cu-, and Ag-) aerogels have been synthesized and characterized [12, 23].

Low-temperature glassy carbon (LTGC) is a carbonaceous material that has been used extensively in electrochemical and separation sciences [26, 27, 28, 29, 30, 31]. Separation studies with a diethynyl aromatic precursor to low temperature glassy carbon have demonstrated that markedly different retention properties can be obtained by thermally processing the precursor to various temperatures [26, 28, 30, 31]. The differences arise from different chemistries that are produced at different processing temperatures. Olesik and colleagues examined the behavior of a silicon low-temperature glassy carbon (Si-LTGC) using liquid chromatography [26, 30]. Shearer and Olesik examined a fluorine-containing low temperature glassy carbon (F-LTGC) using liquid chromatography [31]. Giardina et al. studied the solid phase microextraction of compounds using the Si-LTGC and F-LTGC [28]. The LTGC used in the aforementioned studies was developed by Callstrom et al., and is able to convert to a glassy carbon structure at relatively low temperature (between 200 and 400 °C) and is soluble in common organic solvents [29]. Due to unsaturation in the precursor, the polymer precursors are able to cross-link and are converted to glassy carbon with minimal mass loss [27].
Carbon aerogels are an attractive media for solid-phase extraction (SPE). As mentioned in the introduction to this dissertation, some desirable attributes of extraction media are that the solids used in SPE have surface areas between 200 and 800 m$^2$g$^{-1}$ [32]. It is important that SPE sorbents exhibit good chemical stability, as acidic, alkaline, or organic solvents are used to desorb the solutes [32]. The chemical stability of carbonaceous media and the high surface area are the two main reasons that carbon aerogels are an attractive material for use in solid phase extraction, as these attributes lend to low limits of detection. The employment of low-temperature glassy carbon adds another layer to the chemical stability and selectivity of separation media. The selectivity from the low-temperature glassy carbon arises as a function of processing temperature, which is related to the amount of graphitic character of the resulting polymer [26, 27, 28, 30, 31].

Herein, the synthesis, characterization and application of R:F cryogels and fluorinated low temperature glass carbon (F-LTGC) coated R:F cryogels as an extraction medium are described. Several characterization techniques were employed to determine physicochemical properties of the cryogels. Finally, the ability to extract several organic compounds from the liquid phase was examined. The results of the extractions by R:F cryogels and F-LTGC coated cryogels thermally processed from 200-1000 °C is compared.
3.2 Experimental

3.2.1 Materials

Resorcinol (1,3-dihydroxybenzene) (white, 99%) and anhydrous sodium carbonate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Formaldehyde (37.7%, methanol stabilized), HPLC-grade water, acetone (Reagent Grade), and t-butanol (ACS Grade) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). All reagents were used without further purification. The fluorine-containing low temperature glassy carbon, F-LTGC, was synthesized in house using a previously documented procedure [29].

3.2.2 Methods

3.2.2.1 Sol-gel Synthesis

The R:F cryogels were synthesized using the reaction conditions described by Pekala et al. [1]. Solutions of resorcinol (approximately 0.29 M) and formaldehyde (approximately 0.57 M) were prepared in HPLC grade water. Resorcinol was mixed with formaldehyde in a mole ratio of approximately one resorcinol: two formaldehyde (1:2 R:F). A basic catalyst, sodium carbonate, was added in a mole ratio of approximately 50:1 with respect to resorcinol (R:C). Modifications to the pH of the resulting sol were performed by using dilute nitric acid to provide a sol with pH between 5.5 and 7.5. These pH values are utilized as precipitation has been observed below pH 5.5 and significant retardation of polymerization above pH 7.5 has been reported [2]. Once the pH of the sol has been modified, the resulting sol was separated into 15 mL vials. Once separated, in
the case of the vials, the sol was degassed and purged with argon, to minimize the
dissolved oxygen in the sol. The vials were capped and allowed to rest at ambient
temperature for approximately 24 hours. After 24 hours, the sealed-purged vessels were
placed in an oven and the temperature was ramped from ambient temperature (~21 °C) to
85 °C at a rate of 1 °C per minute. The final temperature, 85 °C, was maintained for 3-7
days. The sol undergoes a color change, from clear-colorless to clear-red, during the
elevated temperature cure. This color change is associated with the transition of the
mixture from the sol phase to the gel phase [1].

3.2.2.2 Aging of Hydrogels

Once the gels cured at 85 °C, they were removed from the vial by scoring and
breaking the vial. The resulting resorcinol: formaldehyde (R:F) gels were carefully
placed in an agitated bath of 0.125 % (v/v) trifluoroacetic acid (TFA) at 45 °C for three
days. The 3-day TFA bath assists in completing the condensation of the residual
hydroxymethyl moieties in the gel by forming ether linkages between resorcinol
molecules [1]. Up to this point in synthesis, the gels are referred to as hydrogels, as there
is an aqueous phase in the pores of the gel.

3.2.2.3 Solvent Exchange

The aqueous phase was removed from the pores of the gels and replaced with
acetone in order to make removal of all solvent from the pores easier. The aqueous phase
in the gels is replaced by acetone by the process of diffusion. The aqueous phase will
migrate out of the pores, high concentration, to the bulk solvent, low concentration. To ensure this process occurs to completion, the hydrogels were exposed to four liters of fresh acetone, maintained at 45 °C, every day for four days thus replacing the aqueous solution contained in the gels with the organic solvent. The R:F hydrogels were converted to xerogels [2].

3.2.2.4 Supercritical Drying

Once the gels’ pores were completely saturated with acetone, they were removed from the acetone bath for drying. The drying began by placing the R:F xerogel in a stainless steel high pressure vessel that is equipped with an ISCO 260 D (ISCO, Inc., Lincoln, NE, USA) syringe pump filled with CO$_2$. The vessel was slowly flushed with CO$_2$ to expel the atmospheric gases. Once the atmospheric gases were removed from the vessel, the pressure was slowly ramped to 8.963 x 10$^6$ Pa (1300 psi). This results in the vessel being filled with liquid CO$_2$. The CO$_2$ in the vessel was exchanged with fresh CO$_2$ every four hours to remove the acetone as it was replaced in the pores by the liquid CO$_2$. After 24 hours, the temperature and pressure were altered to change the phase of the CO$_2$ from the liquid phase to supercritical. The use of supercritical phase CO$_2$ is important as it minimizes capillary pressure that is apparent when liquid CO$_2$ converts to gaseous CO$_2$ when the pressure is decreased. The conversion of supercritical CO$_2$ to gaseous CO$_2$ does not display this phenomenon. This lack of capillary pressure will permit the pore structure of the gels to remain, thus rendering the desired R:F aerogel.
3.2.2.5 Freeze Drying

In order to perform freeze drying, the acetone in the pores of the gels was displaced with $t$-butanol. This was achieved by decanting the acetone and replacing with $t$-butanol. The $t$-butanol is used to minimize damage to the pore structure of the gel as the volume change of frozen $t$-butanol is small [10, 11]. The displacement was performed at 45 °C to increase diffusion of the solvents. The $t$-butanol was replaced with fresh solvent twice a day for two days. Once the solvent exchange was performed, the gels were submerged in liquid nitrogen and vacuum was pulled over the gels to promote sublimation of the $t$-butanol, thus rendering capillary pressure negligible. Vacuum was pulled using a glass vacuum dessicator (Fisher Scientific, Fair Lawn, NJ, USA) equipped with a mechanical pump. The dessicator was cooled in an acetone: dry ice bath (T = -77 °C). Vacuum was pulled over the gels for a minimum of 24 hours, ensuring all the solvent was eliminated from the pores.

3.2.2.6 Carbonization

The resulting R:F cryogels were converted to carbon cryogels by pyrolysis at 800 °C. The procedure for pyrolysis is as follows. The R:F cryogels were placed in a quartz tube furnace that was continually purged with 95:5 N$_2$:H$_2$, and the temperature was ramped from ambient to 800 °C at a rate of 1 °C/minute. The final temperature was maintained for a minimum of 2 hours.
3.2.2.7 Coating

After the gels were carbonized, they were modified with a fluorine-containing low temperature glassy carbon precursor. The modification of the gel was accomplished using encapsulation, as described by Olesik and colleagues with a few modifications [28, 31]. Briefly, the precursor polymer was dissolved in toluene, such that approximately a 3 % (w/w) precursor to carbon cryogel ratio was obtained. The precursor was slowly forced out of solution, by heating the solution until the toluene would boil and evaporate from the solution. As the toluene boiled away, dodecane was added to the solution. The addition of dodecane forces the F-LTGC precursor out of solution, and onto the carbon cryogel that is in slurry in the solution. Once all the toluene has boiled out of solution, the solution was removed from heat and permitted to cool to room temperature. After cooling, the liquid was decanted leaving the F-LTGC coated cryogel. After the solvent was completely removed from the cryogel, by placing the gel in an elevated temperature (100 °C) for a minimum of 24 hours, the F-LTGC precursor coated cryogels were thermally processed to polymerize the precursor. The F-LTGC precursor coated cryogels were thermally processed to several different temperatures, 200 °C, 310 °C, 400 °C, 600 °C, 800 °C, and 1000 °C. These temperatures were chosen based on the chemical properties of the fluorinated precursor experimentally determined in previous studies [28, 31].
3.2.3 Characterization

The R:F gels were characterized at different points throughout the drying process. Changes in the chemical characteristics of glassy carbon were monitored using Raman spectroscopy (HoloSpec f/1.6i; VPT System Kaiser Optical Systems, Inc., Ann Arbor, MI, USA). Spectra were obtained using a 532 nm excitation laser operated at 4.9 mW. The duration of exposure and number scans varied in order to obtain spectra with an acceptable signal to noise ratio. All obtained spectra were baseline corrected and analyzed using Grams/32 version 6.00 (Galactic Industries Corporation, Salem, NH, USA).

The R:F cryogels were visualized by employing scanning electron microscopy (SEM). The microscope used for the observation was a Hitachi S3000H (Hitachi High Technologies, America, Inc., Pleasanton, CA, USA). The SEM images were collected using an accelerating voltage of 10-15 keV. The images were analyzed using the software with which the SEM is equipped.

X-ray photoelectron spectroscopy, XPS, was performed to determine the surface chemistry of the freeze-dried cryogels and fluorinated low temperature glassy carbon, F- LTGC, modified cryogels. The instrument used to perform the analysis was a Kratos Axis Ultra XPS, (Kratos Analytical Inc., USA, Chestnut Ridge, NY, USA), equipped with a magnesium source. Survey scans were performed to determine the chemistries present at the surface of the cryogel to be used for further studies.

Brunauer-Emmet-Teller (BET) surface area analysis was performed using a Micromeritics 1310 surface area analyzer (Micromeritics, Norcross, GA, USA). The
surface area analysis was performed by equilibrating liquid nitrogen (77 K) in a sample chamber that contained 10-100 mg of the cryogel or carbon cryogel to be analyzed. Langmuir sorption isotherms were also obtained using the Micromeritics surface area instrument.

3.2.4 Solid-Phase Extraction

Upon successful synthesis of freeze-dried carbon cryogels and cryogels, extraction of polar herbicides, polycyclic aromatic hydrocarbons, doubly halogenated benzenes, and di- and tri-chlorinated phenols was performed. Approximately 125 μg/mL solutions of the analyte to be extracted were made in methanol. The analyte solutions were separated into 2 mL aliquots, and approximately 20 mg of carbon cryogel was introduced to the solution. The solutions were then permitted to equilibrate for a 24 hours at room temperature while continuously being shaken with a multi-wrist shaker (Fisher Scientific, Fair Lawn, NJ, USA). After the 24 hour equilibration, the solution was separated from the cryogel by decanting. After decanting, the liquid was concentrated to 1.5 mL under nitrogen, before being introduced for gas chromatographic analysis.

3.2.5 Gas Chromatography-Flame Ionization Detection

The gas chromatography was performed with a Hewlett Packard 5890i equipped with a flame ionization detector and a Hewlett Packard 7683 autosampler. The operating conditions for the analysis of solute extracted with the freeze-dried gels were as follows:
injection temperature 260 °C, FID temperature 280 °C, GC-oven temperature program 80 °C for one minute, ramp 15 °C/ min. to 178 °C, hold 178 °C for 2 minutes, then ramped to 183 °C at a rate of 2 °C/min., then immediately ramped to 290 °C at rate of 50 °C/min., 290 °C was held for two minutes, and finally the GC oven was permitted to cool to 80 °C before the next run. Calibration curves of the organic compounds were performed to allow the amount of the organic compounds to be quantified. An internal standard, dodecane, was spiked into the solutions of extracted solutes with freeze-dried gels.

Once the analytes were run on the GC, the peak area of the analyte peak was normalized to the peak area of the internal standard. This permits quantitative data to be obtained from the chromatogram without systematic error due to the loss of sample during transfer from the injector to the column. Analysis of the peak area ratios permitted calculations of the percent extracted and the amount extracted. In order to calculate the amount of analyte extracted, a calibration curve was necessary. The equation that defined the calibration curve was utilized to determine the amount of analyte in the supernatant solution that resulted from the extraction. The amount extracted was then calculated by taking the difference of amount of analyte to which the gel was exposed and the amount of analyte in the supernatant solution. The percentage extracted was calculated using equation 3.3.

\[
\%_{\text{extracted}} = \left( \frac{C_0 - C_e}{C_0} \right) \times 100\% \tag{3.3}
\]

These calculations were performed for all extractions with freeze dried gels.
3.2.6 Molecular Modeling

Spartan Student Physical Chemistry Edition Version 1.0.1 (Wavefunction, Inc. Irvine, CA, USA) was employed to visualize three-dimensional structures of the analytes involved in extraction. The 3-D structures were obtained by building each molecule piece-wise and minimizing the potential energy of the molecule. The minimized 3-D structure was then oriented in the most common arrangements possible with a flat surface, which are flat with respect to the largest plane of the molecule and with the dipole moment of the molecule perpendicular to the flat surface. These orientations permit dispersion, dipolar/polarizability, and hydrogen bonding interactions to be observed more readily.

3.3 Results and Discussion

3.3.1 Synthesis

Gels were synthesized under two pH conditions and differences in the properties of the resultant gel were investigated. R:F gels were produced with an R:F of 1:2 and R:C of ~50:1 and an initial pH between 5.5 and 7.5. All solutions were treated as mentioned above. It was observed that initial solutions with a pH value below 6.5 did not produce gels, but the solutions did produce an orange-yellow precipitate. Sols with initial pH values between 6.5 and 7.5 resulted in gels after several days at elevated temperature, 85 °C. Therefore, all gels used in the following studied were produced using initial solutions where the pH value was between 6.5 and 7.5.
<table>
<thead>
<tr>
<th>Material</th>
<th>Length (cm)</th>
<th>Diameter (cm)</th>
<th>Volume (cm(^3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undried/Unpyrolyzed Hydrogel</td>
<td>2.8</td>
<td>1.3</td>
<td>3.5</td>
</tr>
<tr>
<td>Supercritically Dried/Pyrolyzed Xerogel</td>
<td>0.77</td>
<td>0.45</td>
<td>0.12</td>
</tr>
<tr>
<td>Freeze-Dried/Pyrolyzed Cryogel</td>
<td>2.1</td>
<td>1.1</td>
<td>1.7</td>
</tr>
</tbody>
</table>

**Table 3.1**: Size (cm) and volume (cm\(^3\)) of the various forms of RF gels produced for this study. Volume calculated using volume of cylinder equation as produced gels were cylindrical.
Several attempts to synthesize carbon aerogels were hindered by significant shrinkage (>80%) of the gel while performing the supercritical drying. Several other methods of drying were also attempted. Ambient temperature drying was attempted [33, 34], and severe shrinkage of the gel was observed. Therefore, ambient drying was not pursued any further. Freeze drying, using the previously described protocol, was performed, and resulted in gels retaining a high fraction of their original size. Table 3.1 shows how the size of the gels changed after the designated drying method. The table shows that the pre-dried gels are an average of 2.8 cm tall × 1.3 cm in radius, giving a volume of 3.5 cm$^3$. After supercritical drying and thermal processing, the gels had average dimensions of 0.77 cm tall × 0.45 cm in radius, and a volume of 0.12 cm$^3$. This is a difference of 72 %, 65 %, and 97 % difference in the length, radius, and volumes of the pre-dried gel and supercritically dried gels, respectively. The freeze-dried and thermally processed gels were determined to have lengths of 2.0 cm × 1.1 cm radius, or 29 % smaller in length and 15 % smaller in radius. These dimensions give rise to an average volume of 1.7 cm$^3$, a difference of 50 % in the volume from the pre-dried gel.

The shrinkage observed when synthesizing gels through supercritical drying was attributed to incomplete removal of the organic phase, acetone, from the pores of the medium. Incomplete removal of the acetone from the pores was confirmed by the strong odor of acetone detected when moving the gels. Upon thermal processing, severe capillary pressure would be observed as the liquid acetone converts to the gas phase and is removed from the pores. Due to the supercritical drying not removing all the organic phase from the pores before thermal processing, the gels synthesized through
supercritical drying will be referred to as carbon xerogels. The gels produced through freeze-drying produced a material from which all the t-butanol was removed from the pores. Therefore the pores were filled with air before thermal processing. The carbon materials produced through freeze-drying will be referred to as cryogels due to the pores being empty before thermal processing.

3.3.2 Characterization by Raman Spectroscopy

The Raman spectra for two resulting aerogels are shown in Figure 3.2. The gels were produced using a sol with an initial pH of 6.5 and 7.4. The two large bands observed at 1350 cm\(^{-1}\) and 1590 cm\(^{-1}\) are indicative of graphitic carbon (ordered) and glassy carbon (disordered), respectively. The band at 1350 cm\(^{-1}\) has been attributed to a decrease in microcrystal size and increase in amount of disorder, and is referred to as the D-band [35]. The band at 1590 cm\(^{-1}\), the G-band, is due to the E\(_2g\) vibrational mode of graphite crystals and corresponds to relative graphite character of a carbon material [35]. Single crystal graphite would only have the band at 1570 cm\(^{-1}\) present [35]. The size of the microcrystalline unit can be determined from the peak areas (integrated intensity) of the characteristic graphitic carbon bands (1360 cm\(^{-1}\) and 1590 cm\(^{-1}\)) in a Raman spectra using the Equation 3.4 [35, 36, 37].

\[
\frac{C_\alpha}{I_\alpha} = \frac{I_D}{I_G} \quad (3.4)
\]

\(C_\alpha\) is a proportionality constant that is dependent on the source used for excitation in the Raman experiment [37]. The proportionality constant in equation 3.4 at any wavelength
Figure 3.2: Raman spectroscopy of carbon aerogels resulting from different initial pH conditions.  **A** – Raman spectrum of carbon aerogel produced from sol at pH 6.5.  **B** – Raman spectrum of carbon aerogel resulting from sol with initial pH of 7.4.  Both aerogels were dried and thermally processed to 1000 °C.
can be estimated using equation 3.5:

\[
C(\lambda_L) \approx C_0 + \lambda_L C_1
\]  

(3.5)

where \(C(\lambda_L)\) is the constant at a given wavelength, \(C_0 = -126 \text{ Å}, \lambda_L\) is the wavelength in angstroms, and \(C_1 = 0.033\) [37]. This makes the proportionality constant 50 Å for excitation by 532 nm light. For the Raman spectra shown in Figure 3.2, the average microcrystal size was determined to be 3.6 nm for gels produced at pH 6.5 and 4.7 nm for gels produced at pH 7.4. All of the pyrolyzed cryogels and xerogels tested displayed these characteristic bands, and their peak ratios fell between the pH 6.5 and pH 7.4 ratios. The presence of these Raman bands helps confirm that the gels were converted to glassy carbon after pyrolysis.

3.3.3 Characterization by Scanning Electron Microscopy

SEM images of cryogels and xerogels after pyrolysis were obtained. Figure 3.3 exhibits SEM images of supercritically dried gels at different magnifications. It is evident from the SEM image in Figure 3.3A that the surface of xerogel is marked with irregular depressions. Upon further magnification, Figure 3.3B, the surface appears to be covered with pillar-like structures. Figure 3.4 illustrates the microscopic appearance of freeze dried cryogels. From Figure 3.4A, depressions in the carbon cryogel are clearly visible. Thread-like connections at the surface are observed upon increased magnification, Figure 3.4, and an intricate network of carbon on the surface is clearly visible. Low temperature glassy carbon (LTGC) coated carbon cryogels were also imaged. Figure 3.5 shows scanning electron micrographs of F-LTGC coated on carbon
Figure 3.3: Scanning electron micrograph image of supercritically dried carbon aerogel.
Figure 3.4: SEM image of carbon aerogel produced via freeze drying.
Figure 3.5: SEM image of F-LTGC modified carbon aerogel resulting from freeze drying followed by solvent encapsulation and thermal processing to 200 °C (A) and 400 °C (B).
cryogels produced via freeze drying. The image in Figure 3.5A is of a sample of F-LTGC coated cryogel that has been thermally processed to 200 °C. Figure 3.5B represents a sample of coated cryogel that has been treated at 400 °C. It is evident in comparing the images in Figure 3.5 to those in Figure 3.4 that there is minimal difference in the surface of the resulting gel after coating with the F-LTGC oligomer. This means that the coating the surface of freeze dried carbon cryogels with F-LTGC polymer does not significantly compromise or alter the surface of the carbon cryogel.

### 3.3.4 Characterization of Surface Area, Pore Size, and Pore Volume

The results of the surface area analysis demonstrated that the supercritically dried carbon aerogels exhibit a BET surface area of about 2.75 m² g⁻¹. The average pore size was determined to be 50.0 Å. These values, when compared to the BET surface area and pore size of the freeze dried carbon cryogels, 615 m² g⁻¹ and 137 Å, are extremely small. The large difference in these numbers stems from the significant size loss upon venting during the supercritical drying. The total pore volume as determined using BJH desorption is 0.702 cm³ g⁻¹ and 8.32 x 10⁻⁴ cm³ g⁻¹ for the freeze-dried carbon cryogels and the supercritically dried carbon aerogels, respectively. The surface area analysis data are displayed in Table 3.2. The data for the freeze-dried cryogels coated with F-LTGC and thermally processed to 400 °C are also shown. This shows that there is small difference in the textural properties of the freeze-dried carbon cryogels and F-LTGC coated carbon cryogels. The surface area, pore size and total pore volume were also determined for the freeze dried cryogel, R:F gel before carbonization. This shows that upon carbonization in
the nitrogen: hydrogen atmosphere, that there is a significant increase in surface area. This increase in surface area has been observed by several other researchers [10, 11, 13, 21, 33]. The total pore volume has also been shown to increase upon carbonization of carbon cryogels [10, 11, 13, 21, 33]. These changes in surface area and total pore volume are the result of an increase in the mesopore structure of the carbon cryogels, as recorded in literature [10, 11, 13, 21, 33]. Czakkel et al. observed significant increases in surface area for R:F aerogels, supercritically dried (270 m$^2$ g$^{-1}$), R:F cryogels, freeze dried (570 m$^2$ g$^{-1}$), and R:F xerogels, dried with acetone in pores (110 m$^2$ g$^{-1}$), upon carbonization in CO$_2$ [10]. The surface area of freeze dried carbon cryogels were determined to be between 350-525 m$^2$ g$^{-1}$, and increased to between 590-800 m$^2$ g$^{-1}$ after activation in nitrogen atmosphere at 1000 °C [11]. Fairén-Jiménez et al. observed an increase from 740 to 1300 m$^2$ g$^{-1}$ for acid catalyzed carbon aerogels with the latter value representing activation where 20 percent of the mass of the initial gel had been lost [13]. An increase of surface area and pore volume were observed as a function of pyrolysis temperature, where the surface area increased from 420 m$^2$ g$^{-1}$ to 620 m$^2$ g$^{-1}$ and the pore volume increased from 0.056 cm$^3$ g$^{-1}$ to 0.164 cm$^3$ g$^{-1}$ [21]. The surface area and pore volumes measured for the cryogel, carbon cryogel, and F-LTGC coated cryogels used in these studies are similar to those values obtained by other researchers. The Langmuir desorption isotherms for the freeze dried carbon cryogel and F-LTGC coated carbon cryogel were also collected. Figure 3.6 shows the N$_2$ adsorption/desorption isotherms for freeze dried carbon cryogel (processed to 800 °C) and the F-LTGC coated carbon cryogel (thermally processed to 400 °C). An isotherm similar to this was collected for the freeze
<table>
<thead>
<tr>
<th></th>
<th>Freeze-Dried R:F Cryogel</th>
<th>Freeze-Dried Carbon Cryogel</th>
<th>400 °C F-LTGC Coated Carbon Cryogel</th>
<th>Supercritically Dried Carbon Xerogel</th>
<th>F-LTGC Coated Supercritically Dried Carbon Xerogel</th>
</tr>
</thead>
<tbody>
<tr>
<td>BET Surface Area (m² g⁻¹)</td>
<td>408 ± 3</td>
<td>614 ± 2</td>
<td>692 ± 2</td>
<td>2.75 ± 0.28</td>
<td>2.89 ± 0.31</td>
</tr>
<tr>
<td>BJH Desorption Cumulative Surface Area of pores 1.7 – 300 nm (m² g⁻¹)</td>
<td>268</td>
<td>205</td>
<td>298</td>
<td>0.665</td>
<td>1.47</td>
</tr>
<tr>
<td>BJH Desorption Cumulative Pore Volume of pores 1.7 – 300 nm (cm³ g⁻¹)</td>
<td>0.561</td>
<td>0.702</td>
<td>1.12</td>
<td>8.32x10⁻⁴</td>
<td>6.59x10⁻³</td>
</tr>
<tr>
<td>BJH Desorption Average Pore Diameter (4V/Å)</td>
<td>83.8</td>
<td>137</td>
<td>150</td>
<td>50.0</td>
<td>179</td>
</tr>
</tbody>
</table>

**Table 3.2:** Textural properties of aerogels and xerogels used in extraction experiments. [R:F cryogel – gel directly after freeze drying: Freeze dried carbon aerogel – gel produced after thermally processing cryogel to 800 °C: F-LTGC coated carbon aerogel – freeze-dried carbon aerogel after coating with F-LTGC polymer: Supercritically dried carbon xerogel – supercritically dried (partially) thermally processed to 800 °C: F-LTGC coated supercritically dried carbon xerogel – supercritically dried xerogel after coating with F-LTGC polymer.]
Figure 3.6: N\textsubscript{2} adsorption/desorption isotherms for freeze dried carbon aerogel (800 °C) (A) and carbon aerogel coated with F-LTGC (400 °C) (B).
dried cryogel (not thermally treated) and F-LTGC coated carbon cryogels. These isotherms are similar to those observed for carbon aerogel materials in the previously mentioned studies [10, 11, 13, 33]. The shape of both isotherms is Type IV, according the IUPAC definition. Type IV isotherms are indicative of mesoporous materials [21]. A hysteresis loop is observed between \( P/P_0 \) 0.8 – 1.0, and is attributed to capillary condensation during desorption [21]. The steep change in adsorption at low relative pressure is attributed to micropore filling [11, 13, 21]. The presence of micropores and mesopores in the carbon cryogel and coated carbon cryogel is confirmed by the adsorption/desorption isotherm.

3.3.5 Characterization of Surface Chemistry

Surface chemistry was analyzed using XPS, as mentioned in the experimental section. The trend for F-LTGC coated carbon cryogels, shown in Figure 3.7, is similar to the trend that had been observed by Giardina and Olesik with the same F-LTGC [27]. The percentage of fluorine was previously been to be 35%, 34%, 30%, 16%, 7% and 0% for the oligomer, 200 °C, 310 °C, 400 °C, 600 °C and 1000 °C F-LTGC, respectively [27]. Since the F-LTGC used in this study is the same, the amount of surface fluorine remaining on the coated cryogels should be about the same. However, there is a lower percentage of surface fluorine observed on the coated carbon aerogel as the percentage of surface fluorine is 12.3% at 200 °C, 10.3% at 310 °C, 7.2% at 400 °C, 2.5% at 600 °C, 1.2% at 800 °C, and 0.0% at 1000 °C. The suppressed amount of fluorine in the coated cryogels stems from the coating being thin enough that the x-rays employed in analysis are able to penetrate through the film. The film must be less than 50 Å thick, as the
Figure 3.7: Plot of % Fluorine observed at the surface of several surfaces, (square) and % F in precursor, (triangle) % F on surface of coated FDCC. **Error bars representing one standard deviation are contained in the points**
penetration depth of the x-rays employed in XPS analysis is 20-50 Å [38]. These data show that the surface of the freeze dried carbon cryogels have successfully been coated with F-LTGC polymer, and the thickness of the coating is less than 50 Å.

3.3.6 Extraction

Solid phase extraction with supercritically dried carbon xerogels was not pursued due to the significant shrinkage observed after drying/pyrolysis, and the low surface area measured for supercritically dried xerogels does not lend to attaining high extraction capabilities. Therefore, the following discussion will pertain to extractions performed with freeze dried carbon cryogels and F-LTGC coated carbon cryogels, which were performed as described. The gas chromatographic analysis of solutions post extraction produced chromatographic peak areas that correlate to the amount of analyte remaining in solution after 24 hours of exposure to the separation medium while shaking at room temperature. The difference in the amount of analyte initially in the solution and the amount remaining in the solution post-extraction correlates to the amount of analyte extracted by the separation medium. The peak area ratio with respect to dodecane, the internal standard, was used to quantify the amount of analyte extracted by a given carbon cryogel or coated carbon cryogel. A 4-point calibration curve for each analyte was constructed, and each resulted in a correlation of linearity of ≥ 0.984. This high linear correlation of the calibration curves allows for reliable prediction of the amount of analyte remaining in solution after the 24 hour extraction was completed.
The adsorption capacity was calculated for each analyte extracted by each phase. The equation used to calculate the adsorption capacity is shown in Equation 3.7:

\[ q_e = \frac{V(C_0 - C_e)}{m} \]  

(3.7)

where V is the volume of solution to which the adsorbent has been exposed, \(C_0\) is the initial concentration of analyte in solution (mg/L), \(C_e\) is the concentration of solution at equilibrium (mg/L), and m is the mass of the adsorbent in grams. The normalization to the mass of adsorbent used will permit relative adsorption ability to be quantified for each analyte extracted by all FDCC and coated FDCC materials employed in this study.

The analytes used in this study, Figure 3.8, were chosen due to their environmental relevance. Atrazine and simazine are representative \(s\)-triazine molecules, and have been used as pesticides [39]. Pyrene and phenanthrene are representatives of polycyclic aromatic hydrocarbons, which also are important environmentally, as they are persistent hydrophobic contaminants of soil [40]. Phenolic compounds used in the production of plastics, pesticides, paper, etc., are often released into the environment. Chlorophenols are byproducts of water treatment [41]. Carbon aerogels have been used for the extraction of analytes from solutions. Carbon aerogels had adsorption capacities of 40-100 mg/g for four dye molecules [24]. The dyes and their respective adsorption capacities are: rhodamine B (100 mg/g), acridine orange (58 mg/g), crystal violet (50 mg/g), and methylene blue (38 mg/g) [24]. The adsorption capacities displayed in this work fall in the ranges mentioned above.
Figure 3.8: Chemical Structures of analytes used for extraction by Carbon Xerogels, F-LTGC coated Carbon Xerogels, Freeze Dried Carbon Aerogels, and F-LTGC coated Freeze Dried Carbon Aerogels.
3.3.6.1 Extraction of Analytes with Freeze-Dried Carbon Cryogels

The adsorption capacities of freeze-dried carbon cryogel (FDCC) after carbonization at 800 °C in 95:5 N\textsubscript{2}:H\textsubscript{2} for the analytes are shown in Figure 3.8. The FDCC demonstrates the ability to adsorb both polar and non-polar analytes from the methanol solution to which they were exposed. The ability of carbonaceous media to extract a polar, nonpolar, and ionic species has been observed by several other researchers [42, 43, 44]. The adsorption capacities of the FDCC varied from 14.4-29.4 mg/g for the analytes studied here; the largest adsorption capacity for FDCC was 29.4 mg/g, for dichlorobenzene.

The average adsorption capacity for all studied analytes is 23.6 mg/g. Figure 3.8 shows that the adsorption capacities of the majority of the analytes are similar. The adsorption capacity for atrazine and simazine are both statistically smaller than the adsorption capacities for the remaining analytes. The difference in the adsorption capacities is due to the \textit{s}-triazine molecules not being confined to a planar orientation. Differences in chromatographic behavior that follow steric differences in molecules have previously been observed [45].

3.3.6.2 Extraction of Analytes with F-LTGC coated FDCCs

The average adsorption capacity of the 200 °C F-LTGC coated FDCC is 20.3 mg/g. While this value is lower than the average adsorption capacity of the bare FDCC, a t-test comparison at the 95% confidence interval proves the two means are statistically similar, indicating no apparent change in the average sorption conditions through coating
Figure 3.9: Adsorption capacity for freeze-dried carbon cryogel (FDCC) carbonized at 800 °C. The analytes studied are: atrazine (ATRA), simazine (SIM), pyrene (PYR), phenanthrene (PHEN), 1,2-dichlorobenzene (Cl2-B), 1,2-dibromobenzene (Br2-B), 2,4-dichlorophenol (Cl2-P), and 2,4,6-trichlorophenol (Cl3-P). **Error bars represent one standard deviation**
**Figure 3.10:** Adsorption capacity for F-LTGC coated FDCC thermally processed to 200 °C. The analytes studied are: atrazine (ATRA), simazine (SIM), pyrene (PYR), phenanthrene (PHEN), 1,2-dichlorobenzene (Cl2-B), 1,2-dibromobenzene (Br2-B), 2,4-dichlorophenol (Cl2-P), and 2,4,6-trichlorophenol (Cl3-P). **Error bars represent one standard deviation**
Figure 3.11: Adsorption capacity for F-LTGC coated FDCC thermally processed to 1000 °C. The analytes studied are: atrazine (ATRA), simazine (SIM), pyrene (PYR), phenanthrene (PHEN), 1,2-dichlorobenzene (Cl2-B), 1,2-dibromobenzene (Br2-B), 2,4-dichlorophenol (Cl2-P), and 2,4,6-trichlorophenol (Cl3-P). **Error bars represent one standard deviation**
Figure 3.12: Adsorption capacity of analytes F-LTGC coated FDCC.  (A) Atrazine and Simazine; (B) Pyrene and Phenanthrene; (C) Dichlorobenzene and Dibromobenzene; (D) Dichlorophenol and Trichlorophenol. **Error bars represent one standard deviation**
with F-LTGC. The average adsorption capacity of the 1000 °C F-LTGC coated FDCC is 29.6, and is statistically larger than the average adsorption capacities of the bare FDCC and 200 °C F-LTGC coated FDCC.

Figure 3.12 shows the relationship between the adsorption capacities of the F-LTGC coated FDCC and processing temperature. There is an observable temperature dependence of adsorption capacity seen in Figure 3.12 A, C, and D. It is clear that for temperatures between 400-600 °C there is a shift in the adsorption capacity that increases with temperature. This change in adsorption capacity seems to follow the previously observed temperature range at which the F-LTGC oligomer converts from a perfluorinated surface to a graphitic surface [27, 28]. The molecules displayed in Figure 3.12A, C and D all have a slight dipole moment, which implies that polar analytes interact differently with the perfluorinated surface than the graphitic surface. The observed trend can also be attributed to the molecules being halogenated, as shown in Figure 3.8. Perfluorinated media are known to interact favorably with halogenated compounds. The lack of change in adsorption capacity with the nonpolar analytes, pyrene and phenanthrene, is not surprising, as the perfluorinated surface and the graphitic surface are both hydrophobic.

By comparing the data in Figures 3.9, 3.10 and 3.11, it is clear that the selectivity of the bare FDCC is different than the selectivity of the F-LTGC coated FDCC. The adsorption capacities for simazine, dichlorobenzene and trichlorophenol all increase at a greater rate than the other compounds for F-LTGC coated FDCC. However, the selectivity trends between bare FDCC and F-LTGC coated FDCC for similar classes of
compounds remain the same. For example, the adsorption capacity for simazine is always larger than the adsorption capacity for atrazine, the adsorption capacity for pyrene is always larger than that for phenanthrene, the adsorption capacity for dichlorobenzene is larger than the adsorption capacity for dibromobenzene, and the adsorption capacity for trichlorophenol is always larger than that for dichlorophenol.

As previously mentioned, the adsorption capacity with simazine is statistically larger than that with atrazine for all media employed. The difference can be attributed to differences in the structure of the two molecules. Figure 3.8 shows that atrazine and simazine differ in that atrazine has one amine that has a terminal isopropyl group and the amine groups in simazine both terminate with ethyl moieties. These differences in the structure lead to different polarities and three dimensional structures. The shape of the molecules will help dictate the orientation of the dipole with respect to a flat surface. A flat molecule is able to get closer to an adsorbent surface, which will cause a molecule to experience stronger dispersion interactions. As dispersion forces are often a dominant retention mechanism, the proximity to the surface results in higher adsorption of flat molecules [46]. The terminal isopropyl group in atrazine does not permit a planar arrangement of the molecule. Therefore, simazine should be sorbed more strongly to a surface. Similar reasoning has been reported for increased adsorption of analytes previously with LTGC surfaces [27, 28]. Carbon based media and highly crosslinked divinyl benzene media have displayed little difference in selectivity between atrazine and simazine [43, 47].
Figure 3.13: Three dimensional structures of analytes obtained using Spartan.
A larger average adsorption capacity is observed for dichlorobenzene than dibromobenzene. The difference in the adsorption capacity could arise due to dispersion and dipolar interactions. A space-fill representation of the molecules demonstrates that both molecules are going to be able to participate in approximately equal dipolar interactions, Figure 3.11. The dipole moment of dichlorobenzene is 3.20 Debye, and larger than the dipole moment of dibromobenzene (2.56 Debye). The larger dipole permits stronger dipole-dipole interactions. Another source of the observed selectivity is the difference in the electronegativity of the halogens. Chlorine is more electronegative than bromine, which permits strong electrostatic interactions with the surface. The difference in electronegativity is also responsible for the difference in the polarity of the two molecules.

The relationship of the change in adsorption capacities of pyrene and phenanthrene, two polycyclic aromatic hydrocarbons (PAHs), and processing temperature is shown in Figure 3.11B. One would expect the larger pyrene to participate in more dispersion interactions, which would give rise to a higher adsorption capacity. The only phase that displays an adsorption capacity for pyrene larger than phenanthrene is the F-LTGC thermally processed to 1000 °C. The use of carbonaceous media have also been used to extract PAHs from liquid solutions [41, 48]. Extractions performed with multiwalled carbon nanotubes and activated carbons have also demonstrated very little difference in selectivity between phenanthrene and pyrene [41, 48].

As previously mentioned, a higher adsorption capacity with trichlorophenol was obtained than with dichlorophenol. This observation is not surprising as trichlorophenol
should be able to participate in more dispersion interactions and also more n-lone pair electron interactions. Previous work using F-LTGC media have demonstrated that π-electron interactions and n-lone pair interactions are important in retention in solid phase microextraction and reversed-phase liquid chromatography [28, 31]. For the phases studied herein, the adsorption capacities with the halphenolic compounds are not statistically different. However, a statistically larger adsorption capacity for trichlorophenol is also observed for the F-LTGC coated FDCC phases thermally processed at temperatures ≤ 400 °C. The explanation for this large difference is that the F-LTGC surface with a high percentage of surface fluorine permits stronger interaction with the larger, more polarizable, trichlorophenol. Perfluorinated stationary phases are known to be selective for halogenated compounds. The extraction of chlorophenols has also received attention in literature. The findings that more trichlorophenol was able to be extracted by the FDCCs used was supported by the results of studies where chlorophenols were extracted in solid phase extraction [49] and stir bar extraction [50]. Gawdzik et al. found enhanced selectivity for trichlorophenol than dichlorophenol was extracted with carbonized divinyl benzene [49]. Kawaguchi et al. were able to extract trichlorophenol and dichlorophenol with similar efficiencies using polydimethylsiloxane coated stir bars [50].

3.4 Conclusions

This chapter demonstrated the synthesis and modification of mesoporous carbon separation media. Carbon media synthesized via two drying processes, supercritically
and freeze drying, was investigated. This work also demonstrates that the surface of freeze dried carbon cryogels can be coated using a solvent encapsulation method without compromising the surface area of the carbon medium. The ability to extract organic compounds from liquid mixtures was investigated and analyzed using gas chromatography equipped with flame ionization detection. The extraction performance of FDCCs and F-LTGC coated FDCCs thermally processed at 200 °C and 1000 °C were compared. The adsorption capacity of analyte extracted by the 1000 °C F-LTGC is greater than that observed using 200 °C F-LTGC coating for most analytes. It is apparent that dispersion interactions play a role in determining the amount of analyte extracted, as noticed when comparing extraction data of structurally similar analytes. More planar molecules were often removed from solution more efficiently. It was also observed that there is a benefit to extracting atrazine, simazine, and dichlorophenol with F-LTGC coated FDCC. Statistically higher average adsorption capacities for F-LTGC coated FDCCs thermally processed at temperatures ≥600 °C were observed than for bare FDCCs and F-LTGC coated FDCCs thermally processed at ≥ 400 °C. The adsorption capacities with the s-triazines, dihalogenated benzenes, and halogenated phenols displayed marked differences as a function of processing temperature for F-LTGC coated phases. These changes are attributed to the change from perfluorinated to graphitic chemistry that occurs with the F-LTGC oligomer at these temperatures.
3.5 References


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CHAPTER 4

FABRICATION OF MICROMETER AND NANOMETER CARBON FEATURES

4.1 Introduction

The fabrication of micrometer features has been accomplished using photolithography for several years in the semiconductor industry [1, 2, 3]. Photolithography employs chemical and energetic processes to create micrometer features. Electromagnetic radiation is used to modify the chemistry of a photosensitive polymer, or photoresist, in such a way as to change the solubility of the photoresist. Alkaline and acidic solutions can also be used to etch solid surfaces in order to produce micrometer features [1,3]. Electron beam lithography has been used for the fabrication of nanometer features [2, 4]. Soft lithographic processes permit the fabrication of micrometer features in elastomeric polymers, and have been gaining increased exposure [5]. Soft lithography also includes the use of the microfabricated elastomeric material as a template by which other polymeric micrometer and nanometer features can be fabricated [6]. Photolithography utilizes the energy of photons to produce micrometer features with attainable resolution described by Equation 4.1:
\[
\begin{align*}
\frac{3}{2} \left( \lambda (s + z) \right) \\
\end{align*}
\]

Where \( w_{\text{min}} \) is the minimum line width, \( \lambda \) is the wavelength of light used, \( s \) is the separation distance between the mask and the photoresist, and \( z \) is the thickness of the photoresist. The process of photolithography is shown in Figure 4.1. In the performance of photolithography, first a photoresist polymer is coated onto a surface. A positive or negative photoresist is used in order to accomplish the technique, and the choice of photoresist type is determined by the type of features desired by the user. A positive photoresist is defined as a polymer that increases in solubility upon exposure to an electromagnetic radiation source, and a negative photoresist exhibits the opposite property, exposure to electromagnetic radiation decreases solubility. A mask is employed in order to produce the desired pattern in photolithography. The mask is composed of a material that is opaque with respect to the electromagnetic radiation employed, and has been machined to contain the desired pattern. Two methods of photolithography can be performed; contact printing, in which a mask is placed directly on the surface to be patterned, and proximity printing, where a mask is placed near the desired substrate surface. The photoresist not covered by the mask is then exposed to electromagnetic radiation. The exposed photoresist is then dissolved using a developer solution, which is specific for a given photoresist. As seen in Figure 4.1, a positive photoresist produces a positive image of the mask; the use of a positive photoresist is manifest in recesses in the photoresist surface due to removal of the photo-exposed polymer (left side). The use of a negative photoresist, as shown in the right side of Figure 4.1, produces features where the photoresist was exposed to electromagnetic
Figure 4.1: Process of photolithography and microfabrication with casting of an elastomeric polymer. The top middle represents photolithography. The microfabrication process with a positive photoresist is displayed on the left side, and the result of using a negative photoresist is shown in the right side.
radiation. Photolithography is easily employed, but there is a high cost, due to the necessary use of expensive cleanroom facilities and photomasks are necessary to produce micrometer or smaller structures [1]. The processes of coating, exposing, and developing are photolithographic processes, and the processes after development of the photoresist are microfabrication. Some microfabrication processes are described in the following text.

A microfabrication technique, anisotropic wet etching, also referred to as chemical etching, can be performed by employing an alkaline or acidic etchant to react surface material away, thus leaving a recess where the etchant was allowed to react. Anisotropic etching requires alignment of structures with crystal planes when using silicon wafers [1]. The reason for this is that the crystal planes etch at different rates. Alkaline etchants, potassium hydroxide (KOH) and tetramethyl ammonium hydroxide (TMAH), etch the (100) plane, respectively, 200 and 30 times faster than the (111) plane [1]. It is necessary to mask off the area of the substrate that one does not want to etch. In alkaline etching of silicon, silicon dioxide, and silicon nitride, chemically vapor deposited films are used as a masking material.

Isotropic etching is often performed when using glass, SiO₂, as the substrate. Hydrofluoric acid is the most common acidic etchant used [3]. Solutions of HF that range from 1-50 % are most often employed, and the etch rate increases as a function of the concentration of etchant. The chemical reactions that describes the etching of SiO₂ with HF are listed in reactions 4.1, 4.2, and 4.3:

\[
\text{SiO}_2 + 6\text{HF} \rightarrow \text{H}_2\text{SiF}_6 + 2 \text{H}_2\text{O} \quad [4.1]
\]
The hydrofluoric acid, HF, reacts with the silica, SiO₂, to form dihydrogen silicon hexafluoride species. The formation of H₂SiF₆ is the desired product of the reaction with HF, as it allows the most control over etching of the silica surface when performing isotropic etching. Reaction 4.2 shows that another HF species, HF₂⁻, can react with the silica surface. HF₂⁻ is present at higher concentrations, and etches the silicon dioxide surface 4.5 times faster than HF. Reaction 4.3 shows what occurs when ammonium fluoride (NH₄F) is used to buffer the HF solution, thus maintaining a constant pH and constant concentrations of HF and HF₂⁻, the etching species. The etch rate is more easily maintained with the employment of the ammonium fluoride buffer, as the formation of HF₂⁻ does not change as the etching progresses [3].

Soft lithographic processes have become more prevalent for the fabrication of microfluidic devices and sensors [6]. The term “soft lithography”, initially coined by Whitesides and colleagues described a set of techniques for fabricating structures in elastomeric material, for modifying the chemistry of surfaces, and controlling flow adjacent to surfaces [6]. Soft lithography is relatively inexpensive, easy to perform, and does not require cleanroom control of the laboratory environment to produce micrometer sized features. Whitesides et al. describe three processes for performing soft lithography. The first procedure described, casting, is accomplished by pouring (poly)dimethyilsiloxane (PDMS) over a pattern, and permitting the PDMS to polymerize [6]. This method permits simple fabrication of microfluidic channels by removing the
cross-linked PDMS from a master and sealing the resultant patterned-PDMS on a flat surface. This technique has been employed for fabrication of PDMS microfluidic devices [5, 6, 7]. A master, the product of photolithography or electron beam lithography, has microfabricated features on a substrate that can be used as a mold to produce the features in a second polymer. Another fabrication technique is spin-coating [6]. Spin-coating is performed by placing a small volume of solution containing the desired polymer on a master, and rotating the master at high revolutions per minute. The high rate of rotation spreads the solution over the entire surface of the master, and also permits deposition of the polymer into recesses on the master. The spinning also promotes evaporation of the solvent. For viscous polymer solutions, spin-coating permits a uniform layer to be deposited over a master. Upon removal of the master, one is able to obtain the negative image of the features patterned on the master. A third technique is stamping. Stamping involves casting a PDMS layer of a master that has the positive image of the desired features, producing a negative image of the desired features in the PDMS. The features can then be produced by using the resulting PDMS as a stamp to transfer patterns to surfaces [6]. This process is termed microcontact printing, and contains one technique employed in the following body of work, micro-transfer molding (µTM).

Double stamp micro-transfer molding (µTM ) is accomplished by spin-coating a PDMS mold with a polymer solution. Upon completion of the spin processing, a film of polymer remains on the surface and in the patterned recesses of the PDMS master. The fabrication of the stand alone polymer features is obtained by employing a multi-step stamping process. The first stamp is used to remove the polymer from the top surface of
Figure 4.2: Schematic demonstrating process of micro-transfer molding (\(\mu\)TM).
the PDMS, the sacrificial layer of polymer. After the removal of the sacrificial layer, the polymer that remains on the PDMS mold is that held in the recesses of the mold. The polymer is removed from these recesses by applying pressure to the top of the mold and heating the substrate to which the features are to be transferred. The process of μTM is displayed in Figure 4.2 [8].

Replica molding, the second soft-lithographic method employed in this study, is accomplished using similar techniques as μTM. The main difference, as observed in Figure 4.3, is that the polymer-containing solution is placed directly on the substrate that is being patterned with the desired features. The features are obtained by placing a PDMS mold over the polymer-containing solution, followed by the application of pressure and heat [6].

Nanometer features are easily obtained by employing electron beam lithography. Electron beam lithography is a photolithographically based technique that utilizes a focused beam of high energy electrons to bombard a photoresist surface. The focusing elements in an EBL instrument allow for direct writing of features with lateral dimensions of 20 nm [2, 4].

Low-temperature glassy carbon (LTGC) has been used extensively in electrochemical and separation sciences [9, 10, 11, 12, 13, 14]. It has been demonstrated that a diethynyl aromatic precursor polymer can exhibit markedly different retention properties as a function of processing temperature [9, 11, 12, 13]. The differences arise from different amount of graphitic character that is produced at different processing temperatures. Olesik and colleagues examined the behavior of a silicon low-temperature
Figure 4.3: Schematic demonstrating the process of replica molding (RM).
glassy carbon (Si-LTGC) using liquid chromatography [9, 13]. Shearer and Olesik examined a fluorine-containing low temperature glassy carbon (F-LTGC) using liquid chromatography [14]. Giardina studied the extraction of compounds using the Si-LTGC and F-LTGC [11]. The LTGC used in the aforementioned studies was developed by Callstrom and McCreery, and is able to convert to a glassy carbon structure at relatively low temperatures (between 200 and 400 °C) and is soluble in common organic solvents [12]. Due to unsaturation in the precursor, the polymer precursors are able to cross-link and are converted to glassy carbon with minimal mass loss [10].

The ability to selectively modify patterned surfaces with a diethynyl aromatic precursor to glassy carbon using soft lithography was examined. The viability of applying micro-transfer molding and replica molding of micrometer features was investigated. The efficacy of discrete and continuous features fabricated through micro transfer molding and replica molding is examined. The effect of processing temperature on the vertical dimensions of replica molded features is studied. The following work was performed as a stepping stone to fabrication of carbon-based micrometer and nanometer fluidic devices with possible applications in analytical chemistry, biomedicine, and engineering.

4.2 Experimental

4.2.1 Materials

HPLC-grade toluene was obtained from Fisher Scientific (Fair Lawn, NJ, USA) and used as received. Silicon-containing (Si-LTGC) and fluorine-containing (F-LTGC)
low-temperature glassy carbon precursors were synthesized in-house using a previously published method [10, 11]. Two silane-coupling agents, 3-aminopropyltriethoxysilane (APTES) and 3-(trimethoxysilylpropyl)methylamine, (γ-MAPS) were obtained from Gelest, Inc. (Morrisville, PA, USA) and used as received. Polydimethyl siloxane (PDMS), Silastic T-2 and T-2 curing agent were obtained from Dow Corning (Midland, MI, USA). Hexamethyldisilazane, HMDS, was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Four-inch silicon wafers were obtained from Wafernet (San Jose, CA, USA) and were cleaved into appropriately sized pieces with a diamond-tip pen (Fisher Scientific, Fair Lawn, NJ, USA). Fused silica microscope slides were obtained from Fisher Scientific (Fair Lawn, NJ, USA) and Technical Glass Products (Painesville Township, OH, USA). Silicon was cleaved to the desired dimensions, and the fused silica and quartz were etched with a diamond tip pen and fractured to produce appropriately sized substrates.

4.2.2 Fabrication of Micrometer Features

Initial studies tested the ability to coat the diethynyl aromatic silicon-containing diethynyl aromatic precursor onto silica (SiO₂) micrometer features. The silica features were fabricated using poly-L-lysine (PLL) [15]. The details of the fabrication of SiO₂ microfeatures is as follows: four inch silicon wafers were coated with a monolayer of hexamethyldisilazane, using a gas-phase deposition oven; a positive photoresist, Shipley S1813, was spin processed on the modified silicon wafer to a thickness of 1.4 μm; a photolithography mask was then used to expose the photoresist using the manufacturer’s
protocol; the wafers were divided into 2 mm x 2 mm pieces, and then exposed to a 0.05% (w/v) solution of PLL and the solution was permitted to evaporate at 50 °C in an oven leaving a layer of PLL; liftoff of the photoresist and PLL on the photoresist was performed by sonication in acetone for 30 seconds, leaving PLL on the surface in the areas that did not have photoresist after photolithographic processing [15]. The silica features were then fabricated using a mixture of hydrolyzed tetramethylorthosilicate (TMOS), glycerol, and 0.01 M phosphate buffered saline (PBS) (1:3:8 v/v/v) that was permitted to react with the PLL on the silicon surface through a silicification reaction for 3 minutes; finally the TMOS, glycerol, PBS mixture was aspirated from the surface and the surface was thoroughly rinsed with deionized water thus fabricating silica features on the silicon wafer [15].

Once the silica features were fabricated, the micrometer features were coated with a LTGC precursor in two ways. One method was to perform a simple coating process, which consisted of dipping a wafer that had been patterned with silica features into a solution of LTGC precursor that had been dissolved in methylene chloride. A second method was to slurry coat the patterned silica with LTGC, using a similar technique as previously described by Giardina and Olesik [11]. Briefly, the LTGC precursor was dissolved in methylene chloride/n-heptane (7:3) and n-heptane was added as the methylene chloride was boiled away to maintain a constant volume. The solubility of the LTGC precursor decreased as solvent polarity decreased, which forces the LTGC precursor out of solution.
Soft-lithographic approaches, micro transfer molding (μTM) and replica molding (RM), were also attempted. In order to perform these techniques, silicon masters needed to be fabricated. Briefly, the silicon wafers were lithographically patterned using S1813 photoresist [16]. The S1813 photoresist was spin coated onto a silicon wafer to achieve a thickness of 1.5 μm. The photoresist was then patterned by exposing the photoresist covered with a photomask to ultraviolet (UV) light. The resulting photoresist-patterned silicon wafer served as a master onto which PDMS was cast. The resulting patterned PDMS will be referred to as a ‘master’. Several masters were produced using the previously described technique. Some produced masters were comprised of continuous and discontinuous patterns of several shapes with sizes that ranged from 10 μm to 250 nm. Also masters that contained line patterns of sizes between 1 μm and 5 μm wide were designed and patterned.

4.2.3 Fabrication of Nanometer Features

Electron beam lithography (EBL) was performed using a Leica EBPG-5000 electron beam lithography system (Leica Microsystems, Inc., Bannockburn, IL, USA) to directly write nanometer scale features in a photoresist coated on silicon wafers. This was performed by Aimee Bross, from the OSU Nanoscale Patterning Laboratory of the Nanoscale and Material Processing Center in the Department of Electrical and Computer Engineering. The photoresist used to make the nanometer features was an epoxy-based SU-8 photoresist. E-beam lithography utilizes a high energy beam to electrons to directly expose a photoresist with resolution down to approximately 20 nm [2, 4].
4.2.4 Soft-Lithographic Techniques

In order to perform the micro transfer molding and replica molding, molds of the patterned silicon wafers needed to be made. Cross-linked PDMS was made by mixing PDMS elastomer and PDMS cross-linker in a ratio of 10:1. The mixture was then vigorously stirred by hand to ensure a homogenous mix of elastomer and plasticizer. The mixed solution was then poured over micrometer/nanometer patterned silicon wafer. A vacuum desiccator was employed to remove the dissolved gas from the PDMS. The degassed PDMS was then cured using one of two methods. One method allowed the mixture to cure at 60 °C in an oven overnight. The second method utilized room temperature for 24-48 hours until the PDMS had cured and was no longer fluid.

Once the PDMS was cured, it was removed from the silicon master, thus rendering a negative image of the desired features in the PDMS. The PDMS was then used as a stamp to fabricate micrometer scale features. These micrometer scale features were fabricated through two soft-lithographic methods, micro-transfer molding (μTM) or replica molding (RM). These methods are displayed in Figures 4.2 and 4.3, respectively.

The negative features on the PDMS were filled with a solution of polymer, in micro-transfer molding (μTM). The solvent was driven off through high speed spinning. Next, a lift-off of the sacrificial polymer layer, down to the top surface of the PDMS mold, was performed using light pressure and heat. Finally the desired features were removed from the recesses of the PDMS mold using heat and pressure. The features were transferred onto the desired substrate, in this case: quartz, fused silica, or silicon wafer.
The process of replica molding (RM) is similar to μTM, but differs in that the recesses were filled and removed in one step. Briefly, a small volume, 10-50 μL, of solution of polymer was placed on the desired substrate; quartz, glass, or silicon. Next, the PDMS mold was placed on the drop of solution. Finally, pressure and heat were applied so that the negative features of the mold were filled with polymer solution and the solvent was driven off. The heat and pressure were applied using a laboratory hot plate (Fisher Scientific, Fair Lawn, NJ, USA) equipped with a copper stage on the hot plate’s ceramic top, modifications performed in-house. The modified hot plate was equipped with a lever and chuck, which enable pressure to be applied. The pressure-arm was equipped with a pressure read out (Omegadyne, Inc., Sunbury, OH, USA). The mold was then removed leaving the desired features on the substrate.

Once the features are molded via μTM or RM, they were converted to glassy carbon under a forming gas mixture of 95:5 N₂:H₂ in a quartz tube furnace, Lynberg BlueM (ThermoElectron Corporation, Asheville, NC, USA). The temperature was ramped from ambient to the desired temperature (200-1000 °C). The final temperature was maintained for a minimum of 10 hours while maintaining the forming gas atmosphere. The thermal processing of the LTGC was not modified from what was previously described above or in literature [11, 14].

4.2.5 Characterization

Once the features have been carbonized, they were investigated with scanning probe microscopy (SPM), optical microscopy, and scanning electron microscopy (SEM).
The SPM used in this study was a Veeco NanoMan Dimension 3100 SPM (Veeco Instruments, Inc., Woodbury, NY, USA) operated in the tapping mode. A Leitz Ergolux microscope (Leica Microsystems, Bannockburn, IL, USA) with attached digital camera was employed for optical microscopic studies. The SEM analysis was performed using a Hitachi S3000H (Hitachi High Technologies, America, Inc., Pleasanton, CA, USA). The SEM was equipped with an EDAX, Inc. Sapphire Si(Li) energy dispersive spectroscopy (EDS) detector (EDAX, Inc., Mahwah, NJ, USA), which enabled chemical characterization of the surface.

4.3 Results and Discussion

The micro patterned silica was coated with low temperature glassy carbon, via dip coating and slurry coating. The coated features were then thermally processed and characterized using SEM and optical microscopy. SEM micrograph and optical micrograph images are presented in Figure 4.4. As can be observed from these images, the carbon precursor exhibits an affinity for the patterned silica over the bare silicon substrate. The first SEM micrograph, Figure 4.4A, is an image of the silica fabricated on silicon. The image shows that 10 μm features were fabricated on the silicon wafer using the PLL process described above. The second image, Figure 4.4B, is of the 600 °C Si-LTGC slurry coated onto the silica. The SEM image shows that there is an apparent higher affinity for the silica than for the bare silicon. However, continuous features did not result from execution of the slurry-coating procedure, as was observed using a similar process with silica particles for liquid chromatographic and separation science
Figure 4.4: (A) SEM micrograph of SiO$_2$ fabricated through PLL. (B) SEM micrograph of dip coated Si-LTGC on SiO$_2$. (C) Optical micrograph of Si-LTGC on SiO$_2$. 
Figure 4.5: Scanning probe micrograph image investigating vertical dimensions of the features produced via $\mu$TM with no pressure.

Vertical Height = 850 nm
Figure 4.6: Scanning probe micrograph image investigating vertical dimensions of the features produced via μTM with light pressure

Vertical Height = 750 nm
Figure 4.7: Scanning probe micrograph image investigating vertical dimensions of the features produced via μTM with high pressure.
applications [9, 10, 11, 13, 14]. The Si-LTGC formed micrometer aggregates during dissolution, as observed in Figure 4.4B. Optical images of the slurry coated silica, Figure 4.4C, showed that the carbon was not coated as a film over the entire substrate, and is manifest in the lack of the observed diffraction pattern in small areas on the optical micrograph image. The discontinuities observed in Figure 4.4 were expected with the precursor for Si-LTGC, as it has been observed in the past that the diethynyl aromatic precursor used does not readily wet silica surfaces. The optical and SEM images taken from the study of the ability to affinity coat LTGC led to more studies involving fabrication of micrometer and nanometer features of LTGC.

Initially, the efficacy of performing μTM was investigated. Early attempts to μTM micrometer scale features were performed testing the effect of the relative amount of manual pressure used during the second stamp on the dimensions of fabricated features. Figures 4.5, 4.6, and 4.7 show scanning probe micrograph analysis of the profile and height of features fabricated via μTM using a PDMS stamp that had recesses with dimensions of 1.5 μm. The precise measure of applied pressure was unavailable for this study. Therefore, the amount of pressure was determined based on the exertion of force by the experimenter. Three relative pressures were applied to PDMS stamps placed on a small volume of polymer solution on a substrate; no pressure, medium pressure, and high pressure. The substrate was placed on a hot plate to aid in the evaporation of the organic solvent. No pressure was accomplished by allowing the mass of the PDMS stamp to be the only force pushing the stamp on the solution causing the polymer the solution into the recesses in the PDMS, and is shown in Figure 4.5. The features that
were produced using no pressure demonstrated vertical dimensions of approximately 850 nm. High pressure, Figure 4.7, was applied manually by pressing on a PDMS stamp over a small volume of polymer-containing solution on the substrate of choice with the experimenter’s thumb. The high pressure method resulted in features with vertical dimensions of approximately 650 nm. The third condition, medium pressure, was accomplished by pressing down with the experimenter’s thumb, and resulted in features with vertical dimensions of 750 nm, and shown in Figure 4.6. The tops and recesses of the features produced by the three pressures also exhibited different profiles. The features produced using no pressure exhibited sharp edges and flat recesses. There is an apparent concave profile of the top of the 850 nm features. This concave shape could arise from repulsion of the polymer solution and the PDMS. The medium pressure features exhibited features with the concave tops as observed in the features fabricated with no pressure. The recesses of the features fabricated with medium pressure exhibited a convex profile. This shape arises from the flexibility of the PDMS being transferred through as a result of the downward force. High pressure produced features that displayed relatively flat top profiles and convex recesses. This profile is the result of the flexible PDMS reacting to the intense down pressure. The manual method of applying pressure was abandoned upon the fabrication of a hot plate equipped with a pressure arm and transducer, permitting reproducible pressure being applied to the ‘sandwich’ formed by the substrate-polymer solution-PDMS.

Replica molding was performed using the hot plate-pressure arm apparatus discussed previously. The substrate; a silicon wafer, piece of quartz, or fused silica; was
Figure 4.8: Scanning probe micrograph displaying the vertical dimension of features produce via replica molding before thermal processing.

Vertical Height = 900 nm
Figure 4.9: Scanning probe micrographs displaying the vertical dimension of features produce via replica molding after thermal processing. Vertical Height = 850 nm
placed on the copper-stage equipped hot plate set to 70 °C. Next, 50 µL of 10 % (w/w) Si-LTGC/toluene solution was placed on the substrate. The PDMS mold was then placed on the substrate, over the polymer solution. Pressure was applied, 10-15 psi, to the PDMS using the pressure arm. The pressure was held for approximately 5 minutes. The PDMS was then removed from the substrate, leaving the molded pattern from the recesses of the PDMS on the surface of the wafer. The replica molded features were then investigated using scanning probe microscopy. After SPM characterization, the wafers were thermally processed using the previously described process [10, 11, 14]. The replica molded features were marked in order to allow investigation of the same features before and after carbonization. SPM was performed on the same set of features on a sample before, Figure 4.8, and after pyrolysis, Figure 4.9. This allowed the change in feature dimension to be studied as a function of processing temperature. A plot of the change in feature height versus processing temperature is shown in Figure 4.10. This plot demonstrates that little change in feature height was observed at low processing temperature (200 °C). There is however noticeable height loss at higher processing temperatures (800 - 1000 °C). This loss in height is attributable to the reorganization of the precursor during controlled pyrolysis. A loss in functionality has also been observed with an increase in surface oxygen [10]. All the features were fabricated in the laboratory atmosphere, which would allow significant surface oxygen to be present. Figure 4.10 also shows how the change in step height compares to the percentage of mass loss, as was studied by Giardina using thermo gravimetric analysis (TGA) [1027]. Giardina also
Figure 4.10: Graph of the relationship between vertical dimensions versus thermal processing temperature. (*Error bars represent one standard deviation, and are within the size of the data point)
investigated the amount of surface oxygen as a function of processing temperature. For the Si-LTGC used for these studies, there is chemisorbed surface oxygen present at higher processing temperatures [10]. Rittenhouse suggested that the chemisorbed oxygen on the surface would increase upon exposure to atmosphere post thermal processing [17]. The change in step height observed in this work seems to follow the trends observed with respect to mass loss and loss of surface heteroatom. The reorganization of LTGC oligomers during controlled pyrolysis has been examined previously with Raman spectroscopy [12].

SEM was also used to visualize the features produced through μ-transfer molding and replica molding. The micrographs, Figure 4.11, show that features that range in size from 10 μm to ~600 nm were able to be fabricated using soft lithographic techniques. The ability to micro transfer mold long lines was also performed. SEM micrograph images of the long lines are displayed in Figure 4.12. The successful fabrication of long lines would be a step in the proving the ability to fabricate long channels, which could be used in fluidic devices. Successful fabrication of long lines would be defined as the resultant long lines having little variation in the top profile, as seen in Figure 4.12.

The energy dispersive spectroscopy feature attached to the SEM permitted chemical characterization of the surfaces modified using the various fabrication techniques. The SEM images and corresponding EDS spectra, Figure 4.13, display that the surface chemistry of the substrate has been altered through surface modification and microfabrication. The first EDS spectrum shows the chemical characteristics of the thin portion of the micro-transfer molded carbon features. The spectrum shows that the
Figure 4.11: Scanning electron micrograph of 5 μm features fabricated through replica molding (RM).
Figure 4.12: Scanning electron micrograph of 1 μm lines fabricated through replica molding (RM).
Figure 4.13: Scanning electron micrograph of 600 nm lines fabricated through replica molding (RM).
Figure 4.14: Scanning electron micrograph image of long lines fabricated via replica molding.
Figure 4.15: Scanning micrograph image of replica molded features. EDS spectra of the glass substrate (A) and the Si-LTGC replica molded feature (B).
Figure 4.16: Scanning probe micrograph of nanometer features fabricated via replica molding.
surface is primarily composed of oxygen and silicon. An EDS spectrum of the tallest part of the stamped feature, Figure 4.13B, shows that a significant amount of carbon was found to be present. The presence of the carbon and increased amount of silicon demonstrated that the silicon wafer surface had been modified using μTM.

Nanometer features were also produced using the previously described soft-lithographic techniques. Upon receipt of nanometer features fabricated through electron beam lithography, a PDMS mold was prepared using the previously described method. Nanometer features were then replica molded. The features were stamped with the hotplate set to 70 °C, and applying ~10 psi of pressure using the pressure arm. The solution used to replica mold nanometer features was 5 % (w/w) Si-LTGC/toluene. This solution provided nanometer features that were suitable for analysis. More concentrated solutions were attempted, but provided features that did not result in discernibly discreet features, but produced features that contained particles in the film. Figure 4.14 shows that the nanometer features produced were approximately 100 nm apart and were approximately 20 nm in vertical distance. The replica molded features did not have the square shapes that the replica molded micrometer features displayed. This deviation from the square feature shape may be attributed to deflection of the PDMS during stamping process. It is logical to think that the deflection of PDMS would be magnified as the size of the features being stamped gets smaller.
4.4 Conclusions

This chapter discusses the ability to employ the Si-LTGC and F-LTGC as a material that can be used to fabricate micrometer and nanometer scale features. Initial attempts to use affinity-based fabrication on silica surfaces did not produce continuous carbon features. Therefore, this approach was abandoned for soft-lithographic processes which proved to reproducibly produce continuous carbon features. Micro-transfer molding was utilized to produce micrometer sized features. The features produced were discrete and continuous. Replica molding was also used to produce discrete and continuous micrometer features. Of the two soft-lithographic processes employed, replica molding was chosen as the technique of choice to fabricate micro- and nanometer features due to the ease of reproducibility. This research presents the first low temperature glassy carbon micrometer and nanometer features fabricated via soft lithography. The effect of thermal processing on feature height was studied and compared to mass loss and surface chemistry. Nanometer features were replica molded from electron beam lithographically written nanometer features. This work is the first step in the development of low temperature glassy carbon based microfluidic devices, as it is important to prove that micrometer size features are able to be made. An application of replica molded carbon features in microfluidics will be presented in a subsequent chapter.
4.5 References


CHAPTER 5

CAPILLARY ELECTROPHORESIS AND CHIP ELECTROPHORESIS WITH LOW-TEMPERATURE GLASSY CARBON

5.1 Introduction

Analyte separations may be attainable by applying an electric potential across capillaries or channels. The applied electric field provides the driving force with which analytes and mobile phase are moved through the column. Electrophoresis and electroosmosis are the major fluid movement processes that occur in channels and capillaries. Jorgenson et al. were among the first to conduct free solution electrophoresis in capillary columns [1]. Microfluidics have been increasingly studied since the seminal paper on the topic was published by Manz et al. [2]. Capillaries and microchips provide a platform that allows low volumes of samples and solvents to be analyzed with high resolution [3].

The high resolution attainable with capillary electrophoresis and microfluidic chips arises due to the means of operation. The movement of species in an electric field is the sum of the electrophoretic mobility of the species and the electroosmotic flow of the bulk solution. The equations that explain these mobilities are shown in equations 5.1, 5.2 and 5.3:
Equation 5.1 describes the mobility of species in an applied electric field is dependent on two processes, electroosmotic mobility ($\mu_{eof}$) and electrophoretic mobility ($\mu_{ep}$) of all species involved. The electrophoretic mobility, defined in Equation 5.2, is dependent on two molecular properties, charge ($q$) and Stokes’ radius ($r$) of the analyte. The viscosity of the mobile phase ($\eta$) plays into both the electrophoretic and electroosmotic mobilities. The electroosmotic mobility, seen in Equation 5.3, is also dependent on the permittivity in a vacuum ($\varepsilon_o$), the dielectric of the mobile phase ($\varepsilon$) and zeta potential of the surface ($\zeta$). The mobile phase dielectric and surface charge of the surface of the capillary are dependent on pH, and therefore there is significant pH dependence when employing capillary electrophoretic techniques is observed.

The dielectric of the liquid, often a buffer, and the zeta potential of the surface give rise to an electric double layer, when an electric field is applied. The electric double layer, shown in Figure 5.1, exists due the charge at the surface of the capillary or channel. In many cases, there is a negative charge on the surface of commonly used chips and capillaries, as the substrate of choice is glass or fused silica, which has surface silanols. Cations and anions in the buffer associate near the surface forming an ordered layer that renders the surface essentially neutral. Due to the electric double layer, bulk flow of materials is observed and called the electroosmotic flow. Therefore differences in
Figure 5.1: Electric double layer and bulk solvent in fused silica capillary with electric field.
electrophoretic mobility lead to separation of analytes. The direction of the
electrophoretic mobility of analytes is dependent upon the polarization of their charge.
Anions will migrate toward the positively-charged cathode and cations will migrate toward
the negatively-charged anode.

Capillary electrophoresis is not limited to being performed in fused silica
capillaries. Coated or packed capillaries can also be used as the column in capillary
electrophoresis [3]. Surface coatings have been used to reduce interactions from the
silanols present on the surface of fused silica capillaries. The reduced interaction of the
surface silanols will often result in better separation efficiencies, peak shapes, and
recovery of analytes. This means that it is undesirable for an analyte to interact with the
wall of the surface of the column material when performing electrically driven
separations. Dynamic and chemically bonded coatings are employed to modify the
surface of fused silica. Dynamic coatings are obtained by rinsing the capillary with a
solution containing the coating reagent, and can require significant maintenance. The
maintenance that is often required is regeneration of the coating on the surface and
possibly addition of the coating reagent [3]. Chemically bonded coatings are typically
more robust than dynamic coatings. In order to obtain bonded coatings, the reactivity of
the silanol surface of fused silica is utilized to bind a polymer to the surface of the fused
silica. Bonded coatings are often obtained by performing a series of sequential reactions
to prepare the fused silica surface for further modification with a desired polymer [3].

Capillary electrophoresis and capillary microchip electrophoresis have been
demonstrated as effective tools for separating biomolecules and ionic species [1, 4, 5, 6,
The most typical substrate used in capillary electrophoresis is fused silica and modified fused silica [3, 4, 5, 6, 7, 8, 9, 10]. Fused silica (glass) and polydimethylsiloxane (PDMS) are the most typical platforms utilized in microchip capillary electrophoresis [10, 11, 12, 13].

As mentioned in a previous chapter, microfabrication techniques have been employed in the semiconductor industry [14, 15, 16]. Many microfabrication processes are based upon photolithography, and limited to rigid polymers or photoresists. Whitesides and colleagues coined the term soft lithography to describe the fabrication of micrometer features in flexible polymers [17, 18, 19]. The previous chapter described in detail the soft lithographic processes of micro-transfer molding (μTM) and replica molding (RM). Replica molding, the soft lithographic technique employed in these studies, is performed in a multi-step process. First, a mask must be fabricated via photolithography or electron beam lithography. In photolithography, a photoresist material is patterned with UV light to produce micrometer scale features and patterns on a silicon wafer. After photolithography, a solution of polydimethylsiloxane (PDMS) and cross-linker are poured over the micrometer-patterned silicon wafer. The PDMS is permitted to harden forming a ‘stamp’ that has the negative image of the desired features transferred to the surface of the PDMS. The stamp is then used to fabricate the micrometer features on a desired substrate in one step, by filling the PDMS stamp with a solution containing the desired polymer and applying heat to remove the solvent. This process is pictorially shown in Figure 5.2.

The following body of work focuses on the use of the low-temperature glassy
Figure 5.2: Soft Lithography employed for the fabrication of microchannels to be used for fluidic applications, replica molding (RM).
carbon diethynyl aromatic precursor as a material to be used for fabrication of micrometer/nanometer features for use in capillary electrophoresis and microfluidic devices. The behavior of the LTGC microfluidic chips will be investigated with a fluorescent dye molecule, rhodamine 590 chloride. The application of LTGC as chemically bonded modifications to fused silica in capillary electrophoresis will be studied using four proteins; albumin, lysozyme, myoglobin, and cytochrome c; and a molecule used as an electroosmotic flow marker with fused silica, mesityl oxide.

5.2 Experimental

5.2.1 Materials

HPLC-grade toluene and ACS grade methylene chloride were obtained from Fisher Scientific (Fair Lawn, NJ, USA) and used as received. Bovine serum albumin, cytochrome c (from equine heart), lysozyme (from chicken egg white), and myoglobin (from equine heart) were obtained from Sigma Aldrich company (St. Louis, MO, USA). Silicon-containing (Si-LTGC) and fluorine-containing (F-LTGC) low-temperature glassy carbon precursors were synthesized in-house using a previously published method [20]. Two silane-coupling agents, 3-aminopropyltriethoxysilane (APTES) and 3-((trimethoxysilylpropyl)methylamine, (γ-MAPS) were obtained from Gelest, Inc. (Morrisville, PA, USA) and used as received. Polydimethyl siloxane (PDMS), Silastic T-2 and T-2 curing agent were obtained from Dow Corning (Midland, MI, USA). Hexamethyldisilazane, HMDS, was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Four-inch silicon wafers were obtained from Wafernet (San Jose, CA, USA).
Fused silica microscope slides were obtained from Fisher Scientific (Fair Lawn, NJ, USA) and Technical Glass Products (Painesville Township, OH, USA). Silicon was fractured to the desired dimensions, and the fused silica and quartz were etched with a diamond tip pen (Fisher Scientific, Fair Lawn, NJ) and fractured to produce appropriately sized substrates.

5.2.2 Chemical Modification of Fused Silica Surfaces

Fused silica was modified through a multistep process. The first step in the modification of the fused silica was activation of the fused silica surface by flowing a solution of 0.1 M NaOH through the column at 10 µL per minute. Next, 0.1 M HCl was introduced to the fused silica and flowed for 30 minutes at a rate of 10 µL per minute. After the acid treatment, toluene was introduced to the fused silica and flowed through the fused silica for 30 minutes. After toluene treatment, a solution of either APTES (10% w/v) or γ-MAPS (10% v/v) in toluene was made and introduced for 60 minutes. The fused silica that had been modified with silane-coupling agent was dried overnight by flowing nitrogen continuously. After the silane-modified fused silica had dried, a 3% w/v solution of the desired LTGC, silicon-containing or fluorine-containing, in methylene chloride was flowed through the modified fused silica at a rate of 10 µL/minute. After drying the fused silica, it was further modified with LTGC polymer using a variation of the dynamic coating method as described by Yin et al. [21]. The LTGC solution was flowed through the fused silica until no discontinuities in the flow were observed, thus leaving the fused silica filled with the LTGC/methylene chloride solution. One end of
the LTGC solution-filled fused silica was sealed by dipping it in silicone DC-11 stationary phase. The sealed end of the fused silica was then placed in liquid nitrogen. Once the end was in liquid nitrogen, a mechanical pump was used to pull vacuum on the non-sealed end of the fused silica. The applied vacuum will cause the solvent, methylene chloride, to evaporate as the solvent front moves toward the lower pressure and deposit the LTGC on the surface of the fused silica.

### 5.2.3 Fabrication of Micrometer Features

Soft-lithographic approaches, micro-transfer molding (μTM) and replica molding (RM), were also attempted. In order to perform these techniques, silicon masters needed to be fabricated. Briefly, the silicon wafers were lithographically patterned using S1813 photoresist [22]. The S1813 photoresist was spin coated onto a silicon wafer to achieve a thickness of 1.5 μm. The pattern was obtained by exposing the photoresist covered with a photomask to ultraviolet (UV) light. The resulting photoresist-patterned silicon wafer served as a master onto which PDMS was cast. The resulting patterned PDMS will be referred to as a ‘master’.

Cross-linked PDMS was made by mixing PDMS elastomer and PDMS cross-linker in a ratio of 10:1. The mixture was then vigorously stirred by hand to ensure a homogenous mix of elastomer and plasticizer. The mixed solution was then poured over the patterned silicon wafer. A vacuum desiccator was employed to remove the dissolved gas from the PDMS. The degassed PDMS was then cured using one of two methods. One method allowed the mixture to cure at 60 °C in an oven. The second method utilized
room temperature overnight to ensure the PDMS had cured.

Once the PDMS was cured, it was removed from the silicon master, thus rendering a negative image of the desired features in the PDMS. The PDMS was then used as a stamp to fabricate micrometer scale features. The micrometer channels were fabricated via replica molding (RM). Replica molding is displayed in Figure 5.2.

The process of replica molding (RM) is accomplished by filling the recesses in a master followed by removal of the master in one step. Briefly, a small volume of solution of polymer is placed on the desired substrate. Next, the PDMS mold is placed on the drop of solution. Finally, pressure is applied so that the negative features of the mold are completely filled with polymer solution and heat is applied so the solvent is evaporated. The mold is then removed from the substrate leaving the desired features on the substrate.

5.2.4 Carbonization

Once the fused silica was modified with the low temperature glassy carbon precursor and the micrometer features were molded via RM, they were thermally converted to glassy carbon under a forming gas atmosphere of 95:5 N₂:H₂. The temperature was ramped from ambient to the desired temperature (200-1000 °C). The final temperature was maintained for a minimum of 10 hours.

5.2.5 Fabrication of Fluidic Devices

Carbon channels were fabricated through replica molding onto silane-modified
glass or quartz. Various sized channels were fabricated. The fabricated channels were 100 μm wide and ranged from 20-100 μm deep. The length of the microfabricated channels in all cases is 2.5 cm. Once the channels were thermally processed, a device was fabricated by placing the channel between two pieces of PDMS [23]. The surfaces were reversibly bound by cleaning the surfaces with methanol, then heating the device at 60 °C for 10-15 minutes [10]. The device was also held together by using a home-built plexi-glass apparatus.

5.2.6 Characterization

Once the features have been carbonized, they were investigated with scanning probe microscopy (SPM), optical microscopy, and scanning electron microscopy (SEM). The SPM used in this study was a Veeco NanoMan Dimension 3100 SPM (Veeco Instruments, Inc., Woodbury, NY, USA) operated in the tapping mode. The SEM analysis was performed using a Hitachi S3000H (Hitachi High Technologies, America, Inc., Pleasanton, CA, USA).

5.2.7 Electrophoretic Conditions

Microchip electrophoresis was performed using a Spellman CZE 1000R high voltage power supply (Hauppauge, NY, USA) to provide the drive potential for all electrophoretic applications. The buffer used in these studies was a 10 mM phosphate buffer (pH = 2.5). Analyte solutions were prepared daily in water, and appropriate dilution were made with water or the operating buffer. A Harvard model 33 dual syringe
Figure 5.3: SEM micrograph images of 100 μm wide channels produced via replica molding.
pump (Fisher Scientific) was used to aid in the drive of fluid for the chip studies. The syringe pump was operated in the constant volume mode at a rate of 0.01 µL/minute. Two detectors were employed for initial separation studies, a fluorescence detector and a ultraviolet-visible (UV-vis) detector. UV absorbance measurements were obtained with a Thermo Separations Products UV 1000. Fluorescence detection was performed using a Spectrovision FD-300 fluorescence detector. The detectors were used by aligning a portion of fused silica with the UV opaque polyimide removed with the optical path of the detector. For microchip studies, the fused silica was chosen so the cross-section area of the channel and fused silica were matched, thus minimizing extra channel band broadening. The fused silica on the inlet side of the channel was modified with the LTGC used for the channel to minimize the effect of having the analytes being exposed to significantly different surfaces. The tail fused silica was minimized in size to reduce broadening due to increased volume in the column going to the detector.

5.3 Results and Discussion

5.3.1 Soft Lithographic Fabrication of Microchips with LTGC

Once the channels were stamped and carbonized, they were observed using SEM. Some sample images are shown in Figure 5.3. The SEM micrographs show channels that are 100 µm wide and 20 µm deep were fabricated using the replica molding technique described above. The channel has a continuous edge on either side of the channel. Figure 5.3B displays a channel rotated approximately 45° from perpendicular to obtain a profile of the channel. It can be seen that the fabricated channel exhibits some
Figure 5.4: Set-up used to test ability to seal LTGC channels with PDMS blanks with fluorescence detection.
discontinuity at the substrate surface and at the top edge. The discontinuity at the top edge could be due to incomplete dewetting from the PDMS stamp surface.

5.3.2 Fabrication of Fluidic Device

Once channels were fabricated, the ability to seal them with a PDMS coverslip was investigated. Figure 5.4 shows the initial configuration employed to study the ability to seal the carbon channels. Briefly, a piece of PDMS was cut to a size that would appropriately cover the channel and reservoirs. The PDMS cover had two holes placed to allow access to the reservoirs. The PDMS was placed on the top surface of the channel. Next a few drops of fluorescein isothiocyanate (FITC) dissolved in phosphate buffered saline (PBS) was placed in one reservoir of the PDMS. Vacuum was pulled on the other reservoir, allowing the PBS to fill the channel. The FitC labeled PBS was then observed through the PDMS-cover using fluorescent microscopy. Figure 5.5 shows an image of the FitC in PBS in the microchannel with no apparent leaks outside the channel. This demonstrates that the channel is successfully sealed by the PDMS.

Once sealing the channel was demonstrated, the fabrication of a device could be performed. Initially, a very similar design was employed to fabricate devices to be used by employing electrically driven flow, as tygon tubing was attached through the holes in the PDMS cover. The current-voltage response was examined using this design. As expected, the current increased as the applied voltage was increased until the circuit was no longer complete. At 15 kV applied, Joule heating was sufficient to cause discontinuity
Figure 5.5: FITC in PBS buffer flowing in LTGC channel sealed with PDMS. Fluid is driven by capillary forces.
Figure 5.6: Plexi-glass holder used to hold channel between two pieces of PDMS.
Figure 5.7: Apparatus used to conduct microchip electrophoresis studies with flow-through fluorescence detection.
of the buffer in the channel and the observed current fluctuated due to arcing. While conducting these experiments, it was observed that the use of the somewhat rigid fused silica led to a necessary change in the design of the chip apparatus, as the mass of the PDMS was not sufficient enough to seal the channels. The fused silica would cause the PDMS to pull away from the surface of the channel.

To rectify this issue, a holder was fabricated using plexi-glass. The design of the plexi-glass holder is shown in Figure 5.6, it was fabricated such that a recess for the chip to be placed on PDMS and covered with another piece of PDMS. The pressure on either side of the chip prevents the PDMS from pulling away from the chip as a result of the fused silica’s resistance to the bend. The microchip capillary electrophoretic set-up employed for measurements is displayed in Figure 5.7. The figure is not to scale and is meant to show the orientation of the components of the electrophoretic instrument employed. As previously mentioned the detector was either a UV-vis detector or a fluorescence detector. The electrophoretic instrument for capillary electrophoresis was similar to that shown in figure 5.7, with the only difference being that the capillary from the inlet was fed directly into the detector.

5.3.3 Fabrication of LTGC-modified fused silica

The LTGC modified fused silica was visualized via optical microscopy and scanning electron microscopy. Figure 5.8 shows the image of unmodified fused silica. An SEM micrograph shown in Figure 5.9, displays the result of reacting the surface of the fused silica with a silane-coupling agent and dynamic coating of the surface with a
Figure 5.8: SEM micrograph images of unmodified fused silica
Figure 5.9: SEM micrograph images of LTGC-modified fused silica
Figure 5.10: SEM micrograph images of LTGC-modified fused silica (diameter is approximately 50 μm).
LTGC precursor polymer. The LTGC in Figure 5.9 was thermally processed to 200 °C. The thickness of the LTGC coating in Figure 5.9 is approximately 3 μm. This means that the diameter of the fused silica would be changed by a total of ~6 μm. An SEM micrograph of the LTGC utilized for capillary electrophoresis is displayed in Figure 5.10. The thickness of the coating is difficult to discern from this image, but it can be determined that there is a coating on the fused silica. The coating appears continuous with droplets on the surface of the fused silica. The thickly coated fused silica was not employed for capillary electrophoretic studies, as the diameter of the coated fused silica would be different enough to affect the internal volume, making it to be less than the unmodified fused silica. The difference in internal volume would make comparison of the retention characteristics more tedious.

5.3.4 Capillary Electrophoresis with LTGC microchips

The capillary and channel was filled with 100 mM or 10 mM phosphate buffer (pH = 2.5) using a syringe pump. Once the system was filled with buffer, the ability to run the channel using electrically driven flow was examined, but it was observed that fluid was not consistently transferred from the fused silica into the channel using a concentrated solution of rhodamine 590 chloride (rho6G), which is deep red in appearance. The concentrated solution of rho6G was transferred electroosmotically driven through the fused silica, but not transferred into the channel reproducibly. This observation is attributed to the hydrophobicity differences between the fused silica and the carbon. The carbon is inherently more hydrophobic than fused silica. A syringe
Figure 5.11: Pressure assisted electropherograms of $2.04 \times 10^{-3}$ g/mL rhodamine 590 G on an Si-LTGC chip (A) and a F-LTGC chip (B). Conditions: $V_{\text{app}}$ (1 kV), $\lambda_{\text{ex}}$ (533 nm), $\lambda_{\text{em}}$ (555 nm), Buffer (10 mM phosphate at pH 2.5).
pump was used to apply enough pressure to permit the solution to pass from the fused silica into the channel and back out toward the detector through the remaining fused silica. The syringe pump was run at 0.01 μL/minute.

Figure 5.11 shows pressure-assisted electropherograms for a solution of rhodamine 590 chloride in phosphate buffer on a Si-LTGC channel (A) and an F-LTGC channel (B). Pressure was applied to aid the aqueous solution in entering the carbon channel. The electropherograms show the intensity of fluorescence at 555 nm versus time as the electrophoretic run progressed. A qualitative observation easily made from the electropherograms is that the resulting peak shape of the analyte on the materials is different. The Si-LTGC demonstrates a sharp peak, which is expected in an electrophoretic experiment. The rhodamine peak on the F-LTGC chip is broader than that on the Si-LTGC. This is more than likely attributed to some retention of the analyte on the F-LTGC. The peak height of the electropherogram is also lower on the F-LTGC chip. This supports an argument for retention as well. A comparison of the peak areas and retention times for rhodamine solutions of different concentrations is shown in Table 5.1. With the exception of the most concentrated rhodamine solution (2.04 × 10^{-3} g/mL) the peak area on the F-LTGC chip is lower than the peak area on the Si-LTGC chip. This information also lies in support of a retention interaction of the rhodamine with the F-LTGC surface. However, this data does not provide any information as to any retention on the Si-LTGC surface or F-LTGC surface compared to glass chips, as glass chips were not made or tested. The data in Table 5.1 also demonstrates the difficulty in obtaining reproducible results from run to run. The data shown in Table 5.1 are the average of a
minimum of three runs. Both sets of data demonstrate the expected decrease in peak area as a function of concentration of the rhodamine.

5.3.5 Capillary Electrophoresis with LTGC-modified fused silica

The electrophoretic behavior of fused silica and fused silica modified with either the Si-LTGC or F-LTGC polymers was investigated using capillary columns. Table 5.1 displays the electrophoretic retention and peak area of the different columns and the analytes for which they have been used.

Two 50 μm (inner diameter) capillary columns were investigated using mesityl oxide, which has been used by other researchers to determine the electroosmotic flow velocity with fused silica and glass microchips. The average retention time for the mesityl oxide on fused silica was determined to be 12.98 minutes, meaning that it would take that time in order for a neutral analyte to be moved through the column and be observed at the detector. The average retention time for mesityl oxide on F-LTGC modified fused silica was determined to be 11.49 minutes. Figure 5.12 shows electropherograms of mesityl oxide on a fused silica column (A) and F-LTGC coated column (B). Both columns had an effective length of 25 cm and a total length of 40 cm. The diameter and lengths were held constant to permit comparison of migration through the columns. If two columns have the same diameter, effective length, total length, and use the same buffer, then migration differences are dependent on the charge, charge density, and zeta potential of the surface of the column. Mesityl oxide, a neutral molecule at pH 2.5, will mark the electroosmotic velocity of the buffer. Therefore,
<table>
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<th>Column</th>
<th>Compound</th>
<th>(T_r)</th>
<th>Peak Area</th>
<th>(D_c) (um)</th>
<th>(V_{app})</th>
<th>(L_{eff}) (cm)</th>
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<td>Fused silica</td>
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<td>325669</td>
<td>100</td>
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<td>30</td>
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<td></td>
<td>2.04x10^{-5} g/mL Rhodamine6G</td>
<td>2.529</td>
<td>52892</td>
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**Table 5.1**: Average retention times and peak areas for analytes studied via electrophoresis.
neutral species would detected at 13.0 minutes for fused silica and 11.5 minutes for F-LTGC, assuming no adsorption of the analyte by the surface of the column. Sorption of mesityl oxide would be determined by a significant decrease in the peak area for the analyte. As can be seen in Table 5.1, the peak area of mesityl oxide on fused silica is approximately three times larger than the peak area of the mesityl oxide on F-LTGC. This difference implies that the mesityl oxide is adsorbed by the F-LTGC surface. The applied voltage with both columns was 15 kV, and corresponded to a current of approximately 15-18 µA with both columns. The same applied voltage and produced current permits the data on each column to be compared, as the same set of conditions makes the only variable the surface of the column. The decreased retention time observed with the F-LTGC column implies that the electroosmotic flow is faster than in the F-LTGC, despite the adsorption of mesityl oxide to the F-LTGC surface. The decreased peak area could be attributed to irreversible binding of the analyte, mesityl oxide, to the F-LTGC surface. The surface of the fused silica and F-LTGC modified fused silica was regenerated between runs by flowing dilute sodium hydroxide followed by the buffer used in analysis.

The electrophoretic retention of four proteins has also been examined using fused silica and a Si-LTGC modified fused silica as the column in capillary electrophoresis. In all cases the column was ~100 µm in diameter and an effective length of 25 cm. The applied voltage with each column was 10 kV which resulted in a current of ~30 µA using a 10 mM phosphate buffer at pH 2.5. There is noticeable difference in the retention time for albumin, cytochrome c, and myoglobin on the fused silica and Si-LTGC modified
Figure 5.12: Electropherograms of mesityl oxide on a fused silica capillary (A) and a F-LTGC modified fused silica capillary (B). Conditions: $V_{\text{app}}$ (10 kV), $\lambda$ (254 nm), Buffer (10 mM phosphate at pH 2.5), $d_c$ (50 μm), $L_{\text{eff}}$ (25 cm).
fused silica. The retention time with these three proteins was significantly smaller on the Si-LTGC than for the fused silica. The retention of lysozyme was determined to be slightly longer on Si-LTGC than on the unmodified fused silica. The peak area for all the proteins was determined to be significantly smaller for the Si-LTGC than for the fused silica. From this information, one can infer that the proteins are adsorbed to the LTGC surface. Electropherograms for cytochrome c is shown in Figure 5.12 on fused silica (A) and Si-LTGC modified (B) columns. The decreased peak height and peak area are attributed to the sorption of the analyte on the Si-LTGC surface. The top electropherogram shows the electropherogram for cytochrome c on fused silica, and the bottom electropherogram demonstrates the absorbance versus time for cytochrome c on Si-LTGC. The electropherograms are shown on the same scale to show the difference in the peak height and ratio for the two capillary columns tested. All proteins explored in this study demonstrated the same traits, as is observable in Table 5.1.

Figures 5.14, 5.15, and 5.16 show electropherograms on 100 μm fused silica (A) and 100 μm fused silica coated with Si-LTGC (B) for albumin, lysozyme, and myoglobin, respectively. These electropherograms do not show sharp symmetrical peaks, as is expected in electrophoresis [3]. However, they do demonstrate the different behavior of the fused silica and Si-LTGC modified fused silica. In nearly all cases, the retention time on Si-LTGC coated fused silica is smaller than the retention time on fused silica. This was observed with the F-LTGC coated fused silica as well, and can be attributed to faster electroosmotic flow in the LTGC coated fused silica which results from a larger zeta potential at the surface. This means that the LTGC coating is covering
Figure 5.13: Electropherograms of cytochrome c on a fused silica capillary (A) and a Si-LTGC modified fused silica capillary (B). Conditions: $V_{\text{app}}$ (10 kV), $\lambda$ (254 nm), Buffer (10 mM phosphate at pH 2.5), $d_c$ (100 $\mu$m), $L_{\text{eff}}$ (25 cm).
Figure 5.14: Electropherograms of albumin on a fused silica capillary (A) and a Si-LTGC modified fused silica capillary (B). Conditions: \( V_{\text{app}} \) (10 kV), \( \lambda \) (254 nm), Buffer (10 mM phosphate at pH 2.5), \( d_c \) (100 \( \mu \)m), \( L_{\text{eff}} \) (25 cm).
Figure 5.15: Electropherograms of lysozyme on a fused silica capillary (A) and a Si-LTGC modified fused silica capillary (B). Conditions: $V_{app}$ (10 kV), $\lambda$ (254 nm), Buffer (10 mM phosphate at pH 2.5), $d_c$ (100 $\mu$m), $L_{eff}$ (25 cm).
Figure 5.16: Electropherograms of myoglobin on a fused silica capillary (A) and a Si-LTGC modified fused silica capillary (B). Conditions: $V_{app}$ (10 kV), $\lambda$ (254 nm), Buffer (10 mM phosphate at pH 2.5), $d_c$ (100 $\mu$m), $L_{eff}$ (25 cm).
some of the negative charge from the silanols associated with fused silica. The peak shape for albumin on fused silica, Figure 5.14A, appears to be symmetrical and broad. The peak for albumin in figure 5.14B is barely discernable above the baseline of the chromatogram because of its breadth and low intensity. The breadth of these albumin peaks is due to the albumin molecules spreading equally in the forward and backward direction. The peaks in figure 5.15 exhibit significant peak tailing, and arises from species being attracted to the negative potential at the head of the column. The myoglobin peaks obtained via capillary electrophoresis with fused silica and Si-LTGC coated fused silica also displayed peak tailing. A second peak is also observed on fused silica, and possibly with Si-LTGC on fused silica. This secondary peak implies that there are two myoglobin species in solution, and that one of these species is more positively charged than the other. Two isoforms of myoglobin have been observed in gel electrophoresis and capillary electrophoresis [24, 25].

5.4 Conclusions

This chapter demonstrates the ability to employ the Si-LTGC and F-LTGC as a coating material for capillary electrophoresis and a substrate material in a microchip electrophoretic platform. Microchip capillary electrophoresis has been demonstrated with both the Si-LTGC and F-LTGC polymers. The two substrates demonstrated different retention characteristics as monitored by fluorescence detection. The F-LTGC seemed to show a slightly faster electroosmotic flow and higher degree of analyte retention on the surface of the chip. The low temperature glassy carbon precursors were
also used for capillary electrophoresis, and compared to the behavior of unmodified fused silica. The LTGC demonstrated faster analysis with all analytes except lysozyme. The peak area for all analytes was smaller for all analytes on the LTGC as compared to the unmodified fused silica, which leads to an assumption that the analytes are sorbed to the LTGC surface. This body of work is merely a starting point for the employment of LTGC in electrophoretic applications, and much more work is necessary to discern the optimum conditions of operation for each of the LTGC as processed to 200 °C.
5.5 References


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6.1 Summary of Work

Fluorine and silicon functionalized low temperature glassy carbons have been explored in three separation techniques. The first technique employed was liquid chromatography, where the retention mechanism was examined by employing Abraham’s solvation parameter model. Solid-phase extraction was used to separate environmentally important molecules from solutions with a mesoporous carbon material and F-LTGC coated mesoporous carbon. Finally, electrophoretic studies were performed using LTGC-modified fused silica and replica molded microchips.

The retention mechanism of F-LTGC was probed using oligomer that had been thermally processed at two temperatures, 200 °C and 400 °C, using a solvation parameter model. The solvation parameter models derived demonstrated that F-LTGC thermally processed at 200 °C demonstrates a similar retention mechanism as octadecysiloxane phases. A retention mechanism similar to glassy carbon was obtained for 400 °C F-LTGC. It is evident from these results that the same F-LTGC material can be used to produce liquid chromatographic columns that have tunable retention behavior. The two
liquid chromatographic stationary phases studied here exhibit retention properties that are similar to commercially available and experimental stationary phases. The commercially available phases that are similar to the 200 °C F-LTGC are alkyl phases; like C₈ and C₁₈; Fluophase, and some perfluorinated phases [1, 2, 3, 4, 5, 6], while the 400 °C phase shows retention properties similar to commercially available aromatic media [4, 6] and experimental media that is carbonaceous [1, 2]. Solvation parameter models for the F-LTGC phases are similar to those presented for the respective phases mentioned above. The ability to produce stationary phases with multiple stationary phases with varying retention mechanisms provides another controllable parameter. It is apparent that the F-LTGC oligomer coated onto typical chromatographic supports can produce various retention mechanisms. Therefore, a researcher is able to tune the retention of the column being used to promote optimum solute-stationary phase interactions. The data presented in this dissertation lead to possible arguments that a LTGC stationary phase could be a stationary phase that is capable of being used to separate compounds in several different modes of chromatography.

Mesoporous carbon materials were used in extraction studies. The results of these experiments demonstrate the selectivity for the analytes studied changes when the surface of the mesoporous carbon is coated with F-LTGC. The F-LTGC coated mesoporous carbon demonstrated a slightly higher selectivity for one molecule of each pair studied. The differences in selectivity observed with F-LTGC coated mesoporous carbon materials in this study show promise, as little selectivity among some of these compounds has been previously described [7, 8, 9, 10, 11, 12, 13]. These differences in selectivity
must arise due to the importance of different interactions with the F-LTGC surface and mesoporous surface. At this point, it is difficult to state where this stands as an extraction material in comparison with commercially available extraction media, as this work is preliminary. However, it seems that the mesoporous carbon media will be usable as a phase for extraction that is applicable to a wide range of analytes. The carbon cryogels and F-LTGC coated carbon cryogels demonstrated similar selectivities as other carbonaceous extraction media [14, 15, 16, 17]. A major difference between the F-LTGC coated carbon cryogels and many other commercially available carbon media is that several commercially available carbon media contain added anion exchange sites [18]. A second difference is the presence of fluorine heteroatoms. The addition of the F-LTGC coating to carbon cryogels permits the retention mechanism to be controlled such that the material can display similar behavior as octadecylsiloxane, polydimethylsiloxane and divinylbenzene phases when the material has been thermally processed at temperatures less than 600 °C [14, 19] Retention similar to carbonized polymers, carbon nanotubes, and activated carbon can be obtained with phases thermally processed at temperatures greater than 600 °C [15, 16, 17]. These observations add to the field of separations by demonstrating that the F-LTGC is capable of being used to extract a wide variety of analytes by controlling the temperature at which the polymer has been processed.

The results of the electrically driven separations with LTGC demonstrate that analysis with LTGC may be faster than with fused silica. However, significant interaction with LTGC surface was observed for all analytes. These interactions were manifest in decreased peak areas for peaks with the same concentration of each analyte at
the same injection conditions. Direct comparison of this work to others is not available due to the fact that similar chip platforms are required and more quantitative measurements on the electrophoretic behavior of the Si-LTGC and F-LTGC is required. The observation that different electrophoretic migration times for the same analytes was obtained shows that there may be some merit in furthering micro-/nano-fabricated devices with glassy carbon.

6.2 Future Work

Some future work that may be interesting to pursue would be to further study the liquid chromatographic figures of merit for F-LTGC in non-traditional modes liquid chromatography. These studies could undertake determining the retention behavior of F-LTGC stationary phases with supercritical fluids and enhanced fluid mobile phases. It would also be beneficial to determine van Deemter plots and other chromatographic figures of merit for the LTGCs in these modes of chromatography. Enhanced fluidity mixtures are obtained by mixing an organic solvent with a soluble gas, such as carbon dioxide [20]. By adding carbon dioxide to methanol, the resulting solution has similar polarity as methanol, but lower viscosity than methanol [20]. The most obvious studies with enhanced fluid mobile phases would be to examine the effect that varying the relative percentages of organic modifier and carbon dioxide. Another study that may be useful in permitting wider range comparison would be to extend the range of solutes used to study the retention mechanism and to extend the processing temperature range to
higher temperatures to determine the behavior of the material with increased graphitic character.

An unanswered question that remains for studying the extraction behavior of mesoporous carbon materials is how strongly the extracted analytes are retained. This question could easily be answered by attempting to remove the sorbed analytes and quantifying the solutions to determine the percent recovered. The percent recovered is calculated by taking the ratio of the difference between the amount of analyte adsorbed ($C_e$) and the amount of analyte recovered ($C_R$) to the amount of analyte adsorbed, and is shown in equation 6.1.

$$%_{\text{recovered}} = \left(\frac{C_R - C_e}{C_e}\right) \times 100\%$$ (6.1)

The percent recovered is often obtained by removing analytes sorbed to extraction media by using a solvent in which the analytes are very soluble, by employing Soxhlet extraction, or by thermally desorbing analytes. By determining the percent of analyte recovered, more direct comparison with other extraction media will be attainable. It could also prove beneficial to examine the extraction behavior of a wide range of solutes; volatile organics, environmentally important species, biological molecules; to examine which types of molecules can be most effectively removed from solutions. If recovery studies prove that a high fraction of extracted analyte is capable of being recovered, then the employment of mesoporous carbons a preconcentration step in gas or liquid chromatography could prove useful, and also possibly find a market commercially.

The most interesting future for the materials studied in this dissertation is to determine the behavior of LTGC in capillary electrochromatography (CEC), as
differences in liquid chromatographic behavior and electrophoretic behavior have been observed with LTGC phases in this work. Capillary electrochromatography is a mode of separation that combines components of liquid chromatography and electrophoresis [21].

In CEC, a potential is applied across a packed bed, and permits high efficiency separations by employing plug-like flow and minimizing the multiple path and mass transfer terms from the van Deemter equation [21]. Separations in CEC arise due to differences in adsorption, like in standard liquid chromatography, and electrophoretic mobilities of the analytes [21]. CEC can be performed using instrumentation very similar to capillary electrophoresis.
6.3 References


BIBLIOGRAPHY


