Sol-gel Resorcinarene Sorbent for Capillary Microextraction Coupled to Gas Chromatography

Abdullah Awadh Alhendal

University of South Florida, aalhenda@mail.usf.edu

Follow this and additional works at: https://digitalcommons.usf.edu/etd

Part of the American Studies Commons, and the Analytical Chemistry Commons

Scholar Commons Citation

Sol-gel Resorcinarene Sorbent for Capillary Microextraction Coupled to Gas Chromatography

by

Abdullah A. Alhendal

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science Department of Chemistry College of Arts and Sciences University of South Florida

Major Professor: Abdul Malik, Ph.D.
Kirpal Bisht, Ph.D.
Xiao Li, Ph.D.

Date of Approval:
July 8, 2011

Keywords: Sample Preparation, Preconcentration, Trace analysis Solid Phase Microextraction, Solvent-free extraction

Copyright © 2011, Abdullah A. Alhendal
DEDICATION

I would like to devote this work my wife “Maalei” for her unlimited support and encouragement. This work was not possible without her beside me. Also, I would like to dedicate this work for my parents, their love and prays made a difference in my life.
AKNOWLEDGEMENTS

I would like to acknowledge Dr. Malik for this patience, guidance, and kindness. Dr. Malik has been like a older brother to me for the last two years and this work was not possible with his vision and encouragement. Also, I would like acknowledge my committee members, Dr. Bisht, K. and Dr. Li, X. for their cooperation and helpful support through this work. I would like to thank my friend Ali Husain for his participation in this work. I would like to mention my colleagues, Dr. Fang, Dr. Shearow, Dr. Segro, Erica Turner, MinhPhuong Tran, Chengliang Jiang, Sheshanka Kesani, Emre Seyyal, Youcelyne Larose, Michael Mclean. I would like to thank Mr. Jay Bieber for his help on getting the scanning electron microscopy images. My regards to my friends whom always have been supportive to me.
# TABLE OF CONTENTS

**LIST OF TABLES** ........................................................................................................ iii

**LIST OF FIGURES** ....................................................................................................... v

**LIST OF SCHEMES** ..................................................................................................... vi

**LIST OF ABBREVIATIONS, SYMBOLS, AND ACRONYMS** ....................................... viii

**ABSTRACT** .................................................................................................................. ix

**CHAPTER 1. INTRODUCTION** ..................................................................................... 1

1.1 Sample Preparation in Analytical Chemistry ......................................................... 1

1.2 Solid Phase Microextraction (SPME) ...................................................................... 5

1.3 Capillary Microextraction (CME) and its Coupling to Chromatography ......................... 11

1.4 Theory and Applications of Sol-gel Coating Technology ......................................... 12

1.5 Extraction Phases Synthesis and Immobilization by Sol-gel Chemistry ......................... 17

1.6 Sol-gel Sorbent Phases with Cavity Ligands ........................................................... 19

1.7 Sol-gel Resorcinarene Coating for Capillary Microextraction .................................... 21

**CHAPTER 2. EXPERIMENTAL** .................................................................................... 25

2.1 Equipment ............................................................................................................... 25
2.2 Chemicals and Materials.........................................................................................31
2.3 Preparation of Sol-gel Resorcinarene Coated Capillary .............................35
  2.3.1 Pretreatment of Fused Silica Capillary .........................................................35
  2.3.2 Preparation of Octahydroxyl Resorcinarene with Aliphatic \( (C_6H_5) \) Ligand .............................................................36
  2.3.3 Preparation of Sol Solution ........................................................................36
  2.3.4 Preparation of Sol-gel Silica Resorcinarene Coated Capillary ..............37
  2.3.5 Preparation of Aqueous Samples for CME-GC Analysis .................39
2.4 Capillary Microextraction – Gas Chromatography Experiments ..........40

CHAPTER 3. RESULTS AND DISCUSSION....................................................................42
  3.1 General .............................................................................................................42
  3.2 Chemical Reactions .......................................................................................44
  3.3 Sol-gel Resorcinarene Coating Characterizations ......................................53
  3.4 Evaluation of Extraction Performance for Sol-gel Resorcinarene coated capillaries in CME-GC ......................................................60
  3.5 Experimental Determination of Extraction Profile for of Sol-gel Resorcinarene Coated Capillaries ...............................................78

CONCLUSION........................................................................................................80

REFERENCES ........................................................................................................82
LIST OF TABLES

Table 1. List of alcohols used as solutes in CME-GC aqueous samples .........................32
Table 2. List of Phenols used as solutes in CME-GC aqueous samples .........................32
Table 3. List of Amines used as solutes in CME-GC aqueous samples .........................33
Table 4. List of Ketones used as solutes in CME-GC aqueous samples .........................33
Table 5. List of Polycyclic Aromatic Hydrocarbons (PAHs) used as analytes ..............34
Table 6. Names, functions, and structures of the sol solution ingredients used for the preparation of the sol-gel resorcinarene coating on the inner surface of the fused-silica capillary .................................................................45
Table 7. Run-to-run reproducibility and detection limits data for polycyclic aromatic hydrocarbons ..................................................................................................................72
Table 8. Run-to-run reproducibility and detection limits data of phenols ....................73
Table 9. Run-to-run reproducibility and detection limits data of alcohols .................73
Table 10. Run-to-run reproducibility and detection limits data of amines ...................74
Table 11. Run-to-run reproducibility and detection limits data of ketones ...............74
Table 12. CME-GC-FID peak area data for capillary-to-capillary reproducibility on sol-gel resorcinarene coating ............................................................77
LIST OF FIGURES

Figure 1. Schematic illustration of an SPE device ................................................................. 4
Figure 2. Schematic illustration of an SPME Syringe with Coated Fiber ................................. 6
Figure 3. Schematic illustration of an SPME-GC injection port for thermal desorption after extraction of aqueous sample ................................................................. 8
Figure 4. Schematic illustration of the sol-gel coating on the inner surface of fused silica capillary .......................................................................................................................... 18
Figure 5. Chemical Structure of Octahydroxy Resorcinarene .............................................. 22
Figure 6. Schematic illustration of the purging/filling system ................................................ 26
Figure 7. Schematic illustration of liquid sample dispenser with the coated capillary mounted in the bottom .................................................................................................................. 28
Figure 8. Schematic illustration for the process of extracted analytes in the GC injection port by the coated capillary connected to GC column ................................................. 30
Figure 9. Schematic illustration of the fused silica capillary immersed in the sol solution in the purging/filling system ............................................................... 38
Figure 10. Schematic illustration for connecting the coated capillary to the GC capillary column with a quartz press-fit connector ................................................... 41
Figure 11. FTIR spectra representing (A) pure resorcinarene, (B) sol-gel silica-based resorcinarene sorbent .................................................................................................................. 54
Figure 12A. Sol-gel resorcinarene coated capillary cross-sectional SEM view .......... 57
Figure 12B. SEM image of the surface of sol-gel resorcinarene coated capillary .......... 58
Figure 12C. High magnification SEM image of the sol-gel resorcinarene coated capillary (cross-sectional View) ................................................................. 59
Figure 13. CME-GC analysis of an aqueous sample of PAHs (100 ppb) using a sol-gel resorcinarene coated capillary .................................................. 61
Figure 14. CME-GC analysis of an aqueous sample of ketones (100 ppb) using a sol-gel resorcinarene coated capillary .................................................. 63
Figure 15. CME-GC analysis of an aqueous sample of alcohols (100 ppb) using a sol-gel resorcinarene coated capillary .......................................................... 64

Figure 16. CME-GC analysis of aqueous sample of mixture of analytes (100 ppb) using a sol-gel resorcinarene coated capillary ....................................................... 66

Figure 17. CME-GC analysis of aqueous sample of mixture of analytes (100 ppb) using a sol-gel resorcinarene coated capillary after the exposure to high temperature ................................................................. 68

Figure 18. Chart illustrate the increase in the peak area obtained by CME-GC-FID for identical sample after the exposure to high temperature ....................... 69

Figure 19. Capillary microextraction profile of 1-dodecanol, decane, heptanophenone (100μg/L) ......................................................................................... 79
LIST OF SCHEMES

Scheme 1. Sol-gel Hydrolysis and Condensation ................................................. 13
Scheme 2. Mechanism of Acid-Catalyzed Hydrolysis ........................................ 14
Scheme 3. Mechanism of Base-Catalyzed Hydrolysis ......................................... 14
Scheme 4. Condensation of hydrolyzed sol-gel precursors and creation of three-
dimensional sol-gel network ........................................................................ 15
Scheme 5. Condensation of resorcinarene with TEOS ....................................... 46
Scheme 6. Hydrolysis of silica precursor .............................................................. 47
Scheme 7. Polycondensation of the hydrolyzed precursor and the resorcinarene
molecules ........................................................................................................ 49
Scheme 8. Immobilization of the sol-gel hybrid organic-inorganic silica-based
resorcinarene network to the inner surface of the fused-silica
capillary by condensation reactions ............................................................... 50
Scheme 9. Deactivation of free hydroxyl groups during sol-gel reactions ............ 52
**LIST OF ABBREVIATIONS, SYMBOLS, AND ACRONYMS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTEX</td>
<td>Benzene, Toluene, Ethylbenzene, Xylene</td>
</tr>
<tr>
<td>C&lt;sub&gt;18&lt;/sub&gt;</td>
<td>Octadecyl</td>
</tr>
<tr>
<td>CE</td>
<td>Capillary Electrophoresis</td>
</tr>
<tr>
<td>CME</td>
<td>Capillary Microextraction</td>
</tr>
<tr>
<td>CME-GC</td>
<td>Capillary Microextraction – Gas Chromatography</td>
</tr>
<tr>
<td>FID</td>
<td>Flame Ionization Detector</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared</td>
</tr>
<tr>
<td>GC</td>
<td>Gas Chromatography</td>
</tr>
<tr>
<td>HMDS</td>
<td>1,1,1,3,3,3-hexamethyldisilazane</td>
</tr>
<tr>
<td>HO-TSO</td>
<td>Hydroxyl terminated silicone Oil</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>HS-SPME</td>
<td>Head space – Solid Phase Microextraction</td>
</tr>
<tr>
<td>LLE</td>
<td>Liquid – Liquid Extraction</td>
</tr>
<tr>
<td>PAHs</td>
<td>Polycyclic Aromatic Hydrocarbons</td>
</tr>
<tr>
<td>PDMS</td>
<td>Poly(dimethylsiloxane)</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>Reversed-Phase High-Performance Liquid Chromatography</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative Standard Deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
<tr>
<td>SFE</td>
<td>Supercritical Fluid Extraction</td>
</tr>
<tr>
<td>SPE</td>
<td>Solid Phase Extraction</td>
</tr>
<tr>
<td>SPME</td>
<td>Solid Phase Microextraction</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic Acid</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>$V_f$</td>
<td>Coating volume</td>
</tr>
<tr>
<td>$V_s$</td>
<td>Sample volume</td>
</tr>
<tr>
<td>avg</td>
<td>Average</td>
</tr>
<tr>
<td>$d_f$</td>
<td>Film thickness</td>
</tr>
<tr>
<td>i.d.</td>
<td>Inner diameter</td>
</tr>
<tr>
<td>n</td>
<td>Analytes mass extracted at equilibrium</td>
</tr>
<tr>
<td>o.d.</td>
<td>Outer diameter</td>
</tr>
<tr>
<td>ppb</td>
<td>Parts per billion</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>ppt</td>
<td>Parts per trillion</td>
</tr>
<tr>
<td>psi</td>
<td>Pound per square inch</td>
</tr>
</tbody>
</table>
ABSTRACT

For the first time, octahydroxy methylresorcinarene with four hexyl groups on the lower rim was utilized in the in-situ preparation of a silica-based sol-gel organic-inorganic hybrid coating for sample preconcentration by capillary microextraction (CME). Tetraethoxysilane (TEOS) was chosen as a sol-gel precursor to create a cross-linked sol-gel network via acid-catalyzed hydrolytic polycondensation reactions. Sol-gel chemistry helped in the in situ preparation of resorcinarene-containing extraction phase in the form of a surface coating. It also provided an effective means to chemically bind the coating to the inner surface of fused silica capillary via condensation of the hydroxyl groups in the sol-gel network with the silanol groups on the fused silica capillary inner surface. These chemically bonded sol-gel coatings demonstrated excellent thermal stability (up to 350 °C). The sol-gel resorcinarene coatings successfully extracted traces of polycyclic aromatic hydrocarbons (PAHs), ketones, phenols, amines, and alcohols from aqueous samples providing parts per trillion level detection limits (0.828 – 46.01 ng/L) in GC using a Flame Ionization Detector (FID). CME was performed by passing the aqueous samples through the resorcinarene coated microextraction capillary (10 cm). The extracted analytes where then thermally desorbed into the GC column connected to the exit end of the sol-gel microextraction capillary via a press-fit quartz connector. Peak
area relative standard deviation (RSD %), a measure of the extraction performance reproducibility for the coated capillary, was found in the range of (1.1 % - 8.3 %). The sol-gel resorcinarene sorbent was characterized by FTIR spectrum which indicated the presence of hydroxyl groups in the coating even after the sol-gel reactions were completed which explains the affinity of the resorcinarene sol-gel coating toward polar analytes. Scanning Electron Microscopy (SEM) images of the coating revealed the porous morphology and thickness of 3.5 – 4.0 µm for the coating. The sol-gel resorcinarene coated capillary provided excellent extraction performance for wide range of analytes.
CHAPTER 1

INTRODUCTION

1.1 Sample Preparation in Analytical Chemistry

The analytical process consists of several steps; sampling, sample preparation, separation and quantitation, and data acquisition and evaluation. Each step is important and depends on the one before it. An error at any step can affect the rest of the analytical steps. Nowadays, analytical chemistry is closely related to other fields of science that require sample preparation. The original definition of sample preparation is making the test sample ready to the separation or quantitation step, since most of the samples can’t be directly analyzed as collected. Samples with complex matrices need to undergo special pretreatment and clean up processes to eliminate the interferences that affect the final result of analysis [1]. For example, human biological samples such as blood, urine, plasma, and salvia must be treated by different methodologies to be compatible with the separation (chromatographic and non-chromatographic) instruments to be used for analysis. Environmental monitoring of different types of toxic and hazardous contaminations is required at high level of sensitivity and accuracy. This can be achieved only when the sample is pretreated to eliminate any matrix interference, and to obtain a
preconcentrated sample of the target molecules in a clean medium [2]. The sample preparation step is dependent on the nature of the sample matrix and the nature target analyte. Extraction is one form of sample preparation that is well studied and developed in the analytical chemistry laboratories to serve different scientific fields including chemistry, medicine, environmental science, as well as a number of important industrial areas such as chemical, biochemical, pharmaceutical, biomedical, clinical, biotechnological, etc. Extraction of target molecules leads to higher sensitivity and detectability by various analytical instruments that would not have the same performance without the extraction step. Biological and clinical samples analyses went through drastic improvements by the introduction of modern extraction technologies. Solid phase extraction (SPE) [3,4] made a significant contribution to sample enrichment, pretreatment, clean up, and preconcentration as it was introduced as a substitute for the conventional liquid-liquid extraction (LLE) [5] which is labor intensive, hard to be automated, and uses large volumes of organic solvent. SPE uses sorption phase that adsorb the analytes by intermolecular forces, and retain the target molecule from the sample. Porous solid extraction phase is immobilized or packed in a specially designed cartridge serves as the solid extraction phase and as the carrying phase a gas, fluid, or liquid containing the analyte(s) pass through extracting phase cartridge (see figure 1), the analyte of interest is retained in on the surface of the extraction phase material by intermolecular forces. Depending on the nature of the target analyte, the extraction phase should be properly selected to achieve the highest sorbent-analyte interactions. Release of the extracted analytes prior to analysis can be achieved by different means but mainly is done by solvent elution. Although SPE technique reduces the consumption of organic
solvents as compared to LLE, but, hazardous organic solvents are still used for the elution step. Besides, SPE technique is exhaustive (i.e. the entire amount of the target analyte(s) must be extracted and analyzed) and thus is more time consuming, complex structure of SPE extraction phase complicate the desorption mechanism and may lead to carry-over phenomena. Hyphenation to chromatographic techniques is very hard for SPE cartridges which makes it difficult to perform online extraction for the target analyte(s) and may lead to loss of analytes between the different steps. Plastic cartridges used for SPE have adsorptive character that might interfere the extraction process, and finally SPE cartridges are disposable after one use which increases the laboratory expenses. It is, therefore, imperative to develop a method which is portable, more reproducible with higher recoveries, fast, non-exhaustive, and above all of importance, a solvent-free method, to replace SPE in sample preparation.
Figure 1. Schematic of an SPE device.
1.2 Solid Phase Microextraction (SPME)

One of the most important advancements in the field of sample preparation, preconcentration, and trace level analysis, is the introduction of solid phase microextraction (SPME) technique by Pawliszyn and coworkers [6]. SPME provided a solvent-free pathway for sample preparation, extraction, and preconcentration using a sorbent-coated fiber [7] offering an environmentally friendly, green alternative to conventional extraction techniques such as liquid-liquid extraction (LLE) [5] and solid-phase extraction (SPE) [3] commonly used for the analysis of organic compounds. SPME technique eliminated many of the disadvantages associated with the conventional sample preparation methods. But, the elimination of solvent-consumption was the most important advancement offered by SPME for the analytical and environmental laboratories. The principle of SPME is based on the sorption/desorption equilibrium of the target analytes between the sample matrix and the extracting phase (coating) on the outer surface of the substrate fiber (see figure 2). The coating (which is typically 1 cm long on the cylindrical surface of the fiber) is either immersed directly in the sample (direct immersion SPME) or placed in the sample head-space (headspace SPME) to extract the analytes from their original matrices. To achieve this process in a convenient manner, the fiber is installed on a specially designed syringe-like device (SPME syringe) to provide simplicity and portability that is essential for on-site analysis and can’t be achieved by conventional extraction methods.
Figure 2. Schematic illustration of an SPME syringe with a coated fiber.
To perform the SPME of organic compounds, the plunger of the SPME syringe is depressed to expose the sorbent coating and bring it in contact with the target solutes which eventually are sorbed on the coating by different intermolecular interactions (Van der Waals forces, electrostatic forces, hydrogen bonding, etc) between the coating and the solutes. The sorbed analyte undergo a series of sorption and desorption phenomena on the surface of the coating until a sorption/desorption equilibrium (extraction equilibrium) is established between the original sample and the extraction phase. This is the most important factor that made SPME a uniquely powerful extraction technique. The sample/coating equilibrium of the target analytes is reached after a certain time, the extraction process is completed as the equilibrium is reached. After the extraction process, the coated fiber is retracted into the needle. The syringe needle is used to protect the coated fiber and facilitate the penetration of the rubber septum in the GC injection port to desorb the extracted solutes for their analysis and detection. As soon as the SPME syringe is placed in GC injection port (see figure 3) maintained at elevated temperature, the plunger is depressed to expose the SPME coating to the heated zone inside the injector to facilitate the desorption of the analytes into the carrier gas transporting them to the GC column. Thus, analytes are thermally desorbed from the SPME coating and introduced into the GC capillary column for separation and detection.
Figure 3. Schematic illustration of SPME-GC analysis: (A) extraction by SPME; (B) SPME syringe placed in the GC injection port for thermal desorption.
SPME analysis is a non-exhaustive method, meaning that the whole sample does not need to be analyzed, and just by analyzing a small portion of the sample extracted at equilibrium. It is possible to determine the concentration of the analyte in the population from which the sample was taken. This advantage is very important when many samples are tested (for example, thousands of samples are tested for the drinking water monitoring). Also, as SPME analysis uses only trace amount of the target analyte, the use of SPME for living system should not make any disturbance or interference to the biological processes, SPME is uniquely suited for many medicinal, biological, and pharmaceutical applications [8]. The amount (n) of extracted by SPME coating can be calculated by the following equation [7]:

\[
K_{fs} = \text{distribution constant between the extraction coating and the sample matrix}
\]

\[
V_f = \text{fiber coating volume}
\]

\[
V_s = \text{sample volume,}
\]

\[
C_o = \text{initial molar concentration for a given concentration sample.}
\]

For large volume samples since \(V_s\) is much larger than \((K_{sf} \times V_f)\) which can be neglected, Eq.1 can be further simplified

\[
n = K_{fs}V_fC_o
\]  

Eq. 2

This simplification leads to the conclusion that the analysis by SPME is independent to the sample volume. The amount of the analyte extracted by SPME at
equilibrium is directly proportional to the initial molar concentration of the sample. Diffusion of the analytes from the sample matrix to the coating is affected by several factors that have been studied and discussed by Pawliszyn and coworkers ([6,7]. These factors include extraction conditions and temperature, mode of agitation, desorption condition, nature of coating, and nature of sample matrix, etc. The nature of the selected coating depends on the nature of the target analytes. For polar, moderately polar, nonpolar, to ionic analytes a series of extraction coatings have been successfully synthesized and tested. Many of coatings proved to be useful in SPME and are partly responsible for the rapid growth of SPME in analytical, environmental, forensic, and clinical laboratories [8, 9, 10]. Siloxane (≡Si-o-Si≡) based polymers showed excellent results as extraction phases for SPME applications. A recent study showed that polydimethylsiloxane (PDMS) coating retained the sorptive properties toward volatile organic compounds even after exposure to complex lipid matrices [11]. PDMS coating is the most widely used polymer for SPME applications. It showed affinity toward nonpolar and moderately polar analytes. Polyacrylate (PA) polymers [12] present an excellent choice for the extraction of polar analytes. Mixed phases are applied to enhance the surface area and the selectivity of the extraction phases. PDMS/Polydivinylbenzene (DVB), PDMS/Carbon Molecular Sieve (CAR), CW/DVB (CW: Carbowax, commercial name for polyethyleneglycol), and DVB/CAR/PDMS are some of the commercially available mixed phases available for fiber-based SPME.
1.3 Capillary Microextraction (CME) and its Coupling to Chromatography

Capillary microextraction (In-tube SPME), the capillary format of SPME where the coating is placed on the inner walls of a capillary [13, 14, 15], overcomes some of the problems associated with fiber-based SPME technique like, the susceptibility to mechanical damage: scraping of the sorbent coating, breakage of the fiber, bending of needle during operation. In contrast to conventional fiber-based SPME, in CME the extraction phase is immobilized on the inner walls of fused silica capillary by different methods of coating technologies avoiding some of the drawbacks of fiber SPME. CME allows for an efficient means to hyphenate the extraction method directly to high performance liquid chromatography (HPLC) and provide on-line preconcentration and analysis of the extracted solutes. In some biological applications, storage of the sample might lead to decomposition and change in the concentration of the target analyte which would complicate the analysis process and make the conventional off-line pretreatment of sample inaccurate and less sensitive. Hyphenation of CME to chromatographic instruments (especially HPLC) allowed for sample extraction, preconcentration, and enrichment and led ultimately to higher sensitivity, selectivity, and efficiency for the trace level analysis [17].

The term In-tube SPME is widely replaced by capillary microextraction. CME is more diverse and general thus it can be used to include both solid and liquid extraction phases. CME offers a substrate’s higher surface area on the inner surface of fused silica capillary providing higher loading of the extraction phase. Eventually, larger amount of the extraction phase provides higher loading capacity of the target analytes and also lead to better sensitivity and detection limits. Like SPME, CME is an equilibrium extraction
method. As the analyte pass through the coated fused silica capillary, the analyte will interact with the inner coating and reach distribution equilibrium. Depending on the chromatographic technique coupled with the coated fused silica capillary, desorption of the extracted analytes from the extraction phase coating is performed prior the chromatographic analysis. Thus, if CME is coupled to HPLC, desorption of the extracted analytes is accomplished by the organic modifier-rich mobile phase. If CME is coupled to GC, then thermal desorption the target analytes to the flow of GC mobile phase leading to their introduction into the separation column.

1.4 Theory and Applications of Sol-gel Coating Technology

Sol-gel employs two main reactions to produce the sol-gel network: hydrolysis and condensation (scheme 1). Hydrolysis is the first reaction whereby the alkoxide groups in the alkoxy-based sol-gel precursor are displaced with hydroxyl groups. Silica based sol-gel precursors can be hydrolyzed under either acidic or basic condition. Sol-gel precursors are hydrolyzed in the presence of water molecules as the hydrolyzing reagent. Acid-catalyzed hydrolysis mechanism is a bimolecular nucleophilic substitution ($S_{N2}$), the alkoxy groups are protonated by the acid and acquire a positive charge that withdraw the electron density from the silicon atom making silicon susceptible to nucleophilic attack by the oxygen atom in water.
Scheme 1. Sol-gel hydrolysis and condensation

\[
\text{Si-(OR)}_4 + n \text{H}_2\text{O} \xrightleftharpoons{\text{Hydrolysis}} \text{(HO)}_n\text{Si-(OR)}_{4-n} + n \text{ROH} \quad (a)
\]

Esterification

\[
\equiv\text{Si-OR} + \text{HO-Si} \equiv \xrightarrow{} \equiv\text{Si-O-Si} \equiv + \text{ROH} \quad (b)
\]

Alcohol Condensation

Alcoholysis

\[
\equiv\text{Si-OH} + \text{HO-Si} \equiv \xrightarrow{\text{Water Condensation}} \equiv\text{Si-O-Si} \equiv + \text{H}_2\text{O} \quad (c)
\]

Hydrolysis
As water attack silicon, a pentacoordinate transition state is formed and later an alcohol is produced as a bi-product (scheme 2)

Base-catalyzed hydrolysis of alkoxy silane precursors occur as in basic conditions water dissociate and produce hydroxyl groups that attack the silicon atom similarly like the acid-catalyzed mechanism to hydrolyze the alkoxy silane precursor. For base-catalyzed hydrolysis, hydrogen bonding between solvent and the alkoxy group makes it a better leaving group:

Scheme 2. Mechanism of acid-catalyzed hydrolysis

Scheme 3. Mechanism of base-catalyzed hydrolysis
The rate of hydrolysis is affected by the steric factor of the alkoxy groups as the bulky
groups reduce the susceptibility of nucleophilic attack on silicon atom [17]. The second
reaction of sol-gel process is condensations. Water-condensation or alcohol condensation
are possible for hydrolyzed sol-gel precursors, polycondensations can occur between
hydrolyzed precursors, nonhydrolyzed precursors, condensed precursors producing three-
dimensional cross-linked inorganic network:

Scheme 4. Condensation of hydrolyzed sol-gel precursors to a three-dimensional inorganic network.
The sol-gel pathway for the synthesis of chromatographic stationary phases provided remarkable convenience since the sol-gel reactions can be conducted at room temperature providing an easy, simple, and concerted coating procedure leading to the creation of a polymeric coating, immobilization by chemical bonds to the inner surface of fused silica capillary, and deactivation of the residual silanol groups all at one step. Sol-gel coating technology made a breakthrough in the field of stationary phase creation and immobilization. That method provided a control over the film thickness providing optimum performance [18]. Thus, thick stationary phases \( (d_f > 1 \, \text{µm}) \) for GC column results in high retention and longer analysis, and thin coating for GC stationary phases results in higher separation efficiency. The control over the coating thickness can be achieved by the manipulation of the coating time and the concentration of the sol ingredients. Among other advantages that sol-gel coating technology provided for the stationary phase synthesis is the high thermal and chemical stabilities that can’t be achieved by the conventional coating methods. Sol-gel hybrid organic-inorganic network is created and immobilized by the convenient sol-gel coating technology. Beside the interactions with the analytes, the organic component in the sol-gel hybrid network provides elasticity to the sol-gel coating while the chemical stability and hardness are attributed to the inorganic components [19]. These characteristic advancements by sol-gel coating technology solved most of the drawbacks of the conventional coating technologies where the coating is only physically deposited or partially cross-linked to inner walls of the capillary column. In our group, sol-gel coating technology was utilized for the creation of stationary phases for open tubular columns for Gas Chromatography (GC) [18], Capillary Electrophoresis (CE) [20], and Capillary Electrochromatography
(CEC) [21] by the addition of organic components to the sol-gel cross-linked network. Sol-gel pathway allowed for in-situ preparation of capillary column via reproducible, easy and time-saving method. In our group, sol-gel coating technology was developed for the synthesis of the capillary microextraction phases on the inner surface of fused silica capillary [22] as explained in next section.

1.5 Synthesis and Immobilization of Extraction Phases by Sol-gel Chemistry

Malik and coworkers pioneered sol-gel extraction phases for SPME and [23] succeeded in solving the major problems associated with solvent and thermal stabilities of conventional fiber-based SPME coating. Sol-gel capillary microextraction (sol-gel CME) [22], (also called In-tube sol-gel SPME), allows for chemical immobilization of thin coatings on the inner surface of fused silica capillary (see figure 4) via condensation of silanol groups located on the fused silica surface with the hydroxyl groups of the hybrid organic-inorganic sol-gel network. Chemical bonds provide these sol-gel coatings with excellent thermal and solvent stabilities as compared to the conventional coating technology were the coated sorbent is physically deposited on a substrate. In our group, we have successfully designed a number of sol-gel extraction phases for CME hyphenated to GC or HPLC with distinguished characteristics providing excellent extraction performance of nonpolar, moderately polar, and highly polar analytes. We developed sol-gel silica-, titania-, and zirconia-based coatings [24, 25, 26, 27, 28] for capillary microextraction with thermal stability (350°C and higher) as thermal stability is
required for GC applications, and solvent stability which is required for liquid chromatography applications.

Figure 4. Schematic illustration of the sol-gel coating on the inner surface of fused silica capillary.
Extractions were performed on polycyclic aromatic hydrocarbons, aldehydes, ketones, fatty alcohols, amines derivative, phenols. Recently, our group [29] developed sol-gel germania-based hybrid organic-inorganic coating that possessed remarkable stability and served as a sorbent for CME-GC and GC stationary phase capillary column. Also, fused silica capillaries with germania-based extraction phase were used in CME hyphened with HPLC [30]. The unique ability of germania-based precursors to be incorporated to the silica network is due to the fact that germania represents an isostructural to silica. We developed a sol-gel germania-based hybrid organic-inorganic CME coating that possessed stability and resistance for high temperature solvents (200°C) [30] and such advantage can be exploited in high temperature liquid chromatography. Also, sol-gel germania-based CME coating provided high resistance and reproducibility used at extreme pH conditions (0.0 ~ 14.0) [31], pH-resistant extraction coating is required in many biomedical, environmental, industrial, and pharmaceutical applications where the sample analysis requires low or high pH-value media.

1.6 Sol-gel Sorbent Phases with Cavity Ligands

Among many different types of sol gel coatings used for SPME, sol-gel hybrid organic-inorganic coating with a cavity ligand serving as organic component in sol-gel coatings presents special appeal as sorbents due to the presence of molecular cavity in its structure. Crown ethers possess a molecular cavity shape with a polar interior region [32] and have been utilized in capillary GC column because of the high selectivity they can
offer to the stationary phase once they are is attached to the polysiloxane backbone. Different types of crown ether have been introduced as efficient extraction phases for SPME coupled with GC with flame ionization detector (FID) and found to be stable at high temperature (350 °C) with excellent extraction recoveries (up to 95.3%) for various types of analytes (both polar and nonpolar) [33, 34]. Sol-gel hydroxyl-terminated crown ether SPME fibers are well studied and proved to be effective in the area of trace analysis by providing low relative standard deviation (RSD%), and efficient extraction of low concentration of target analytes (ng/mL) [35].

The extraction performance of conventional SPME fiber can be enhanced by the attachment of a high surface area ligand on the sorbent matrix as shows by Zeng and coworker [36] who successfully attached β-cyclodextrin ligand to a hydroxyl terminated silicone oil (HO-TSO) providing enhanced surface area of the extraction phase created via condensation to sol-gel network with cyclodextrin-based extracting phases that are known to offer high selectivity towards phenols and amines [37].

Calixarenes are macrocyclic molecules synthesized by cyclic condensation of formaldehyde and para-substituted phenols. Several derivatives of calixarene were attached to silica gel particles to produce novel stationary phases with unique characteristics because of the presence of molecular cavity that provided high selectivity toward target analytes in HPLC [38, 39, 40]. Also, calixarene extraction ability was widely investigated since it was introduced by Zeng as a sol-gel based extraction phase for SPME applications towards polycyclic aromatic hydrocarbons (PAHs), aromatic amines, and BTEX (Benzene, Toluene, Ethybenzene, and Xylene) [41]. Calixarene macrocyclic molecules were anchored by sol-gel chemistry reactions to form a highly
sensitive, and selective coating that was utilized in SPME-GC applications for the analysis of phenols, amines, methyl esters, propranolol, chlorobenzene, etc. showing high thermal and solvent [42, 43, 44, 45, 46]. Volatile analytes can be extracted by headspace SPME [7]. Benzoxy-terminated calyx[6]arene was involved in headspace determination of phthalates in real beer samples, high sensitivity and selectivity of the calixarene based coating was attributed to the π-π and electrostatic interactions. The best extraction results were obtained for analytes having structures matching with the cavity region of the used calixarene [47].

1.7 Sol-gel Resorcinarene Coating for Capillary Microextraction

Resorcinarene (see figure 5) can be synthesized as a cyclic tetrameric structure by acid-catalyzed condensation of four molecules of aldehyde with four molecules of resorcinol with the loss of four water molecules as proposed by Niederl and Vogel in 1940 [48] and later established by Erdtman and coworkers who resolved the crystal structure in 1968 [49]. The eight hydroxyl groups on the upper rim (see figure 4) provided resorcinarene molecules with several functional abilities and have been investigated in the fields of metals phase transfer [50, 51] because of the interactions of the polar upper rim of the resorcinarene with the metals.
Figure 5. Chemical Structure of octahydroxyl resorcinarene
The target metals are surrounded by hydrophobic shield that transfers it to the organic medium. Resorcinarene macrocycles were intensively investigated in the applications of host-guest interactions. The enantioselectivity was introduced to resorcinarene by manipulating the conformation of the resorcinarene with diamido bridges that provides chirality and rigidity to the structure [53]. Because of the complexation ability of resorcinarene, it has been widely tested for the preconcentration, trapping, encapsulation, and inclusion of metal ions [54, 55, 56, 57, 58]. Chirality of resorcinarene can be initiated by the different methods, chiral monomers (ex. chiral monomeric amides) can be used in the synthesis of resorcinarene to produce a chiral macrocycles [59], or by chemical modification of the hydroxyl groups on the upper rim [60]. Enantioselectivity of resorcinarene-based chromatographic stationary phases were examined by the separation of amino acids by a modification of resorcinarene with L-valine-tert-butylamide ligands bonded via the hydroxyl groups on the upper rim, and via the unsaturated alkyl ligands on the lower rim, resorcinarene was connected to polysiloxane polymer [61, 62]. Zhang et. al. [63] who introduced the utilization of phenyl-pentyloxy resorcinarene stationary phase for GC for the separation of positional isomers. Synergistic effect of a mixed stationary phase (mixed-CSP) [64] of cyclodextrin and resorcinarene for GC column provided a number of remarkable properties due to the combination of advantages of these macromolecules such as the substituted functional groups, selectivity via the cavity interaction, and thermal stability. The mixed stationary phase was able to successfully separate positional isomers and Grob test mixture. A novel mixed-CSP was introduced by Ruderisch and coworkers [65] that exploited enantioselectivity of the mixed components, the mixed-CSP successfully resolved recemic mixtures, and amino acids derivatives
mixtures. Resorcinarene stationary phase was used in the separation of cis-,trans isomers of thioxanthene and showed an excellent selectivity among the structural isomers. When compared with conventional stationary phases, the resorcinarene provided a better selectivity toward hydrophilic analytes [66]. Efforts have been given to the modification of the hydrophobic alkyl-bonded silica particles in-order to enhance the functionality of HPLC columns by the addition of polar substitutes to increase the selectivity of the reversed-phase RP-HPLC columns [67]. Resorcinarene macrocyclic molecule provided an excellent opportunity for chemists to attach functional polar moieties on the upper rim by the reaction of the polar substituent with the hydroxyl groups. Isopropylcarbamate resorcinarene-bonded silica stationary phase was synthesized and successfully applied in the separation of a mixture of uracil, phenol, anthracene, and naphthalene. As compared to the commercially available octadecyl (C$_{18}$) bonded silica stationary phase with additional polar groups, the modified resorcinarene-based stationary phase showed a higher affinity and longer retention time of phenol (polar analyte) as a proof of polarity enhancement for the RP-HPLC column [67].

In the present thesis, we are showing a simple, all-in-one step preparation of a sol-gel silica-based octahydroxy resorcinarene coating for solvent-free capillary microextraction. To our knowledge, this is the first time that resorcinarene has been utilized as an extracting phase in SPME.
CHAPTER 2.

EXPERIMENTAL SECTION

2.1 Equipments

Sol-gel coating was chemically immobilized on the inner surface of fused silica capillary. Sol solution ingredients were mixed thoroughly by a Fischer model G-560 Genie 2 vortex (Fisher Scientific, Pittsburgh, PA). A Micromax model 3590F microcentrifuge (Thermo IEC, Needham Heights, MA) was employed for the centrifugation (14,000 rpm, 15,682 x g) of various sol solutions. A laboratory-made gas-operated filling/purging device [20] (see Figure 6) was used for several processes: (a) cleaning and purging the new fused-silica capillary with different organic solvents and water, (b) filling the capillary with the sol solution, (c) expelling the unreacted portion of the sol solution from the capillary after predetermined period of time allowed for the sol solution to undergo sol-gel reactions in solution and with the silanol groups on the inner wall of the capillary. The device was also used for purging the coated capillary with nitrogen to dry it after rinsing with solvents. Deionized water (18 MΩ) used for the preparation of aqueous sample, and rinsing of glassware was obtained by using Maxima
HPLC pure water system (ELGA, England). ChromPerfect (version 3.0) computer software was used for the data acquisition.

Figure 6. Schematic illustration of the purging/filling system.
Extraction of organic analytes from aqueous samples was achieved by passing the aqueous sample containing the analytes through the coated capillary which was vertically connected to the lower end of a laboratory-build chamber (a modified Chromaflex AQ column) which served as a liquid sample dispenser. The flow of the sample through the extraction capillary was caused just by the gravity [22] (see figure 7).
Figure 7. Schematic illustration of liquid sample dispenser with the coated capillary mounted in the bottom.
Capillary microextraction – Gas Chromatography (CME-GC) experiments were performed on a Shimadzu model 17A GC system (Shimadzu Corporation, Columbia, MD) with the widely used flame ionization detector (FID) well known detector because of the high sensitivity towards organic compounds [68]. Split/Splitless GC injection port was used for thermal desorption of the extracted analytes from the capillary. The coated capillary is connected to the GC capillary column by a glass connector (Fischer Sci. Pittsburgh, PA) (see figure 8). Most of the extraction capillary (9 cm) is placed inside the GC injector, while only 1 cm of connected end of the capillary to the glass connector is located outside the injector. When the CME-GC experiment is performed, the temperature of the injector is elevated to very high temperature (300 °C) to weaken the intermolecular forces that retain the extracted analytes on the coating surface. After certain time, all the extracted analytes are desorbed from the extraction capillary and introduced to the GC column with the gas mobile phase. In order to have highly resolved and sharp chromatographic peaks, the analyte(s) zone inside the GC column must be as narrow as possible. Narrowing the analyte(s) zone requires a focusing method that must be performed prior to the GC run. The GC oven was held at 30 °C to maintain a cooled environment for the analytes desorbed from the capillary. Once the analytes are introduced inside the GC column, they will be strongly interacting with GC column stationary phase and thus held and focused at the beginning of the GC column.
Figure 8. Schematic illustration of the desorption of extracted analytes in the GC injection port for their introduction to the GC column for analysis.
2.2 Chemicals and Materials.

Fused silica capillary (250 µm i.d.) with an external protective polyimide coating was obtained from Polymicro Technologies Inc. (Phoenix, AZ). HPLC grade organic solvents (tetrahydrofuran (THF), methanol, methylene chloride), and naphthalene were purchased from Fischer Scientific (Fair Lawn, NJ). Decanophenone, 1-dodecanol, 1-nonanol, and trifluoroacetic acid (TFA 99%), 3-nitrophenol, 4-nitrophenol, 4-chloro,3-methylphenol were purchased from Acros Organics (Pittsburgh, PA). Tetraethoxysilane (TEOS), 1-triacontanol, and 1,2-propanediol, 3,4-dimethylphenol, 2,4,5-trichlorophenol, 2,3-dichlorophenol, o-toluidine, benzanilide, sulfanilamide, heptanophenone, valerophenone, butyrophenone, chrysene, fluorene, p-tolualdehyde were purchased from Aldrich Chemical Company Inc. (Milwaukee, WI). Catechol, 1,1,1,3,3,3-hexamethyldisilazane (HMDS), were purchased from Sigma-Aldrich Inc. (St. Louis, MO). Diphenylamine was purchased from J.T. Backer Chemicals Co. (Phillisburg, NJ). 3,4-Dinitroaniline was purchased from Eastman Kodak Co. (Rochester, NY). Octahydroxyl resorcinarene was synthesized at Dr. Bisht’s laboratory at the University of South Florida.
Table 1. List of alcohols used as solutes in CME-GC aqueous samples

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Chemical Structure</th>
<th>Boiling Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2-propanediol</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>Bp: 187 °C</td>
</tr>
<tr>
<td>Catechol</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>Bp: 245 °C</td>
</tr>
<tr>
<td>1-nonanol</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>Bp: 215 °C</td>
</tr>
<tr>
<td>1-dodecanol</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>Bp: 259 °C</td>
</tr>
<tr>
<td>1-tridecanol</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>Bp: 272 °C</td>
</tr>
<tr>
<td>1-octadecanol</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>Bp: 336 °C</td>
</tr>
</tbody>
</table>

Table 2. List of phenols used as solutes in CME-GC aqueous samples

<table>
<thead>
<tr>
<th>Phenols</th>
<th>Chemical Structure</th>
<th>Boiling Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Nitrophenol</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>Bp: 194 °C</td>
</tr>
<tr>
<td>3,4-Dimethylphenol</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>Bp: 227 °C</td>
</tr>
<tr>
<td>2,4,5-Trichlorophenol</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>Bp: 248 °C</td>
</tr>
<tr>
<td>4-chloro,3-methylphenol</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>Bp: 235 °C</td>
</tr>
<tr>
<td>2,3-Dichlorophenol</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>Bp: 214 °C</td>
</tr>
</tbody>
</table>
Table 3. List of amines used as solutes in CME-GC aqueous samples

<table>
<thead>
<tr>
<th>Amines</th>
<th>Chemical Structure</th>
<th>Boiling Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-Toluidine</td>
<td><img src="image" alt="O-Toluidine" /></td>
<td>Bp: 200 °C</td>
</tr>
<tr>
<td>Benzanilide</td>
<td><img src="image" alt="Benzanilide" /></td>
<td>Bp: 117 °C</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td><img src="image" alt="Diphenylamine" /></td>
<td>Bp: 302 °C</td>
</tr>
<tr>
<td>3,4-Dinitroaniline</td>
<td><img src="image" alt="3,4-Dinitroaniline" /></td>
<td>Bp: 71 °C</td>
</tr>
<tr>
<td>Sulfanilamide</td>
<td><img src="image" alt="Sulfanilamide" /></td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4. List of ketones used as solutes in CME-GC aqueous samples

<table>
<thead>
<tr>
<th>Ketones</th>
<th>Chemical Structure</th>
<th>Boiling Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decanophenone</td>
<td><img src="image" alt="Decanophenone" /></td>
<td>Bp: 168 °C</td>
</tr>
<tr>
<td>Heptanophenone</td>
<td><img src="image" alt="Heptanophenone" /></td>
<td>Bp: 155 °C</td>
</tr>
<tr>
<td>Valerophenone</td>
<td><img src="image" alt="Valerophenone" /></td>
<td>Bp: 105 °C</td>
</tr>
<tr>
<td>Butyrophenone</td>
<td><img src="image" alt="Butyrophenone" /></td>
<td>Bp: 222 °C</td>
</tr>
<tr>
<td>4-Phenylacetophenone</td>
<td><img src="image" alt="4-Phenylacetophenone" /></td>
<td>Bp: 325 °C</td>
</tr>
</tbody>
</table>
Table 5. List of polycyclic aromatic hydrocarbons (PAHs) used as solutes in CME-GC aqueous samples

<table>
<thead>
<tr>
<th>PAHs</th>
<th>Chemical structure</th>
<th>Boiling Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td><img src="image1" alt="Chemical structure" /></td>
<td>Bp: 218 °C</td>
</tr>
<tr>
<td>Fluorene</td>
<td><img src="image2" alt="Chemical structure" /></td>
<td>Bp: 298 °C</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td><img src="image3" alt="Chemical structure" /></td>
<td>Bp: 375 °C</td>
</tr>
<tr>
<td>Anthracene</td>
<td><img src="image4" alt="Chemical structure" /></td>
<td>Bp: 340 °C</td>
</tr>
<tr>
<td>5-bromoacenaphthene</td>
<td><img src="image5" alt="Chemical structure" /></td>
<td>Bp: 335 °C</td>
</tr>
</tbody>
</table>
2.3 Preparation of Sol-gel Resorcinarene Coated Capillary

2.3.1 Pretreatment of Fused Silica Capillary

Pretreatment of the uncoated fused silica capillary was performed in order to (a) clean the inner wall of the capillary from any contaminations, (b) increase the silanol groups (Si-OH) on the inner surface of the capillary. The uncoated capillary was rinsed sequentially with 2 mL each of methylene chloride, methanol, and deionized water respectively, by using the laboratory-made purging/filling device using nitrogen gas pressure (10 psi) [20]. After the cleaning step, both ends of the capillary were sealed with an oxy-acetylene torch, and placed in a GC oven for conditioning at an elevated temperature with traces of water on the inner surface of the capillary. The GC oven was programmed from 40 °C – 350 °C at rate of 2 °C/min, holding the capillary at the final temperature for 2 h. After the temperature ramp, the GC oven was allowed to cool down to room temperature. Then, sealed ends were cut open using an alumina wafer, and then one end of the capillary was connected to the injection port of the GC, and the capillary was further conditioned (40 °C – 350 °C @ 5 °C/min, final temperature maintained for 2h) under helium flow at rate of 1mL/min. After this, the capillary was ready for the sol-gel coating.
2.3.2 Preparation of Octahydroxyl Resorcinarene with Aliphatic (C₆H₁₃) Ligand

Octahydroxyl resorcinarene, the organic substituent in the prepared sol-gel hybrid coating was synthesized by Husain, A. at Dr. Bisht’s laboratory according to the following reference [69]. Briefly, methyl resorcinol was dissolved in ethanol. Hydrochloric acid was added to catalyze the condensation reaction of resorcinol with heptaldehyde which was added drop-wise to the solution. The reaction mixture was refluxed for 12 h at 80°C. The reaction yielded a yellow-colored precipitate which was washed with water-ethanol mixture and dried in oven (see Figure 4).

2.3.3 Preparation of Sol Solution

15 mg of the octahydroxyl resorcinarene was placed in an amber glass vial, and mixed with 100 µL TEOS, the mixture was thoroughly vortexed for 2 min, partially dissolving the resorcinarene compound. Then, 50 µL THF was added to completely dissolve the compound with vortexing for 2 min. The vial was placed in water bath and maintained at 60 °C for 3 hours. 50 µL of the resorcinarene-TEOS solution was transferred to a polypropylene microcentrifuge vial and later a 50 µL of TEOS was added. A 100 µL portion of TFA (1% water) was added later to the mixture with vigorous mixing. An immediate yellow precipitate formed, and it was excluded by centrifugation of the mixture. The clear supernatant was transferred to a new microcentrifuge tube to be used for coating the fused silica capillary.
2.3.4 Preparation of Sol-gel Silica Resorcinarene Coated Capillary

The sol solution was passed through the pretreated capillary connected to the filling/purging device (see figure 9) using low pressure (<5 psi) to avoid fragmentations in the coating. The sol solution was allowed to drip out from the exit end of the capillary. After ~ 5 seconds, the exit end of the capillary was sealed with a rubber septum to hold the sol solution inside the capillary. The sol solution was allowed to reside in the capillary for 30 min to allow sol-gel reactions inside the sol solution as well as the condensation reactions between the hydroxyl groups of the sol-gel three-dimensional network and the silanol groups on the inner wall of the fused silica capillary. The unreacted portion of the sol solution was pushed out of the capillary by passing nitrogen gas through the capillary after removing the rubber septum from the exit end. Nitrogen gas is passed through the coated capillary for 1 hr to dry out the coating from the residual solvents.
Figure 9. Schematic illustration of the fused silica capillary immersed in the sol solution in the purging/filling system.
The coated capillary was then thermally conditioned in the GC oven. For this, one end of the coated capillary was connected to the GC injection port, and the other end remained free in the GC oven. Under the flow of helium gas, the coated capillary was thermally conditioned in the GC oven by programming the oven temperature from 40 °C to 300 °C at rate of 1 °C/min, holding the capillary at the final temperature for 350 min. After conditioning the coated capillary, it was rinsed first by 2 mL each of methylene chloride, methanol, and then with 10 mL deionized water. After rinsing in the GC oven from 40 °C to 300 °C at rate of 5 °C/min, and the capillary was maintained at the final temperature for 120 min. The coated capillary was later connected to the purging/filling system and rinsed with 2 mL each of methylene chloride, methanol, and water respectively. After this step, 10 cm piece of the coated capillary was cut to be used in extraction experiments.

2.3.5 Preparation of Aqueous Samples for CME-GC Analysis

All the used glassware were deactivated by treating the glassware with a 10% HMDS solution in methylene chloride. The HMDS-treated glassware was dried in the oven at 120 °C to avoid any loss of the analytes due to adsorption on the glassware surface. The chemical standards were dissolved in methanol or THF in Amber vials (10 mL vials) to prepare stock solution with 10 mg/mL concentration. Further dilution of the stock solution with methanol or THF was done to obtain a 0.01 mg/mL concentration. A 500 µL of the diluted solution was transferred to a 50 mL volumetric flask and diluted up to the mark with deionized water to obtain 100 µg/L concentration level.
2.4 Capillary Microextraction – Gas Chromatography Experiments

Prior to each extraction experiment, the coated capillary was conditioned in the GC oven, to eliminate any contamination or adsorbed analytes from the previous runs, and that was done by connecting the coated capillary to the injection port of the GC and the GC oven was programmed as the follows (40 °C to 300 °C at a rate of 20 °C/min, and holding the capillary at the final temperature was held for 30 min under flow of helium gas through the capillary. A 10 cm piece of the coated capillary was connected to the bottom of the laboratory-made gravity-fed sample dispenser [22] where the aqueous sample was poured in the chamber and allowed to pass through the capillary using gravity. After an extraction equilibrium is established, (typically it takes 30-40 min) the capillary was disconnected from the sample dispenser and then taken for GC analysis. By using a quartz press-fit connector (see figure 10), the extraction capillary was attached to the GC separation column. The extraction capillary was then connected to the GC injection port. By increasing the temperature of the injection port (300 °C), thermal desorption of the extracted analytes was achieved by weakening the intermolecular forces holding the extracted analytes in the coating. By the flow of helium gas, the analytes were carried to the GC capillary column. Automatic aggregation and focusing of the analyte molecules occurred at the beginning of the column since the GC oven was at maintained at 30 °C. Once the analyte molecules entered front end held at (30 °C), intermolecular interactions with the stationary phase of the GC column would be quite strong to retain the analytes. Once the GC temperature-programmed run was started, the increase in the temperature of the GC oven would decrease the intermolecular forces retaining the analytes on the stationary phase. Therefore, analytes starts to move through
the column with different velocities due to differential intermolecular interactions with the stationary phase which would lead to the separation of the analytes from each other and further detection by FID.

Figure 10. Schematic illustration for connecting the microextraction capillary to the GC column with a quartz press-fit connector
CHAPTER 3

RESULTS AND DISCUSSION

3.1 General

Since the first appearance of SPME technique, the fiber coating was a key of success for the of SPME methodology. In very early reports [6] on SPME technique, the protective polyimide outer coating of the fused silica capillary was used as the extraction phase for the analysis of chlorinated compounds, and even uncoated fused fiber showed adsorptive characteristics toward benzene derivatives (BTEX). The thermal and solvent stabilities of the extraction phase coating is very important for SPME applications. The low stability of the non-bonded SPME extraction phases was one of the main drawbacks of the technique. In 1997, Malik and coworkers [23] introduced sol-gel coating technology to overcome some of the main problems associated with the conventional coating methods such as static coating technology [70] which is designed for creating thin, sub-micrometer thickness coatings that are not suitable for SPME. Sol-gel coating technology facilitates the creation of thick extraction phases for SPME and in-tube SPME (synonymously called Capillary Microextraction). Thicker coating leads to higher capability to extract more amounts of the target analytes, and therefore, achieve lower
detection limits. Sol-gel coating technology also provided an important feature to the SPME extraction phase the high thermal and solvent stability due to the chemical immobilization of the coating film on the inner surface of the fused silica capillary enhancing the thermal (above 350°C) and solvent stabilities. Commercially available SPME coatings are characterized with thermal stability range from 200 °C to 270 °C for PDMS fiber coating [71], PDMS/DVB which is cross-linked and moderately polar phase, can resist elevated temperature up to 270 °C. CW/DVB is a polar extraction phase that has affinity toward polar analytes and optimally operated at 300 °C [72]. Sol-gel SPME coating technology provides a simple pathway for in-situ creation of sol-gel hybrid organic-inorganic cross-linked network that can be synthesized to provide polar or non-polar extraction media. Selectivity and sensitivity can be enhanced for the sol-gel SPME coating because of the variety of the organic ligands that can be attached to the sol-gel network. Zeng and coworker [73] reported triple enhancement for the extraction performance of chlorophenols by sol-gel hydroxyl-terminated silicone oil (HO-TSO) SPME coating modified by the addition of cavity-shaped calixarene as compared to sol-gel HO-TSO coating. Also, the sol-gel coating was characterized by high thermal stability up to 380 °C. The enhanced performance associated with the addition of calixarene to the sol-gel coating is attributed to the well-defined molecular cavity and the selective interaction that this cavity can provide in the extraction of analytes like PAHs [74], amines [75], and organopesticides [76]. Coupling the microextraction method to GC requires a thermally stable extraction phase (coating) to be amenable to the extraction and GC analysis of components with high boiling points that require high desorption and analysis temperatures.
3.2 Chemical Reactions

Sol-gel coating method is a well investigated method for the *in-situ* creation of SPME and CME extraction phases [77]. As the ingredients of the sol-gel coating are mixed together, a colloidal system (sol solution) is formed [17]. In the sol solution, the ingredients (see table 6) are in free motion and able to react with each other.
Table 6. Ingredients names, functions, and structures of the sol solution used for the preparation of the sol-gel resorcinarene coating on the inner surface of the fused-silica capillary.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Function</th>
<th>Chemical structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetraethoxysilane (TEOS)</td>
<td>Sol-gel precursor</td>
<td><img src="image" alt="Chemical structure of TEOS" /></td>
</tr>
<tr>
<td>Octahydroxyl resorcinarene</td>
<td>Organic ligand</td>
<td><img src="image" alt="Chemical structure of resorcinarene" /></td>
</tr>
<tr>
<td>Trifluoroacetic acid (TFA 99%)</td>
<td>Catalyst</td>
<td>CF₃COOH</td>
</tr>
<tr>
<td>Tetrahydrofuran (THF)</td>
<td>Solvent</td>
<td>C₄H₈O</td>
</tr>
<tr>
<td>Water</td>
<td>Hydrolysis reagent</td>
<td>H₂O</td>
</tr>
</tbody>
</table>
Octahydroxyl resorcinarene was allowed to condense with TEOS to ensure the incorporation of the organic moiety in the sol-gel network. Resorcinarene was dissolved with THF and added to TEOS in a closed amber vial which then was placed in a water bath for 5 hours at 60 °C with continuous stirring with a magnetic bar. The condensation of resorcinarene to TEOS is non-restricted to a particular number of condensation reactions. Resorcinarene has eight hydroxyl groups available to condense with the ethoxyl groups on the silica precursor as shown in scheme 5.

Scheme 5. Condensation of resorcinarene with TEOS
Hydrolysis which is one of the key reactions in the sol-gel chemistry occurs with the alkoxide precursors (TEOS) in acidic medium (trifluoroacetic acid was used for the present work) producing an alcohol as the by-product [17] as in scheme 6.

Scheme 6. Hydrolysis of silica precursor

\[
\begin{align*}
\text{H}_3\text{C}_2\text{O} - \text{Si} - \text{O} - \text{C}_2\text{H}_5 + n \text{H}_2\text{O} & \rightarrow \left[\text{H}_3\text{C}_2\text{O}\right]_{4-n} \text{Si} \left[\text{OH}\right]_n + n \text{C}_2\text{H}_5\text{OH}
\end{align*}
\]

TEOS was chosen to build the sol-gel resorcinarene network, and since TEOS has no alkyl substituent, that leads to a more dense coating. It is known that alkyl-derivatives (methyl-, ethyl-, isopropyl etc.) of the alkoxyisilane precursors produce a more open sol-gel structure which is required to resist the shrinkage of the coating upon drying and conditioning [78], because the alkyl chains do not involve in the sol-gel hydrolysis/condensation reactions [79] and thus yield a less dense product. In this context, because of the relatively big size of the resorcinarene molecules, the sol-gel structure is expected to be open and less dense. That assumption led us to use TEOS as a precursor to increase the integrity of the sol-gel coating. A second reason for choosing tetraalkoxyisilane as sol-gel precursor is related to the main objective of this work to examine the capability of resorcinarene alone as the organic substituent in the sol-gel coating in the extraction of the organic analytes. The presence of an alkyl chains may
interfere the extraction process and co-extract the target analyte with the resorcinarene moiety. At convenient room-temperature, the sol solution containing all the components of the sol-gel reactions which undergo the polycondensation reactions that occur simultaneously between hydrolyzed alkoxy precursor molecules and also between hydrolyzed alkoxy precursors and the precursors molecules attached on the resorcinarene macromolecules (see scheme 7). The result of the hydrolysis-condensation-polycondensation reactions is a sol-gel three dimensional hybrid organic-inorganic crosslinked network produced in a single step under mild conditions. The sol-gel resorcinarene network that contains free hydroxyl groups was later allowed to be immobilized on the inner surface of fused silica capillary by condensation with silanol groups to form a chemically bonded coating (see scheme 8) possessing high thermal stability up to 350 °C (thermal stability results will be shown later).
Scheme 7. Polycondensation of the hydrolyzed precursor and the resorcinarene molecules
Scheme 8. Immobilization of the sol-gel hybrid organic-inorganic silica-based resorcinarene network to the inner surface of the fused-silica capillary by condensation reactions.
One point worth to be mentioned is that deactivation of the residual free hydroxyl groups is usually performed during the sol-gel process by reacting the hydroxyl groups with a chemical reagent (polymethylhydrosiloxane (PMHS)) to eliminate the highly adsorptive sites [18] (see scheme 9). But in this work we eliminated this process to have the hydroxyl groups available to interact with the polar analytes. Since for CME-GC analysis, a focusing method is performed to minimize the band broadening of the analyte zone. Thus, after raising the temperature of the GC injection port to 300 °C, the GC oven was left at low temperature (30 °C). Once the extracted analytes are introduced in the GC column, the analytes will be strongly retained by the stationary phase of the GC column and collected and focused at the inlet end of the column.
Scheme 9. Deactivation of free hydroxyl groups in sol-gel reactions
3.3 Sol-gel Resorcinarene Coating Characterizations

FTIR spectra were taken for pure resorcinarene compound (see figure 11A). The components of the sol-gel resorcinarene coating were placed and mixed in a vial and allowed to undergo sol-gel reaction until a solid material is formed. That material is similar in composition to the sol-gel resorcinarene coating. The bulk sol-gel resorcinarene (200 mg) was conditioned and rinsed in a typical method that was applied to the sol-gel resorcinarene coated capillary. The bulk material was taken for FTIR analysis. Figure 11B shows a FTIR spectrum for a sol-gel resorcinarene hybrid material. The FTIR spectrum shows that a major decrease in the hydroxy groups content was noticed after the condensation reactions took place indicating that some of the hydroxy groups on the resorcinarene compound were bonded to the sol-gel network, but still worth to say that not all the hydroxy groups were condensed, which later can explain the affinity of the coating for polar analytes. Although the nature of the coating was expected to be hydrophobic, but the presence of free hydroxy groups led to better extraction of polar analytes by different intermolecular interactions such as dipole-dipole and hydrogen bond interactions. The peak at 1022 cm$^{-1}$ represent the $\equiv$C-O-Si$\equiv$ vibration. Also, hydrogen bonding is possible between the hydroxy groups on the upper rim of resorcinarene leaving some unreacted hydroxy groups that provide hydrophilic character to the sol-gel resorcinarene coating for CME.
Figure 11. FTIR spectra representing (A) pure resorcinarene, (B) sol-gel silica-based resorcinarene bulk material. The peak at 1022 cm\(^{-1}\) in (B) is attributed to C-O-Si vibration. Change in the FTIR spectra of resorcinarene is due to the condensation with the silica precursor.

Figure 11A. Pure resorcinarene
Figure 11B. FTIR spectrum of sol-gel resorcinarene solid material after conditioning and rinsing.
One of the most important advantages of sol-gel coating technology is the ability to create porous coating and manipulate the coating thickness. For microextraction applications, these factors can determine the efficiency of the extraction method. A thick coating allows for high loading capacity of the target analytes and thus leads to better detection limits [18, 23]. Thick porous coatings were produced in this work which explains the high extraction efficiency obtained by the sol-gel resorcinarene coated capillary. The Scanning electron microscopy image in figure 12A shows a cross-sectional view of the of the sol-gel resorcinarene coated capillary. Figure 12B shows the morphology of the surface of the sol-gel resorcinarene coating and it reveals the porous structure of the coating. From figure 12C we can see that sol-gel coating technology helped to create a thick coating with a thickness range of (3.5 µm – 4 µm). Since the introduction of sol-gel coating technology for the creation of stationary or extraction phases, it was known that with the proper manipulation of the sol solution concentration, gelation time, and coating time, the thickness of the sol-gel coating can be controlled. Such thick coating was very useful for CME experiments because of the high loading capacity. Also, it provided the efficiency of sol-gel coating technology for making thick extraction phase coatings with porous morphology.
Figure 12A. SEM cross-sectional view of the sol-gel resorcinarene coated capillary.
Figure 12B. SEM image showing surface view of the sol-gel resorcinarene coated capillary.
Figure 12C. High magnification cross-sectional SEM image of the sol-gel resorcinarene coated capillary.
3.4 Performance of Sol-gel Resorcinarene Coated Capillary in CME-GC.

Sol-gel resorcinarene coated capillary was examined for the extraction of a mixture of analytes from aqueous sample. Polycyclic aromatic hydrocarbons (PAHs) are considered as a toxic and carcinogenic chemical compounds that are widely emitted from natural and industrial sources. US Environmental Protection Agency (EPA) recommended periodical monitoring of the public water sources to ensure that the exposure of PAHs is below the EPA limits for the target pollutants. These tests are established by EPA using different chromatographic techniques and other extraction techniques that provided parts per billion limits of detection. Sol-gel CME technique achieved parts per trillion detection limits which shows the suitability of sol-gel CME in the environmental monitoring of PAHs. Figure 13 shows the GC chromatogram for a mixture of PAHs extracted by sol-gel resorcinarene coated capillary at room temperature from an aqueous sample. The extracted analytes were at 100 ppb concentration level. [80, 81]. Also, PAHs were chosen as an example of nonpolar analytes and to examine the ability of the sol-gel resorcinarene coated capillary for the extraction of nonpolar target analytes.
Figure 13. CME-GC analysis of an aqueous sample of PAHs (100 ppb) using a sol-gel resorcinarene coated capillary.

Capillary microextraction of: 1) naphthalene, 2) fluorene, 3) 5-bromo-acenaphthene, 4) anthracene, 5) fluoranthene from aqueous sample by 10 cm x 250 µm i.d. sol-gel silica resorcinarene coated capillary at room temperature. Extraction for 40 min via gravity chamber. GC conditions: 15 m x 250 µm i.d. DB-5 commercial column; Splitless desorption; injection port temperature 300 °C; GC column temperature programmed from 40 °C – 300 °C at rate of 8 °C / min; helium carrier gas at rate of 1 mL/min and linear velocity of 30 cm/sec; FID 350 °C.
Figures 14 and 15 show the CME-GC analyses for ketones and aliphatic alcohols respectively. The chromatograms show the efficiency of the sol-gel resorcinarene coated capillary for the extraction of ketones (moderately polar) and alcohols (polar) compounds from aqueous samples at the nano-gram per liter level of concentration. Ketones are considered as a moderately polar class of chemical compounds. Also, ketones are considered as industrial pollutants emitted to the air and water in the industrial areas [82] that require an efficient monitoring systems to maintain the high quality of the water sources and thus reduces the human health risks. Powerful and accurate extraction detection methods are required for trace level analysis [83] to provide early detection of hazardous chemicals.
Capillary microextraction of: 1) valerophenone, 2) heptanophenone, 3) benzophenone, 4) decanophenone from aqueous sample by 10 cm x 250 µm i.d. sol-gel silica resorcinarene coated capillary at room temperature. Extraction for 20 min via gravity chamber. GC conditions: 15 m x 250 µm i.d. DB-5 commercial column; Splitless desorption; injection port temperature 300 ºC; GC column temperature programmed from 40 ºC – 300 ºC at rate of 8 ºC / min; helium carrier gas at rate of 1 mL/min and linear velocity of 30 cm/sec; FID 350 ºC.
Figure 15. CME-GC analysis of an aqueous sample of alcohols (100 ppb) using a sol-gel resorcinarene coated capillary.

Capillary microextraction of: 1) 1-nonanol, 2) 1-dodecanol, 3) 1-tridecanol, 4) 1-octadecanol from aqueous sample by 10 cm x 250 μm i.d. sol-gel silica resorcinarene coated capillary at room temperature. Extraction for 20 min via gravity chamber. GC conditions: 15 m x 250 μm i.d. DB-5 commercial column; Splitless desorption; injection port temperature 300 °C; GC column temperature programmed from 40 °C – 300 °C at rate of 8 °C / min; helium carrier gas at rate of 1 mL/min and linear velocity of 30 cm/sec; FID 350 °C.
Extraction of mixtures of analytes belonging to different chemical classes has been always an interest for environmental analyst since environmental samples are known to be complicated and heavily contaminated. One of the most powerful analytical tools for the analysis of such complex samples is through coupling GC to mass spectrometry detector. The high resolution that can be obtained from GC using capillary columns can be very useful for the separation of complex samples. This present work suggests for an excellent procedure that can be applied to GC-MS analysis as needed for the preconcentration and extraction of a sample containing analytes from various chemical classes prior to the GC analysis. Figure 16 shows CME-GC chromatogram of such a mixture of analytes extracted from aqueous sample by sol-gel resorcinarene coated capillary.
Figure 16. CME-GC analysis of an aqueous sample of mixture of analytes (100 ppb) using a sol-gel resorcinarene coated capillary.

Capillary microextraction of: 1) 1-nonanol, 2) valerophenone 3) 1-dodecanol, 4) heptanophenone, 5) fluorene, 6) anthracene from aqueous sample by 10 cm x 250 µm i.d. sol-gel silica resorcinarene coated capillary at room temperature. Extraction for 20 min via gravity chamber. GC conditions: 15 m x 250 µm i.d. DB-5 commercial column; Splitless desorption; injection port temperature 300 °C; GC column temperature programmed from 40 °C – 300 °C at rate of 8 °C / min; helium carrier gas at rate of 1 mL/min and linear velocity of 30 cm/sec; FID 350 °C.
Since the introduction of the sol-gel coating technology for SPME by Malik and coworkers [23] sol-gel coating method gained wide reputation for the ability to produce thermally stable coatings, controllable thickness, and ability to modify the extraction phase properties by the addition of organic polymers or macromolecules [19, 84, 85]. The thermal stability of sol-gel based coatings was proved and tested while ago [23]. Sol-gel coatings for separation and extraction are now well known to have remarkable thermal and solvent stabilities compared to the conventional types of coatings [85]. Figure 17 shows a repetition of the CME-GC experiment showed in figure 16, but after the exposure of the sol-gel resorcinarene coated capillary to a high temperature (350 °C). The sol-gel resorcinarene coated capillary was placed in the injection port of the GC for 1 hour under the flow of helium and the temperature of the GC injection port was maintained at 350 °C to examine the thermal stability of the coating. Unexpected results showed that after the exposure to the very high temperature environment, the CME-GC chromatogram (in figure 17) performed by sol-gel resorcinarene coated capillary achieved a slightly higher peaks than the previous run performed by the same capillary for identical sample and at identical extraction conditions. The increase in the peak areas is indicative to an increase in the extracted mass (see Figure 18) of the analytes. This can be explained that after the exposure to high temperature, the cavities of the resorcinarene molecules were better conditioned and cleaned from any remaining analytes from previous runs. The coating was conditioned and brought to its original status restoring the maximum number of extraction sites. That result not only proved the thermal stability of the sol-gel resorcinarene coating, but also, it indicated that the cavities of the resorcinarene can perform intermolecular interaction and inclusion for the target analytes.
Figure 17. CME-GC analysis of aqueous sample of mixture of analytes (100 ppb) using a sol-gel resorcinarene coated capillary after the exposure to high temperature.

Capillary microextraction of: 1) 1-nonanol, 2) valerophenone 3) 1-dodecanol, 4) heptanophenone, 5) fluorene, 6) anthracene from aqueous sample by 10 cm x 250 µm i.d. sol-gel silica resorcinarene coated capillary at room temperature. Extraction for 20 min via gravity chamber. GC conditions: 15 m x 250 µm i.d. DB-5 commercial column; Splitless desorption; injection port temperature 300 °C; GC column temperature programmed from 40 °C – 300 °C at rate of 8 °C / min; helium carrier gas at rate of 1 mL/min and linear velocity of 30 cm/sec; FID 350 °C.
Figure 18. Chart illustrate the increase in peak area obtained by CME-GC-FID for identical samples after the exposure to high temperature.
The performance of the sol-gel resorcinarene coated capillary was tested for repeatability of extractions of various analytes from aqueous samples at concentration level of 100 ppb using a laboratory-made gravity fed chamber that allowed the sample to pass through the coated capillary for 40 min. Tables 7, 8, 9, 10, and 11 contain experimental data obtained for polycyclic aromatic hydrocarbons, phenols, alcohols, amines, and ketones with excellent run-to-run repeatability (n= 3). For PAHs, the relative standard deviation for the peak area (arbitrary value) was maximum of 6.9 % for fluorene. Detection limits of PAHs ranged from 2.011 to 15.19 ng/L. Although the extraction performance of the coated capillary for analytes of the same class are expected to be relatively similar, but, molecules that can be included in the molecular cavity (6.9 Å) of resorcinarene [86] have lower detection limits such as naphthalene which means that the sol-gel resorcinarene coating have higher affinity toward these molecules. One of the recommended methods for the analysis of PAHs by US EPA is laser-induced fluorescence spectroscopy [87] with a fiber-optic probe at very low temperature (4.2K). Freezing the sample was achieved by using liquid helium in liquid cryogen, which eventually will facilitate the fluorescence detection of PAHs. Using liquid helium for freezing and the heat gun to melt the sample might cause inconvenience and increase the laboratory cost. Detection limits for PAHs reported by Campiglia et. al. [80] using laser-induced fluorescence spectroscopy were in the parts per billion region while in the presented work of sol-gel resorcinarene CME-GC analysis, parts per trillion limits of detection is achieved at convenient room temperature extraction of aqueous samples. Sol-gel crown ether [33] and sol-gel calixarene [73] SPME coatings have been used for the extraction of phenols from aqueous samples and reached the ng/mL level for detection
limits. In this work, sol-gel resorcinarene coated capillary achieved ng/L levels of detection limits for phenols (table 8). The advantage of sol-gel resorcinarene coated capillary as compared to cavity-based coatings is due to the fact that the crown ether ($d_f = 73 \, \mu m$)- [33], calixarene ($d_f = 85 \, \mu m$)-based [73] coating were created on a significantly shorter segment of the fiber (1 cm) which is the regular length for fiber-based SPME coating, while the sol-gel resorcinarene coating created on the inner surface of 10 cm long fused silica capillary. Longer substrate used as a support for the extraction phase eventually leads to larger volume of the extraction phase and thus higher loading capacity for the extracted analytes. The ability to introduce higher amount of the target analytes will increase the GC response as the peak area in the GC-FID chromatogram is directly proportional to the extracted amount of analytes. Higher peaks eventually will reduce the limits of detection for the CME technique as shown in the limits of detection formula:

$$L.\, O.\, D = \frac{3 \times \text{noise} \times \text{initial concentration}}{\text{peak height}}$$  \hspace{1cm} \text{Eq.}$$

Limits of detection for phenols and alcohols were in the range of 1.38 to 15.9 ng/L and 0.83 to 3.83 ng/L respectively. The sol-gel resorcinarene coating was characterized by FTIR and the FTIR spectra showed the presence of the hydroxyl groups in its structure. The presence of the hydroxyl groups helped by dipole-dipole and hydrogen bonding to interact with the alcohols and phenols and retained them. Although the nature of the coating was expected to be nonpolar, but the presence of the molecular cavity which provided $\pi-\pi$ stacking interactions and inclusion interactions. The hydroxyl groups, polar
analytes were extracted at excellent limits of detections that can be seen for the extracted alcohols, amines, and ketones in tables 9, 10, and 11 respectively.

Table 7. Run-to-run reproducibility and detection limits for polycyclic aromatic hydrocarbons in CME-GC-FID analysis.

<table>
<thead>
<tr>
<th>Name of Analyte</th>
<th>Run #1</th>
<th>Run#2</th>
<th>Run #3</th>
<th>RSD%</th>
<th>LOD(ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>284695</td>
<td>280946</td>
<td>278048</td>
<td>1.184</td>
<td>5.729</td>
</tr>
<tr>
<td>Chrysene</td>
<td>163501</td>
<td>158025</td>
<td>160023</td>
<td>1.726</td>
<td>7.558</td>
</tr>
<tr>
<td>Anthracene</td>
<td>258051</td>
<td>254906</td>
<td>249674</td>
<td>1.664</td>
<td>10.04</td>
</tr>
<tr>
<td>Fluorene</td>
<td>161618</td>
<td>153642</td>
<td>176163</td>
<td>6.971</td>
<td>15.19</td>
</tr>
</tbody>
</table>

CME-GC replicate measurement for run-to-run reproducibility using sol-gel resorcinarene coated capillary. Extraction conditions: 10 cm x 250 µm i.d. sol-gel resorcinarene coated fused silica capillary; extraction time, 40 min (using gravity fed chamber). Other conditions: DB-5 commercial column 15m x 250 µm i.d., Splitless desorption, injection port temperature 40-300 ºC; GC column temperature programmed from 40ºC to 300ºC (started after rinsing the temperature of the injection port) at rate of 10ºC / min; helium carrier gas; FID 350ºC.
Table 8. Run-to-run reproducibility and detection limits of phenols in CME-GC-FID analysis.

<table>
<thead>
<tr>
<th>Name of Analyte</th>
<th>Run #1</th>
<th>Run #2</th>
<th>Run #3</th>
<th>RSD%</th>
<th>LOD(ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-nitrophenol</td>
<td>1320194</td>
<td>1382251</td>
<td>1376565</td>
<td>2.523</td>
<td>1.381</td>
</tr>
<tr>
<td>3,4-dimethylphenol</td>
<td>1272378</td>
<td>1392610</td>
<td>1402242</td>
<td>5.337</td>
<td>1.292</td>
</tr>
<tr>
<td>2,4,5-trichlorophenol</td>
<td>1402780</td>
<td>1399139</td>
<td>1370614</td>
<td>1.266</td>
<td>1.191</td>
</tr>
<tr>
<td>4-chloro-3-methylphenol</td>
<td>554192</td>
<td>497233</td>
<td>550585</td>
<td>5.972</td>
<td>2.775</td>
</tr>
<tr>
<td>2,3-dichlorophenol</td>
<td>100174</td>
<td>110053</td>
<td>101083</td>
<td>5.261</td>
<td>15.99</td>
</tr>
</tbody>
</table>

Conditions are similar to the conditions below table 7

Table 9. Run-to-run reproducibility and detection limits of alcohols in CME-GC-FID analysis.

<table>
<thead>
<tr>
<th>Name of Analyte</th>
<th>Run #1</th>
<th>Run #2</th>
<th>Run #3</th>
<th>RSD%</th>
<th>LOD(ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-triacontanol</td>
<td>1361274</td>
<td>1442631</td>
<td>1545632</td>
<td>6.372</td>
<td>0.828</td>
</tr>
<tr>
<td>Lignoceryl</td>
<td>1329060</td>
<td>1386016</td>
<td>1281436</td>
<td>3.930</td>
<td>0.889</td>
</tr>
<tr>
<td>1,2-propanediol</td>
<td>1344202</td>
<td>1340728</td>
<td>1522335</td>
<td>7.405</td>
<td>0.924</td>
</tr>
<tr>
<td>Catechol</td>
<td>745463</td>
<td>698936</td>
<td>700452</td>
<td>6.320</td>
<td>1.993</td>
</tr>
<tr>
<td>1-nonanol</td>
<td>231411</td>
<td>259982</td>
<td>250977</td>
<td>6.758</td>
<td>3.831</td>
</tr>
</tbody>
</table>

Conditions are similar to the conditions below table 7
Table 10. Run-to-run reproducibility and detection limits of amines in CME-GC-FID analysis.

<table>
<thead>
<tr>
<th>Name of Analyte</th>
<th>Run #1</th>
<th>Run #2</th>
<th>Run #3</th>
<th>RSD%</th>
<th>LOD(ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzanilide</td>
<td>59943</td>
<td>57396</td>
<td>58878</td>
<td>2.177</td>
<td>17.42</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>55017</td>
<td>48484</td>
<td>53284</td>
<td>6.475</td>
<td>23.598</td>
</tr>
<tr>
<td>3,4-Dinitroaniline</td>
<td>52100</td>
<td>46133</td>
<td>50729</td>
<td>6.317</td>
<td>26.18</td>
</tr>
<tr>
<td>Sulfanilamide</td>
<td>26985</td>
<td>25878</td>
<td>30301</td>
<td>8.302</td>
<td>46.01</td>
</tr>
<tr>
<td>O-Toluidine</td>
<td>97692</td>
<td>98057</td>
<td>91866</td>
<td>3.623</td>
<td>15.95</td>
</tr>
</tbody>
</table>

Conditions are similar to the conditions below table 7

Table 11. Run-to-run reproducibility and detection limits of ketones in CME-GC-FID analysis.

<table>
<thead>
<tr>
<th>Name of Analyte</th>
<th>Run #1</th>
<th>Run #2</th>
<th>Run #3</th>
<th>RSD%</th>
<th>LOD(ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decanophenone</td>
<td>420987</td>
<td>439604</td>
<td>410903</td>
<td>3.435</td>
<td>2.376</td>
</tr>
<tr>
<td>Heptanophenone</td>
<td>470541</td>
<td>414249</td>
<td>420754</td>
<td>7.076</td>
<td>4.389</td>
</tr>
<tr>
<td>Valerophenone</td>
<td>441337</td>
<td>375053</td>
<td>416904</td>
<td>8.154</td>
<td>6.380</td>
</tr>
<tr>
<td>4-Phenylacetoohenone</td>
<td>222036</td>
<td>220847</td>
<td>210985</td>
<td>2.783</td>
<td>12.83</td>
</tr>
</tbody>
</table>

Conditions are similar to the conditions below table 7
Capillary to capillary performance repeatability in sol-gel resorcinarene capillary microextraction was evaluated on the basis of the reproducibility of GC peak area measurements. To achieve this measurements five sol-gel resorcinarene coated capillaries 10 cm x 250 nm i.d. were prepared from the same batches of chemicals and following identical coating and conditioning procedures. The capillaries were used for the extraction of valerophenone (100 ppb) from aqueous samples at room temperature for 40 min (time required for extraction equilibrium to be established). A total of five extractions were performed by each capillary at identical conditions prior to the GC-FID analysis.

Table 12 shows the peak area measurements and the average, standard deviations, and the relative standard deviation for five sol-gel resorcinarene. Also this table shows the average, standard deviation, and the relative standard deviation for the five capillaries. The relative standard deviation for capillaries were as follows #1 (3.805 %), #2 (6.276 %), #3 (5.86 %), and #4 (6.962 %) the RSD values are quite close and reveal resemblance of the performances of the 4 capillaries, but, still capillary #5 has a outlier relative standard deviation value of 12.77 % and at the present time this value cannot be completely explained except by the possible improper post-extraction purging step where the small portions of the aqueous sample containing the target solute still residing inside the capillary which eventually increase the amount of solutes introduced to the chromatographic system. Q-test was performed to examine whether the RSD value obtained by capillary #5 was an outlier or not. The test showed that the value 12.77 can be accepted at 95% confidence.
The relative standard deviation of the average peak area measurements produced by the five sol-gel resorcinarene coated capillaries was calculated to be 3.257% as shown in table 8. Low RSD value indicate an excellent overall reproducibility and repeatability of the five sol-gel resorcinarene capillaries. Also these results indicate a decent repeatability of the fabrication method of the coated capillary and how the method produces similar coated capillaries as these capillaries produced similar peak area values with low relative standard deviations.
Table 12. CME-GC-FID peak area measurements for capillary-to-capillary reproducibility on sol-gel resorcinarene coating.

<table>
<thead>
<tr>
<th></th>
<th>Cap #1 Peak Area</th>
<th>Cap #2 Peak Area</th>
<th>Cap #3 Peak Area</th>
<th>Cap #4 Peak Area</th>
<th>Cap #5 Peak Area</th>
<th>Cap-to-Cap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run #1</td>
<td>1169154</td>
<td>1337815</td>
<td>1697127</td>
<td>1325284</td>
<td>1446825</td>
<td>Avg 1342271</td>
</tr>
<tr>
<td>Run #2</td>
<td>1283879</td>
<td>1380063</td>
<td>1616069</td>
<td>1103798</td>
<td>1374304</td>
<td>S pooled 103236.7</td>
</tr>
<tr>
<td>Run #3</td>
<td>1233433</td>
<td>1242472</td>
<td>1573008</td>
<td>1250887</td>
<td>1454104</td>
<td>RSD (%) 7.691</td>
</tr>
<tr>
<td>Run #4</td>
<td>1271709</td>
<td>1315810</td>
<td>1485692</td>
<td>1286654</td>
<td>1101158</td>
<td></td>
</tr>
<tr>
<td>Run #5</td>
<td>1207624</td>
<td>1472147</td>
<td>1478025</td>
<td>1295321</td>
<td>1154402</td>
<td></td>
</tr>
<tr>
<td>Avg.</td>
<td>1233163</td>
<td>1349661</td>
<td>1569984</td>
<td>1252389</td>
<td>1306159</td>
<td></td>
</tr>
<tr>
<td>STD</td>
<td>46928.66</td>
<td>84709.51</td>
<td>92005.3</td>
<td>87195.9</td>
<td>166863.7</td>
<td></td>
</tr>
<tr>
<td>RSD (%)</td>
<td>3.805</td>
<td>6.276</td>
<td>5.860</td>
<td>6.962</td>
<td>12.77</td>
<td></td>
</tr>
</tbody>
</table>

Five different 10 cm sol-gel resorcinarene coated capillaries were used for extraction of aqueous sample of valerophenone (100 ppb). Extraction time for 40 min via gravity chamber at room temperature. GC conditions: 15 m x 250 µm i.d. DB-5 commercial column; Splitless desorption; injection port temperature 280 °C; GC column temperature programmed from 40 °C – 300 °C at rate of 10 °C / min; helium carrier gas at rate of 1 mL/min and mobile phase linear velocity of 30 cm/sec; FID 350 °C.
3.5 Experimental Determination of Extraction profiles for Sol-gel Resorcinarene Coated Capillaries.

Figure 12 presents experimental results for the construction of extraction profiles for a sol-gel resorcinarene coated capillary using decane (nonpolar), heptanophenone (moderately polar), and 1-dodecanol (polar) as probe solutes extracted at room temperature from aqueous samples at 100 ppb concentration level. The extraction profile is required for each analyte of interest to determine the time required for the extraction process to reach the equilibrium. Knowing the extraction equilibrium time for the target analytes helped in the optimization of the analysis time and thus increases the laboratory efficiency. Decane extraction requires 40 minutes to reach equilibrium as shown in the figure. Decane equilibrium time is longer than the other analytes. Heptanophenone and 1-dodecanol reached equilibrium within 20 and 30 minutes respectively. Although the nature of the sol-gel resorcinarene coating is expected to be hydrophobic and should facilitate the equilibrium for hydrophobic analytes, but the hydrophilic analytes had shorter equilibrium time. This can be attributed to the presence of the free hydroxyl groups in the sol-gel resorcinarene coating. Molecular level force such as hydrogen bonding and dipole-dipole interactions might be responsible for the affinity of the coating toward the polar analytes.
Figure 19. Capillary microextraction profile of 1-dodecanol, decane, heptanophenone (100µg/L)

Extraction conditions; by 10 cm x 0.25 mm i.d. sol-gel silica resorcinarene microextraction capillary. Extraction conditions: extraction from aqueous samples triplicate extractions for 10, 20, 30, 40, 50, 60, 70 min. GC conditions: Thermal desorption by injection port heating up to 280 °C, programmed GC ramp from 40 °C-300 °C, at rate of 20 °C/min, 15 m x 0.25 mm i.d. DB-5 GC capillary column, FID set at 350 °C, helium carrier gas.
CONCLUSION

Sol-gel reactions provided a simple, fast, and easily reproducible method for the creation of sol-gel silica based octahydroxyl resorcinarene coating chemically immobilized on the inner surface of fused silica capillary. The presence of chemical bonds between the sol-gel coating and the capillary wall provided the extraction phase enhanced thermal and solvent stabilities. In this work, for the first time octahydroxyl resorcinarene was used as an extraction phase for microextraction application. The hydroxyl groups on the upper rim of resorcinarene condensed with the hydroxyl groups of the hydrolyzed sol-gel precursors led to the formation of sol-gel hybrid organic-inorganic three-dimensional network that was allowed to chemically anchor to the inner wall of the fused silica capillary resulting a chemically bonded coating for use in capillary microextraction. Resorcinarene can be a very good candidate as a multi-function organic ligand as the presence of the hydroxyl groups allows for further chemical modifications, and the molecular cavity shape provided superior intermolecular interaction to certain target analytes with structural features compatible with cavity size. Both the upper and the lower rims can be functionalized and incorporated in the sol-gel network to synthesize a functional coating for microextraction and separation applications. The presence of the hydroxyl groups in the sol-gel resorcinarene coating enhanced the hydrophilic character which provided and efficient method for the extraction of polar and moderately polar analytes. EPA reported parts per billion detection limits of PAHs using laser-induced fluorescence spectroscopy. Beside the more convenience and simplicity CME can offer, parts per trillion detection limits (ng/L) was achieved for PAHs. Phenols, alcohols, ketones and amines were extracted by the sol-gel
resorcinarene coated capillary, and revealed sensitivity and efficiency of the sol-gel resorcinarene CME. Low variation in the run-to-run CME-GC experiments that suggest excellent reproducibility achieved by the sol-gel resorcinarene coated capillaries in CME. Capillary-to-capillary performance reproducibility revealed an strong evidence for efficiency of the sol-gel coating method followed for this work. Extraction of mixtures suggest the ability to employ the sol-gel resorcinarene coated capillary for the extraction of complicated samples such as biological, environmental, and petrochemical samples.
REFERENCES


2 - Stoeppler, M. Sampling and Sample Preparation, Springer-Verlag Berlin Heidelberg, 1997

3 - Poole, C.F. Trends in Anal. Chem. 2003, 22, 362-373

4 - Simpson, N. Solid Phase Extraction: Practice, Techniques, and Applications, New York, Mercel Dekker, 2000

5 - Majors, R.E. LC-GC Int. 1997, 10, 93-101


8 - Kataoka, H.; Saito, K. J. Pharmaceutical and Biomedical Analysis, 2010, 54, 926-950


12 - Chai, M.; Arthur, C.L.; Pawlisyn, J. Analyst. 1993, 118, 1501-1505


60 - Iwanek, W. Tetrahedron, 1998, 9, 4289-4290


71 - Supelco SPME Catalog 2009/2010: Chromatography Products for Analysis and Purification, Sigma-Aldrich, St. Louis, MO, USA


