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Novel sol-gel titania-based hybrid organic-inorganic coatings for on-line capillary microextraction coupled to high-performance liquid chromatography

Tae-Young Kim
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Novel Sol-Gel Titania-Based Hybrid Organic-Inorganic Coatings for On-Line Capillary Microextraction Coupled to High-Performance Liquid Chromatography

by

Tae-Young Kim

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy
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DEDICATION

To my family and relatives for their overwhelming support, concern, and love.

Kim, Keum Hun,
Yang, Sung Ae,
Dr. Kim, Soo Chul,
Kim, Chae Kyoung,
Kwon, Young Hee,
Kim, Kwanyoung Christopher,
and many others …
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LIST OF SYMBOLS AND ABBREVIATIONS

a  Activity of analytes
AN  Nucleophilic Addition
ACN  Acetonitrile
AFM  Atomic Force Microscopy
ASE  Accelerated Solvent Extraction
bp  Boiling Point
BMA  Butyl Methacrylate
BTEX  Benzene, Toluene, Ethylbenzene, and Xylene
C\textsubscript{18}-TMS  N-octadecyldimethyl[3-(trimethoxysilyl)propyl] ammonium chloride
CAR  Carboxen
CE  Capillary Electrophoresis
CEC  Capillary Electrochromatography
CERAMERS  Ceramic Polymers
CME  Capillary Microextraction
CN-PDMS  Cyanopropyl-Poly(dimethylsiloxane)
CW  Carbowax
d  Density
D  Translational diffusion coefficient
DATEG/OH-TSO  α,ω-Diallyltriethylene Glycol/Hydroxyl-Terminated Silicon Oil
DCCA  Drying Control Chemical Additive
DI  Deionized
DB (or DVB)  Divinylbenzene
EOF  Electroosmotic Flow
EPA  Environmental Protection Agency
EXAFS  X-Ray Absorption Fine Structure Spectroscopy
FID  Flame Ionization Detector
FTIR  Fourier Transform Infrared Spectroscopy
GAA  Glacial Acetic Acid
GC  Gas Chromatography
HMDS  1,1,1,3,3,3-Hexamethyldisilazane
η  Viscosity of a solvent
HPLC  High-Performance Liquid Chromatography
HS-SPME  Headspace Solid-Phase Microextraction
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<td>IC-CD</td>
<td>Ion Chromatography with Conductivity Detection</td>
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<tr>
<td>i.d.</td>
<td>Inner Diameter</td>
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<td>LC-MS</td>
<td>Liquid Chromatography-Mass Spectrometry</td>
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<td>k_B</td>
<td>Boltzmann’s constant</td>
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<td>K</td>
<td>Distribution constant</td>
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<td>MIP</td>
<td>Molecularly Imprinted Polymers</td>
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<td>MS</td>
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<td>MTMOS</td>
<td>Methyltrimethoxysilane</td>
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<td>ORMOCERS</td>
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<td>------------</td>
</tr>
<tr>
<td>SN</td>
<td>Nucleophilic Substitution</td>
</tr>
<tr>
<td>Se</td>
<td>Surface concentration of adsorbed analytes in the solid extracting phase</td>
</tr>
<tr>
<td>SAM</td>
<td>Self-Assembled Monolayer</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
<tr>
<td>SFC</td>
<td>Supercritical Fluid Chromatography</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>TEOS</td>
<td>Tetraethylorthosilicate (or Tetraethoxysilane)</td>
</tr>
<tr>
<td>TESP-PEO</td>
<td>N-(Triethoxysilylpropyl)-O-Polyethylene Oxide Urethane</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic Acid</td>
</tr>
<tr>
<td>TMOS</td>
<td>Tetramethylorthosilicate (or Tetramethoxysilane)</td>
</tr>
<tr>
<td>TR</td>
<td>Templated Resin</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>VOC</td>
<td>Volatile Organic Compound</td>
</tr>
<tr>
<td>XANES</td>
<td>X-Ray Absorption Near Edge Spectroscopy</td>
</tr>
<tr>
<td>XPS</td>
<td>X-Ray Photoelectron Spectroscopy</td>
</tr>
</tbody>
</table>
Novel sol-gel titania-poly(dimethylsiloxane) (TiO$_2$-PDMS) and titania-silica-$N$-(triethoxysilylpropyl)-O-polyethylene oxide urethane (TiO$_2$-SiO$_2$-TESP-PEO) coatings were developed for capillary microextraction (CME) to perform on-line preconcentration and HPLC analysis of trace impurities in aqueous samples. Due to chemical inertness of titania, effective covalent binding of a suitable organic ligand to its surface is difficult via conventional surface modification methods. In this research, sol-gel chemistry was employed to chemically bind hydroxy-terminated poly(dimethylsiloxane) (PDMS) and $N$-(triethoxysilylpropyl)-O-polyethylene oxide urethane (TESP-PEO) to sol-gel titania and sol-gel titania-silica network, respectively. A method is presented describing in situ preparation of the titania-based sol-gel PDMS and TESP-PEO coatings and their immobilization on the inner surface of a fused-silica microextraction capillary. To perform on-line CME-HPLC, the sol-gel TiO$_2$-PDMS or TiO$_2$-SiO$_2$-TESP-PEO capillary was installed in the HPLC injection port as an external sampling loop, and a conventional HPLC separation column was used for the liquid chromatographic separation. The sol-gel
TiO$_2$-PDMS-coated microextraction capillary was used for on-line CME-HPLC analysis of non-polar and moderately polar analytes, and the sol-gel coatings showed excellent pH (1-13), and solvent (acetonitrile and methanol) stabilities under elevated temperatures (150 °C) over analogous non-sol-gel silica-based coatings. Extraction of highly polar analytes, especially from aqueous phases is not an easy task. However, the sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated capillaries showed excellent capability of extracting underivatized highly polar analytes from aqueous samples. This opens the possibility to employ sol-gel titania-based polar coatings for solvent-free extraction and trace analysis of target analytes in environmental and biomedical matrices. To our knowledge, this is the first research on the use of sol-gel titania (or titania-silica)-based organic-inorganic materials as a sorbent in capillary microextraction. The newly developed sol-gel titania (or titania-silica)-based organic-inorganic hybrid extraction media provides an effective solution to coupling CME with HPLC (CME-HPLC), and this can be expected to become a powerful analytical tool in environmental investigations, proteomic research, early disease diagnosis and biomarker research. Being a combination of a highly efficient solvent free sample preconcentration technique (CME) and a powerful separation method (HPLC), CME-HPLC poses to become a key analytical tool in solving complex chemical, environmental, and biomedical problems involving complex matrices.
CHAPTER ONE

AN OVERVIEW OF SOLID-PHASE MICROEXTRACTION (SPME)

1.1 Introduction to Sample Preparation

Traditional sample preparation is a labor-intensive analytical process, and for a long time it has not been considered as a field of analytical chemistry [1]. More than 75% of analysis time is spent on sampling and sample preparation steps [2]. However, due to increasing awareness of environmental and health concerns, and demands on fast and cost-effective analyses of trace organic compounds, the analytical procedure for sample extraction and preconcentration from various environmental matrices has become very important.

There are several steps in typical analytical procedures as shown in Figure 1.1: sampling, sample preparation, separation, quantitation, statistical evaluation, and decision making. As emphasized in Figure 1.1, analytical steps follow one after another, and the next one cannot start until the preceding one has been completed. Therefore, the overall speed of the analytical procedure depends on the slowest step.

The chemical properties of the analytes are important parameters for the sample extraction, as are the properties of the liquid medium in which it is dissolved and the gaseous, liquid, supercritical fluid, or solid extractant used to effect a separation. Of all the relevant solute properties, five chemical properties are fundamental to understanding extraction theory: vapor pressure, solubility, molecular weight, hydrophobicity, and acid
dissociation. These essential properties determine the transport of chemicals in the human body, the transport of chemicals in the air-water-soil environmental compartments, and the transport between immiscible phases during analytical extraction.
Figure 1.1 Steps in an analytical process. Reproduced from ref. [3] with permission.
1.2 Fundamentals of Sample Extraction

The fundamental concept of a sample extraction is to convert a real matrix into a sample format that is suitable for analysis various analytical techniques. This can be accomplished by employing different treatments and enrichment procedures with following common goals:

- Removing potential interferences from the sample to increase the selectivity of the method.
- Increasing the concentration of the analyte hence the sensitivity of the method.
- Converting the analyte into a more suitable form for detection or separation, if necessary.
- Developing a robust and reproducible method which is independent of variations in the sample matrix.

Conventional sample preparation methods involve time-consuming and labor intensive processes and multi-step procedures that often use large amounts of toxic organic solvents and are prone to analyte losses. These characteristics make such methods very difficult to integrate with sampling and separation methods, especially for hyphenation and automation.

Various traditional sample preparation techniques are used in analytical practice. These include Soxhlet extraction [4], liquid-liquid extraction (LLE) [5], accelerated solvent extraction (ASE) [6], microwave-assisted extraction (MAE) [7], solid-phase extraction (SPE) [8], supercritical fluid extraction (SFE) [9], purge-and-trap [10], membrane extraction [11], static headspace analysis [12], and others [13-16]. Table 1.1 summarizes conventional sample preparation methods for various sample matrices.
Table 1.1 Conventional sample preparation techniques.

<table>
<thead>
<tr>
<th>Sample preparation technique</th>
<th>Type of sample matrix</th>
<th>Solvent-free</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soxhlet extraction</td>
<td>s</td>
<td>no</td>
<td>[4]</td>
</tr>
<tr>
<td>Accelerated solvent extraction (ASE)</td>
<td>s</td>
<td>no</td>
<td>[6]</td>
</tr>
<tr>
<td>Microwave-assisted extraction (MAE)</td>
<td>s</td>
<td>no</td>
<td>[7]</td>
</tr>
<tr>
<td>Supercritical fluid extraction (SFE)</td>
<td>s</td>
<td>yes</td>
<td>[9]</td>
</tr>
<tr>
<td>Liquid-liquid extraction (LLE)</td>
<td>l</td>
<td>no</td>
<td>[5]</td>
</tr>
<tr>
<td>Solid phase extraction (SPE)</td>
<td>l</td>
<td>no</td>
<td>[8]</td>
</tr>
<tr>
<td>Membrane extraction</td>
<td>l</td>
<td>no</td>
<td>[11]</td>
</tr>
<tr>
<td>Purge-and-trap extraction</td>
<td>s, l, g</td>
<td>yes</td>
<td>[10]</td>
</tr>
<tr>
<td>Static headspace analysis</td>
<td>s, l, g</td>
<td>yes</td>
<td>[12]</td>
</tr>
</tbody>
</table>

*a* s: solid samples; l: liquid samples; g: gaseous samples
1.2.1 Extraction of analytes from solid sample matrices

Many solid samples, such as soils, environmental solids, plant material, and polymers are highly insoluble and usually cannot be directly studied. Strong acid is often used to digest the samples, but this procedure often decomposes the target analytes. Therefore, it is necessary to use a better technique to extract the analyte of interest from the sample matrix with high efficiency, specificity, and selectivity to simplify the subsequent separation processes.

1.2.1.1 Soxhlet extraction

Soxhlet extraction technique [4] has been the most widely used method for exhaustive extraction analytes from solid samples. Soxhlet extraction involves placing the solid sample in a porous cellulose sample thimble placed in thimble holder. During operation the thimble is filled with fresh organic solvent from a distillation flask. During the process of extraction, the extracted analytes accumulate in the solvent, and are automatically siphoned into a distillation flask on a regular basis. These steps are repeated until exhaustive extraction of the analytes is achieved. Although the Soxhlet technique uses inexpensive equipment to operate, the processes involved are quite slow and may require the use of large amounts of hazardous organic solvents to ensure complete extraction process; these are hazardous to the environment and human health and are highly costly to dispose of.

1.2.1.2 Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) is a fast and selective technique. Unlike Soxhlet extraction, which requires significant amounts of organic solvents, SFE uses compressed carbon dioxide characterized by low viscosity, high volatility and high solute
diffusion rates. However, the relatively low polarity of carbon dioxide is one of the major problems, making it unsuitable for most polar samples including pharmaceuticals and drugs. Therefore, SFE often requires the use of polar organic modifiers to extract analytes of higher polarity. In addition, using high purity carbon dioxide can be expensive; SFE equipment is generally heavy which makes the technique incompatible with field analyses [17,18]. Also the high pressures required have often caused troubles for automation.

1.2.1.3 Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) utilizes microwaves to facilitate the digestion of solid samples by focusing energy into the sample, resulting both in heating and increased agitation [19]. High efficiency is the major advantage of MAE, which offers fast mass transfer and short extraction time with less solvent. However, MAE often shows limitations in using solvents because, some of them do not absorb microwave. Moreover, cooling and filtration after extraction delays the overall process. Since MAE is an exhaustive technique, the extract often contains impurities that requires cleanup prior to analysis and also requires the use of environmentally hazardous organic solvents.

1.2.1.4 Accelerated solvent extraction (ASE)

Accelerated solvent extraction (ASE) [6] is also known as pressurized fluid extraction (PFE) or pressurized liquid extraction (PLE). It uses high temperature (100 to 180 °C) and high pressure (1500 to 2000 psi) to accelerate the extraction of organic analytes from solid matrices and to enhance the extraction efficiency. The major advantage of ASE is that it uses minimal amount of organic solvent and is fast, fully automated, and easy to use. However, the equipment of ASE is expensive, and
concentration and/or cleanup processes are often required prior to analysis.

1.2.2 Extraction of analytes from liquid sample matrices

Traditionally, partitioning into an immiscible solvent, trapping, or evaporation [5] has been used to isolate analytes from liquid matrices. Collected liquid samples must be representative and maintain compositional integrity prior to analysis. Because of the great diversity of liquid samples, there is no universal sampling technique or typical sampling equipment. Liquid samples can be taken from diverse sources: surface waters, groundwaters, drinking water, industrial waters, physiological fluids, and so on.

1.2.2.1 Liquid-liquid extraction (LLE)

Liquid-liquid extraction (LLE) [5], or solvent extraction, is one of the oldest techniques, but most widely used. It involves the distribution of sample components between two immiscible liquid phases. The most common LLE method for a liquid matrix is to use a separatory funnel to extract any organic compounds from aqueous phase into a nonpolar organic phase by phase separation. This method typically requires using a large volume of organic solvent, and the extraction has to be repeated several times. In addition, drying and cleanup processes are often required, which make the overall process slow and costly.

1.2.2.2 Solid-phase extraction (SPE)

The historical development of solid-phase extraction (SPE) has been traced by various authors [20,21]. The most commonly cited benefits of SPE methods that led to early advances relative to LLE are reduced the amount of solvent required, shorter extraction time, and lower cost. SPE uses the disposable pre-packaged cartridge for the extraction of analytes in solution [20,22,23], achieved through non-equilibrium,
exhaustive removal and accumulation of analytes from a flowing liquid sample matrix via retention on the solid sorbent contained in the cartridge. However, SPE is still a multistep process that is prone to loss of analyte if it is not fully automated and still requires organic solvents for the elution step. SPE is limited to semi-volatile compounds, and the boiling points of the analytes must be above those of the solvents [24]. Also, SPE cartridges are not reusable because the cartridges are usually discarded after one extraction. In addition, SPE suffers from poor reproducibility and high carry over problems [25].

1.2.2.3 Membrane extraction techniques

Membrane extraction techniques [11] involve two simultaneous processes where the analytes are extracted from the matrix by the membrane, and then analytes are removed from the membrane using a stripping phase. This method is only applicable for nonpolar volatile and semi-volatile compounds, but has limited application to polar compounds as well. The other limitations of this technique include system carryover due to slow response of the membrane to changes in concentration, and difficulty to interface this technique with separation instruments.

1.2.3 Extraction of analytes from gaseous sample matrices

Generally volatile organic compounds (VOCs) do not require much sample preparation. They can be directly separated and analyzed by gas chromatography (GC) [26,27]. The whole sample is either gas or solid and liquid matrices containing volatile organic compounds. Unlike other extraction techniques, the volatile analytes do not equilibrate between the gas phase and sample matrix, instead, a flowing gas continuously transfers the analytes from the sample matrix. However, the analytes of interest are often at low concentrations and near their limits of detection. Also, the high diffusion rates in
gases create the problem that the sample is hard to maintain from the collection point to the analyzer. Typical gas phase extractions include static head space and dynamic headspace (also known as purge-and-trap) analyses.

1.2.3.1 Static headspace analysis

In static headspace [12], also known as equilibrium headspace extraction or simply as headspace, the volatile analytes in the sample matrix diffuse into the headspace in a vial, and the concentration of the analyte in the headspace reaches equilibrium with concentration in the sample matrix. Once the equilibrium is established after some time determined from the calibration plot, a small volume of the headspace gas is injected into a GC. Using static headspace method is advantageous due to its ease in initial sample preparation. It is more convenient for qualitative analysis since the sample can be placed directly into the headspace vial and analyzed with no such additional preparation. However, this technique is less sensitive because there is no mechanism for sample preconcentration. Also, this technique only applies to volatile samples.

1.2.3.2 Dynamic headspace analysis

In dynamic headspace, also known as purge-and-trap [10], a carrier gas is bubbled through either solid or liquid samples containing volatile organic compounds (VOCs), and VOCs captured in a sorbent trap. The sorbent trap is then heated to desorb the analytes from the trap for GC analysis. Like static headspace analysis, dynamic headspace technique also relies on the volatility of the analytes for extraction from sample matrices. However, equilibration is not achieved between the analytes and sample matrix. Instead, the analytes are removed from the sample matrix continuously by a flowing gas. The major disadvantages of dynamic headspace analysis are instrument
carryover and incompatibility with separation instruments.

1.3 Solid-Phase Microextraction (SPME)

Conventional sample preparation techniques are time-consuming, labor-intensive, involve multistep processes, and often require large quantities of organic solvents. In addition, most of the conventional sample preparation techniques are not suitable for field analysis, and often require additional processing.

To overcome these inherent problems of conventional sample preparation techniques, solid-phase microextraction (SPME), a solvent-free sample preparation technique, was developed in the early 1990s by Pawliszyn and co-workers [28,29].

In SPME, the extracting phase coated on the surface of a fused-silica fiber or capillary plays an important role in the extraction process. The extraction in SPME is an equilibrium process between the extracting phase and the analyte to be extracted. Once the equilibrium is established, the extracting phase cannot accumulate the analyte from the sample matrix, and there is a direct relationship between sample concentration and the amount of analyte extracted.

The simple format of the classical SPME device based on the Hamilton™ 7000 series microsyringe was introduced as the first SPME device. The metal rod in the microsyringe is replaced with stainless steel microtubing having a slightly larger inner diameter than the outer diameter of the fused-silica rod. Generally, the first 5 mm of the protective external coating is burned off from 1.5 cm long fused-silica rod, and this end is installed in the microsyringe using high temperature epoxy glue to protect from mechanical damage. In this case, fiber is only exposed for extraction and desorption
processes when the plunger is moved. Figure 1.2 illustrates the configuration of the first commercial SPME device [30].

A small piece of fused-silica fiber is coated with a polymeric sorbent covering a small segment (~ 1 cm) of it at one of the ends. The thickness of the SPME coating generally ranges between 10 and 100 µm. Thermally stable polar or nonpolar polymeric sorbents that allow fast solute diffusion are commonly used in SPME. Such polymers include poly(dimethylsiloxane) (PDMS), divinylbenzene (DB), polyacrylate (PA), Carboxen (CAR; a carbon molecular sieve), and Carbowax (CW; polyethylene glycol).

Generally, there are two steps in the SPME process: (a) extraction of the analytes on the fiber coating and (b) desorption of the extracted molecules into an analytical instrument for analysis. Using a polymeric sorbent coated SPME fiber, the analytes present in the sample medium are directly extracted on the coated sorbent of the SPME fiber in the process of reaching extraction equilibrium with the sample matrix. The extracted analytes are then desorbed into an instrument for separation and analysis. The desorption process is typically done by placing the fiber in a GC injection port. It can be also performed in an high-performance liquid chromatography (HPLC) by introducing SPME-HPLC interface. The whole process can be automated and coupled to GC [31,32] or HPLC [33].

There are two basic modes of extraction in SPME: direct (immersion) SPME, and headspace SPME (HS-SPME) [3]. For the analysis of gaseous and relatively clean liquid samples, direct SPME can be applied. However, HS-SPME is better suited for the analysis of solid samples and dirtier liquid samples containing volatile analytes.

When SPME was introduced, it was used to analyze relatively volatile
Figure 1.2 Design of the first commercial SPME device made by Supelco. Reproduced from ref. [30] with permission.
compounds in environmental samples. Now the application of SPME has extended to a wide variety of sample matrices and analytes. To date, SPME has been used successfully to analyze various analytes in solid, liquid, or gaseous sample matrices, such as pesticides [34,35], phenols [36,37], polychlorinated biphenyls (PCBs) [38,39], polycyclic aromatic compounds (PAHs) [34,40], and some inorganic compounds [41].

SPME is very simple, fast, easily automated, portable, sensitive, and inexpensive. Unlike conventional sample preparation methods SPME does not require the use of toxic organic solvents, and only small volumes of sample are needed for analysis. Also SPME can be easily automated with analytical instruments, such as gas chromatography (GC) [31,32], high-performance liquid chromatography (HPLC) [33,42], and capillary electrochromatography (CEC) [43,44].

1.3.1 Principles of SPME

The principles of SPME have been presented by Pawliszyn and co-workers [45-47]. SPME can be used for aqueous or gaseous samples. In both cases, there is proportional relationship (so called, the distribution constant) between the concentration of analyte in the sample and the amount of analyte extracted by the extracting phase when the latter is at equilibrium with sample matrix.

In direct (immersion) SPME, the mathematical relationship of the distribution constant ($K_{fs}$) for aqueous samples is described as following:

$$K_{fs} = \frac{C_f}{C_s}$$

(1-1)

where,

$K_{fs}$: distribution constant between extracting phase and aqueous sample matrix,
C_f: equilibrium analyte concentration in the extracting phase,

C_s: equilibrium analyte concentration in the aqueous sample.

The amount of analyte in the extracting phase on the fiber (n_f) is given by [3]:

\[ n_f = \frac{K_{fs} V_f C_0}{K_{fs} V_f + V_s} \]  \hspace{1cm} (1-2)

where,

\( n_f \): number of moles of the analyte(s) extracted by the extracting phase,

\( V_f \): volume of the extracting phase,

\( V_s \): volume of the aqueous sample,

\( C_0 \): initial concentration of a given analytes in the sample.

Since the volume of the sample (V_s) in the aqueous phase is very large or practically infinite \((K_{fs} V_f \ll V_s)\) compared to the volume of the extracting phase \(V_f\), the term, \(K_{fs} V_f\), in the denominator can be ignored. Therefore, the amount of analyte extracted on the fiber coating can be simply expressed as:

\[ n_f = K_{fs} V_f C_0 \]  \hspace{1cm} (1-3)

Equation (1-3) shows that once the establishment of equilibrium is reached, the amount of analytes extracted in the extracting phase will be directly related to the initial concentration of the analytes in the sample and will not depend on the sample volume. During the extraction process, the concentration of the analytes in the extracting phase rapidly increases first, then more slowly until equilibrium is reached. The amount of extracted analytes on the fiber is proportional to the volume of extracting phase. Therefore, the thicker the extracting phase, the more analytes will be extracted onto the
SPME fiber, however the equilibration time tends to be delayed due to the longer diffusion time for the analytes from the sample to the extracting phase.

The extraction efficiency and sensitivity depend on the distribution constant. To achieve higher selectivity and sensitivity, a high distribution constant is desirable. The distribution constant does not simply depend on the fiber coating material, but also depends on various operating parameters including temperature, pressure, and sample matrix conditions such as pH, salt concentration, and concentration of organic component, which need to be optimized for maximum transfer of analytes to the sorbent phase for extraction.

In headspace SPME (HS-SPME), aqueous sample matrices containing volatile compounds are placed in a sealed container at a constant temperature until equilibrium of the analytes between gaseous and aqueous phases is reached in the closed container. Then the SPME fiber is inserted into the sealed container and exposed to the headspace above the aqueous sample matrix for a certain period of time to extract the analytes in the gaseous phase.

The mass of an analyte extracted by the extracting phase on the fiber is related to the overall equilibrium of the analyte in the three-phase system. Since the total mass of an analyte should remain constant during the extraction:

\[ C_0V_s = C_f^\infty V_f + C_h^\infty V_h + C_s^\infty V_s \]  

(1-4)

where, \( C_0 \): the initial concentration of the analyte in the sample matrix,

\( \infty \): at equilibrium,

\( C_f \): analyte concentration in the extracting phase,
\( V_f \): the volume of the extracting phase on the SPME fiber,

\( V_h \): the volume of the gaseous phase (or headspace),

\( C_h \): the concentration of the analyte(s) in the gaseous phase (or headspace),

\( C_s \): the concentration of the analyte in the aqueous phase,

\( V_s \): the volume of the sample matrix.

The distribution constant \( (K_{fh}) \) between the extracting phase and the headspace can be defined as:

\[
K_{fh} = \frac{C_f^{\infty}}{C_h^{\infty}}
\]  \hspace{1cm} (1-5)

And, the distribution constant \( (K_{hs}) \) between the headspace and the sample matrix can be defined as:

\[
K_{hs} = \frac{C_h^{\infty}}{C_s^{\infty}}
\]  \hspace{1cm} (1-6)

The mass of the analyte extracted by the sorbent phase, \( n_f = C_f^{\infty} V_f^{\infty} \), can be expressed as:

\[
n_f = \frac{K_{fh} K_{hs} V_f C_0 V_s}{K_{fh} K_{hs} V_f + K_{hs} V_h + V_s}
\]  \hspace{1cm} (1-7)

Also, \( K_{fs} = K_{fh} K_{hs} = K_{fg} K_{gs} \), since the extracting phase/headspace distribution constant, \( K_{fh} \), can be approximated by the extracting phase/gas distribution constant, \( K_{fg} \). The headspace/sample distribution constant, \( K_{hs} \), can be also approximated by the gas/sample distribution constant, \( K_{gs} \). If the moisture effect in the gaseous headspace is neglected, the equation (1-3) can be rewritten as:

\[
n_f = \frac{K_{fs} V_f C_0 V_s}{K_{fs} V_f + K_{hs} V_h + V_s}
\]  \hspace{1cm} (1-8)
In contrast to the equation (1-2), the distribution of the analyte between the gaseous (headspace) and the aqueous phases (sample matrix) is considered. Equation (1-8) shows that the amount of extracted analyte is independent of the location of the fiber in the system. It may be placed in the gaseous phase or directly in the aqueous phase as long as all other parameters, such as the volume of the extracting phase, headspace, and the sample matrix, remain constant. Direct SPME and HS-SPME are illustrated in Figure 1.3.
Figure 1.3 Modes of SPME operation: (A) direct (immersion) SPME, (B) headspace SPME (HS-SPME). Adapted from ref. [48].
1.3.2 Conventional SPME coatings

To achieve good selectivity for the analytes of interest, the choice of the most suitable coating is essential. The principle of “like dissolves like” can be applied to SPME fiber selection. Conventional SPME extracting phases (or sorbent coatings) are generally nonbonded, partially cross-linked, or highly cross-linked. They are held on the outer surface of a fused-silica fiber (or on the inner surface of a fused-silica capillary for in-tube SPME) and serve as the extraction medium in which the analytes are preferentially sorbed and/or preconcentrated.

1.3.2.1 Fiber SPME coatings

Currently several coatings with different thicknesses are commercially available: poly(dimethylsiloxane) (PDMS), polyacrylate (PA), the mixed phases of poly(dimethylsiloxane)-poly(divinylbenzene) (PDMS-DVB), Carboxen-poly(dimethylsiloxane) (CAR-PDMS), Carbowax-poly(divinylbenzene) (CW-DVB), and Carbowax-templated resin (CW-TR). Table 1.2 shows the summary of commercially available SPME coatings on fibers.

Poly(dimethylsiloxane) (PDMS) and polyacrylate (PA) were the first coated fibers to be used in SPME. Especially, PDMS fibers are the most popular SPME coatings to date. Due to the difficulties in stabilizing thick coatings through cross-linking reaction, the PDMS fiber with 7 µm coating thickness is the only commercially available cross-linked one. The cross-linked SPME coating is very rugged and is stable up to about 340 °C while other two are not. PDMS is a nonpolar polymer which usually extracts nonpolar analytes such as BTEX compounds (benzene, toluene, ethylbenzene, and xylene) [46,49], alkanes [50,51], polycyclic aromatic hydrocarbons (PAHs) [52,53],
Table 1.2 Commercially available fiber coatings for SPME and their applications.

Adapted from ref. [2,54,55].

<table>
<thead>
<tr>
<th>Fiber Coating</th>
<th>Coating Thickness (µm)</th>
<th>Max. Temp. (for GC use) (°C)</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(dimethylsiloxane) (PDMS)</td>
<td>100&lt;sup&gt;c&lt;/sup&gt;</td>
<td>280</td>
<td>GC/HPLC, Nonpolar organic compounds such as VOCs, PAHs and BTEX</td>
</tr>
<tr>
<td></td>
<td>30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>280</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>340</td>
<td></td>
</tr>
<tr>
<td>Polyacrylate (PA)</td>
<td>85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>320</td>
<td>GC/HPLC, Polar organic compounds such as triazines and phenols</td>
</tr>
<tr>
<td>PDMS-divinylbenzene (PDMS-DVB)</td>
<td>65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>270</td>
<td>GC/HPLC, PAHs, aromatic amines, VOCs</td>
</tr>
<tr>
<td></td>
<td>60&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Carboxen-PDMS (CAR-PDMS)</td>
<td>85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>320</td>
<td>GC/HPLC, VOCs and hydrocarbons</td>
</tr>
<tr>
<td></td>
<td>75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>Carbowax-divinylbenzene (CW-DVB)</td>
<td>70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>265</td>
<td>GC/HPLC, Polar analytes such as alcohols and polar compounds</td>
</tr>
<tr>
<td></td>
<td>65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>265</td>
<td></td>
</tr>
<tr>
<td>Carbowax-templated resin (CW-TR)</td>
<td>50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>HPLC, Anionic surfactants and aromatic amines</td>
</tr>
<tr>
<td>Divinylbenzene-Carboxen-PDMS (DVB-CAR-PDMS)</td>
<td>50/30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>270</td>
<td>GC/HPLC, Odors and flavors</td>
</tr>
</tbody>
</table>

<sup>a</sup>Bonded phase; <sup>b</sup>Partially cross-linked phase; <sup>c</sup>Non-bonded phase; <sup>d</sup>GC application only.
organic compounds (VOCs) [56,57] and some pesticides [58,59]. However, it may also extract more polar analytes if the extracting conditions are optimized such as pH, salt concentration, and temperature.

Polyacrylate (PA) is a more polar coating and suitable for the extraction of more polar compounds such as alcohols [60-62], diols [60,63], aldehydes [62,64,65], ketones [62,65], esters [62,64], amines [66,67], acids [60,63], phenols and their derivatives [37,68] and some pesticides [37,42,69]. However, the extraction time tends to be longer because diffusion coefficients in PA are smaller than in PDMS [37,68,70].

The mixed phase coatings have been more recently introduced. They have complementary properties compared with PDMS and PA, and are prepared with a blend of two phases in which the porous particles are embedded in the partially cross-linked polymeric phase. For example, porous particles of poly(divinylbenzene) (DVB), or Carboxen (CAR) are blended in PDMS, to produce PDMS-DVB and CAR-PDMS, respectively. Similarly, porous particles of poly(divinylbenzene) are blended in Carbowax (CW) to prepare CW-DVB, which are suitable for extracting more polar analytes such as alcohols and ethers [71]. In addition, CAR-PDMS fibers possess a larger surface area and show good extraction capability for organic analytes, such as low molecular weight VOCs from the air [57]. Carbowax-templated resin (CW-TR) is a partially cross-linked phase with bi-polar properties. CW-TR is used for the extraction of surfactants from aqueous samples. Generally speaking, the mixed phase coatings demonstrate better affinity for polar analytes, and provide very high selectivity due to the existence of two sorbents in the coating, but they tend to possess lower mechanical stability. Figure 1.4 demonstrates a graphical scheme for choosing a SPME fiber based on polarity and
The first SPME fibers were developed for GC applications, and they often created problems when applied to HPLC [40]. Currently, some coating fibers have been developed for HPLC applications. The analyte desorption process in HPLC can only be performed when the fiber coating is stable to the addition of organic mobile phases, and can perform without dissolution and swelling. Among commercially available SPME fibers, only the bonded phases are compatible with all organic solvents, and are recommended for use with HPLC.
Figure 1.4 Graphical scheme for choosing SPME fiber coating. Reproduced from ref. [3] with permission.
1.3.2.1.1 Direct (immersion) SPME

In the direct immersion SPME (or simply direct SPME), the sorbent coated fiber is directly immersed into the sample matrix, and the analytes of interest are extracted directly from the sample to the coating. Often some type of agitation, such as rapid vial movement, stirring, and sonication, need to be employed to enhance the extraction from aqueous sample matrix due to slower diffusion coefficients compared to gaseous samples [57,72].

1.3.2.1.2 Headspace SPME (HS-SPME)

Headspace SPME (HS-SPME) would be considered for the same analytes as static or dynamic headspace extractions described in section 1.3. In HS-SPME, the volatile analytes first need to be equilibrated between the sample matrix (either solid or liquid) and the headspace in the closed container. Once the pre-equilibrium is reached, the coated fiber is inserted to extract the analytes in the headspace until the equilibrium is reached between the coating and the headspace. HS-SPME is preferred to protect the fiber coating from especially complex or dirty samples, such as those at very high or low pH, or those with large molecules.

1.3.2.2 In-tube SPME

In conventional fiber-based SPME, still there exist a number of shortcomings that need to be overcome. These include inadequate thermal and solvent stability of conventionally prepared sorbent coatings [52], low sample capacity, difficulties associated with the immobilization of thick coatings, susceptibility of the fiber (especially the coated end) to mechanical damage [73,74], and technical difficulties associated with coupling of fiber-based SPME to liquid-phase separation techniques [42,
In-tube SPME methods [33,76] present a convenient means for coupling SPME to other analytical instruments, such as HPLC, CEC, MS, FTIR etc. It is also suitable for automation, which not only reduces the total analysis time, but provides better accuracy and precision compared to traditional methods.

There are two types of in-tube SPME: static in-tube SPME and dynamic in-tube SPME (also called capillary microextraction (CME) [77]). In static in-tube SPME, the extracting phase is not exposed directly to the sample matrix. Instead, it is contained in the protective tubing (needle) without any flow of the sample through it, and the extraction occurs through the static gas phase present in the needle. However, in dynamic in-tube SPME, the sample is brought into direct contact with the extracting phase by means of a continuous flow of the sample through the tube.

Coupling of in-tube SPME to HPLC is especially important for the analysis of a wide range of less volatile or thermally labile compounds [78] that are not amenable to GC separation. In the open tubular format of SPME, a sorbent coating is applied to the inner surface of a capillary as shown in Figure 1.5. This alternative format provides an effective solution to the problem associated with the mechanical damage of sorbent coating frequently encountered in conventional fiber-based SPME where the coating is applied on the outer surface of the fiber. In this new format of SPME, a segment of wall-coated capillary GC column is commonly used [33,76,78] for the direct extraction of organic analytes from an aqueous medium, and the analytes in the aqueous samples are transported into the capillary, so the analytes can be directly extracted and concentrated in the extracting phase by repeated draw and eject cycles of the sample solution. Figure
1.6 illustrates the comparison of extraction process for the transfer of the analytes in fiber SPME and in-tube SPME.

In order to develop the in-tube SPME-HPLC method, extraction and desorption parameters need to be optimized, including the selection of the extracting phase, pH, extraction flow rate, desorption solvent, and separation conditions. In-tube SPME methods were mainly hyphenated with HPLC-UV and LC-MS. To perform HPLC analysis, the extracted analytes are transferred to the HPLC column by desorbing them with an appropriate mobile phase. In addition, the hyphenation with ion chromatography with conductivity detection (IC-CD) [79] and GC with flame ionization detection (GC-FID) [80] were also reported. Table 1.3 shows a list of capillaries and their applications for in-tube SPME. Classification of sample preparation techniques is illustrated in Figure 1.7.
Figure 1.5 Schematic of coatings in (A) fiber-based SPME and (B) in-tube SPME. Adapted from ref. [81].
Figure 1.6 Extraction of analytes by (A) fiber SPME and (B) in-tube SPME. Reproduced from ref. [82] with permission.
<table>
<thead>
<tr>
<th>Capillary</th>
<th>Detection</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omegawas 250</td>
<td>HPLC-UV</td>
<td>Phenylureas and carbamates</td>
</tr>
<tr>
<td></td>
<td>LC-MS</td>
<td>β-blockers, amphetamines, ranitidines, and heterocyclic amines</td>
</tr>
<tr>
<td>DB-WAX</td>
<td>LC-MS</td>
<td>Phenoxy acid herbicides</td>
</tr>
<tr>
<td>PPY-coated</td>
<td>HPLC-UV</td>
<td>PAHS and aromatic amines</td>
</tr>
<tr>
<td></td>
<td>LC-MS</td>
<td>Organoarsenic compounds, amphetamines, phenylureas and carbamates, β-blockers, amphetamines, catechins, and caffeine</td>
</tr>
<tr>
<td></td>
<td>IC-CD</td>
<td>Inorganic anions</td>
</tr>
<tr>
<td>BP-20 PEG</td>
<td>GC-FID</td>
<td>BTEX and phenols</td>
</tr>
<tr>
<td>Fiber-packed PEEK™</td>
<td>HPLC-UV</td>
<td>Phthalates</td>
</tr>
<tr>
<td>(Polyetheretherketone)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supel-Q PLOT</td>
<td>HPLC-UV</td>
<td>Phthalates, phenols, and isoflavones</td>
</tr>
<tr>
<td></td>
<td>LC-MS</td>
<td>Trimethyllead, thiethyllead, and benzodiazepines</td>
</tr>
<tr>
<td>MIP-packed</td>
<td>HPLC-UV</td>
<td>Propranolol</td>
</tr>
<tr>
<td>Wire-packed DB-1</td>
<td>HPLC-UV</td>
<td>Tricyclic antidepressants</td>
</tr>
<tr>
<td>Fiber-packed DB-5</td>
<td>CE-UV</td>
<td>Tricyclic antidepressants</td>
</tr>
</tbody>
</table>
Figure 1.7 Classification of sample preparation techniques. Reproduced from ref. [83] with permission.
1.4 Sol-Gel Capillary Microextraction (CME) and Sample Preconcentration

Although in-tube SPME (or CME) has great prospects in trace analysis, especially in liquid-phase sample matrices, the technology needs further improvements in a number of areas to achieve its full analytical potential. First, segments of GC columns that are commonly used for sample preconcentration have thin coatings that limit the sorption capacity, and hence, the extraction sensitivity of in-tube SPME. Second, the sorbent coatings in such microextraction capillaries usually are not chemically bonded to capillary inner walls, which limits their thermal and solvent stabilities. Third, conventionally prepared GC coatings that are used in in-tube SPME capillaries inherently possess poor pH stability. This places serious limitations on the range of solutes amenable to CME-HPLC analysis. Low pH stability of in-tube SPME coatings practically excludes the applicability of the technique to high-pH samples or analytes that require high-pH solvent systems for desorption from the microextraction capillary. Therefore, development of methodologies for the creation of high pH- and solvent-resistant sorbent coatings is an important area in the future development of in-tube SPME, which is expected to play a major role in effective hyphenation of this sample preconcentration technique with liquid-phase separation techniques that commonly use organo-aqueous mobile phases with a wide range of pH conditions [84].

Sol-gel chemistry offers a great promise to overcome the inherent shortcomings of conventional SPME and in-tube SPME. Sol-gel chemistry provides a simple and convenient way of developing new forms of sol-gel extraction media for CME analysis via chemical incorporation of organic components into the inorganic polymeric structures. Chemically bonded sol-gel extracting phases can withstand harsh experimental
conditions, such as high temperatures, extreme pHs, and organic solvents. In addition, a simple sol-gel coating technology allows in situ creation of chemically bonded thick and thin coatings. The sol-gel chemistry offers several promising directions in the SPME extracting phase (open tubular coatings and monolithic bed) technology.

### 1.4.1 Principles of CME

For all chemical extraction methods, the fundamental thermodynamic principle is common and involves distribution of analytes between the sample matrix and the extracting phase. When a liquid is used as the extracting phase then the distribution constant \(K_{es}\) defines the equilibrium conditions and enrichment factors achievable in the technique.

\[
K_{es} = \frac{a_e}{a_s} = \frac{C_e}{C_s} \quad (1-9)
\]

where,

- \(K_{es}\): distribution constant between liquid extracting phase and sample matrix,
- \(a_e\): activities of analytes in the liquid extracting phase,
- \(a_s\): activities of analytes in the sample matrix,
- \(C_e\): analytes concentration in the liquid extracting phase,
- \(C_s\): analytes concentration in the sample matrix.

For solid extracting phase adsorption, equilibria can be expressed as:

\[
K_{es}^s = \frac{S_e}{C_s} \quad (1-10)
\]

where,

- \(K_{es}^s\): distribution constant between solid extracting phase and sample matrix,
Se: surface concentration of adsorbed analytes in the solid extracting phase.

The expression (1-10) is similar to (1-9) with the exception that extraction phase concentration is replaced with surface concentration. The interstitial linear extraction phase velocity \( u_e \) can be expressed as [85]:

\[
    u_e = u_s (1+k_0)
\]  

and

\[
    u_s = \frac{L}{t_0}
\]

where, \( u_e \): interstitial linear extraction phase velocity (m/s),

\( u_s \): chromatographic linear velocity of the sample through the tube (sample flow rate) (m/s),

\( k_0 \): ratio of the intraparticulate void volume to the interstitial void space (partition ratio),

\( L \): length of the extraction vessel (capillary) (m),

\( t_0 \): time required to remove one void volume of the extracting phase from the extraction vessel in chromatography (can also be rewritten as \( t_e \) in in-tube SPME) (sec).

Since the partition ratio \( k \) can be defined as:

\[
    k = K_{es}^s \frac{V_e}{V_v}
\]

where, \( V_e \): volume of the extracting phase (cm\(^3\)),

\( V_v \): void volume of the tubing containing the extracting phase (cm\(^3\)),

\( K_{es}^s \): extracting phase/sample matrix distribution constant.
By plugging the equations (1-12) and (1-13) into (1-11), the equation (1-11) can be rewritten in terms of $t_e$:

$$t_e = \frac{L \left( 1 + K_{es} \frac{V_e}{V_v} \right)}{u_e} \quad (1\text{-}14)$$

The equation (1-14) shows that the time for the extraction required to reach the equilibrium is proportional to the length of the capillary and inversely proportional to the linear flow rate of the sample. The increases of the extracting phase/sample distribution constant and the volume of the extracting phase also prolong the extraction time. However, extraction time decreases with an increase of the void volume of the capillary. In other words, thicker coatings with a smaller capillary void volume will take a longer time to establish extraction equilibrium compared to the thinner coatings on a capillary with the same internal diameter.

The CME technique is somewhat different from the conventional SPME methods in terms of the equilibrium process, except dynamic in-tube SPME. In static SPME methods, an equilibrium between the sample matrix and the extracting phase is achieved which directly represents the concentration of the analytes in the sample. However, in flow-through techniques such as dynamic in-tube SPME and CME, the mass transfer equilibrium is achieved between the extracting phase and the analytes in sample matrix, and the analytes can be preconcentrated in the extracting phase. In addition, the sensitivity and selectivity of the extracted analytes in the sorbent phase may be different depending on the type of the extracting phase. Therefore, sorbent coating technology plays an important role in the SPME. All other parameters such as temperature, pH,
pressure, and sample matrix conditions, as described in the conventional SPME methods, also affect the mass transfer and distribution for CME applications. Figure 1.8 illustrates mass transfer of the analytes in the sol-gel CME capillary.
Figure 1.8 Extraction and preconcentration of analytes by sol-gel CME capillary.
1.5 References for Chapter One


CHAPTER TWO
SOL-GEL TECHNOLOGY IN SOLID-PHASE MICROEXTRACTION

2.1 Introduction to Sol-Gel Technology

The sol-gel process [1] represents a powerful synthetic route whereby a colloidal liquid phase (sol) is formed from sol-gel precursors, typically through hydrolytic polymerization reactions that ultimately lead to the formation of a solid phase (gel). In recent years, this term has been used for any solution process involving hydrolysis and formation of a gel starting from precursor materials. In general, hydrous metal oxides or hydroxides can be formed by chemically process of the precursor. The main advantage of the sol-gel approach is the controllability of the entire sol-gel process from the sol-gel precursor to the end product, so called “tailor-made” materials. In addition, due to the inherent flexibility of sol-gel processing the development of highly specialized materials is possible by variation of the sol-gel components, and/or processing conditions [2].

In 1844, Ebelman [3] was the first scientist to describe sol-gel synthesis. He prepared transparent solid using silica ester by slow hydrolysis process at the room temperature. After this, it took almost a century to use the sol-gel technology. Geffcken [4] used alkoxides to prepare oxide films. Later, the Schott Glass Company in Germany developed this process, and which well reviewed by Schroeder [5]. Hurd [6] showed a polymeric structure of silicic acid with continuous liquid phase, which became widely accepted research for the demonstration of the network structure of silica gels in the 1930s. In the 1950s, Roy used sol-gel method to prepare homogeneous powders in
ceramics research [7,8], however that work did not fully explain the concepts of sol-gel reaction mechanisms. A few years later, Dislich [9], and Levene and co-workers [10] developed multicomponent glasses independently using alkoxides by controlling the sol-gel hydrolysis and condensation reactions. Yoldas [11-13], and Yamane and co-workers [14] demonstrated in their papers that monoliths could be prepared by careful drying of gels, which devoted to gain large attention in sol-gel research to date.

The new field, the synthesis of organic-inorganic hybrid materials, was initiated in the 1980s by the pioneering work of Schmidt at the Fraunhofer Institute [15,16], and it was one of the major developments in sol-gel processing. Hybrid materials have been called ORMOSILS (ORGanically MODified SILicates), ORMOCERS (ORGanically MODified CERamics) and CERAMERS (CERAmic polyMERs) or POLYCERAM (POLYmeric CERAMics). Later, Mackenzie and co-workers [17] pointed out many inherent advantages to sol-gel process, such as better homogeneity and purity, easier controllability, enhanced manageability, and so on. Due to unique combinations of properties and numerous inherent advantages, sol-gel process has found growing interest in diverse research areas including nanoparticles, coatings, fibers, monoliths, or bulk materials as shown in Figure 2.1 [18].

Schmidt’s work made a commitment to the new sol-gel application in chemistry, which was started less than two decades ago. In 1987, Cortes and co-workers [19] at Dow Chemical Company reported that they created porous monolithic ceramic beds within small-diameter capillaries using sol-gel technology by polymerizing solutions containing potassium silicate to apply as separation column in liquid chromatography (LC). In 1993, Crego and co-workers [20] reported a procedure for the preparation of sol-gel open-
Figure 2.1 Shape of different products available through processing by sol-gel technology. Adapted from ref. [18].
tubular capillary columns (OTCs) using tetraethyl orthosilicate (TEOS), \( \text{Si(OC}_2\text{H}_5)_4 \), with octadecylsilane (ODS) moieties for reversed-phase liquid chromatography (RPLC). Guo and Colon [21] developed a sol-gel stationary phase for open tubular liquid chromatography (OTLC) and open tubular electrochromatography (OTEC). Malik and co-workers prepared sol-gel coated columns for capillary gas chromatography (GC) [22], and sol-gel coated fibers for solid-phase microextraction (SPME) [23,24].

Recently, Tanaka and co-workers [25-27] developed sol-gel monolithic columns for high-performance liquid chromatography (HPLC), and showed high permeability and high column efficiency compared to the conventional packed column. Their sol-gel monolithic work opened up a new direction of sol-gel research in separation science.

The sol-gel process offers many advantages such as tunable specificity, reactivity, homogeneity and purity, and controllable porosity, so it has found ever increasing application in a variety of disciplines, such as ceramics [28,29], sensors [30-32], optics [33,34], nanotechnology [35-37], and different areas of chemistry [38-43]. The sol-gel process also provides an effective means for the control of the surface area and surface characteristics (i.e., hydrophilic/hydrophobic properties, positive/negative charge, etc.) of sol-gel materials which enables one to create chemically bonded stationary phases on the inner surface of fused-silica capillary with high stability and efficiency for the applications in sample preparation [23,44-48] and separation [22,49,50].

### 2.2 Fundamentals of Sol-Gel Chemistry

In general, by the simultaneous hydrolysis and polycondensation reactions of
metal alkoxide precursors, M(OR)$_x$, followed by aging and drying under ambient conditions are the major key reactions of the sol-gel process as illustrated in Figure 2.2.

During the sol-gel process, sol-gel precursors form colloidal particles in a liquid, a sol, which then transforms into a three dimensional rigid network with pores of submicrometer dimensions and polymeric chains, a gel. Flory [51] classified “gel”s in four categories: (1) well-ordered lamellar structures; (2) completely disordered covalent polymeric networks; (3) predominantly disordered polymer networks formed through physical aggregation; and (4) particular disordered structures.

When the pore liquid is removed as a gas phase from the three dimensional sol-gel network under hypercritical conditions, the network does not collapse and a low density aerogel is produced. When the solvent is evaporated by thermal evaporation at or near ambient pressure and shrinkage occurs, a xerogel is generated. This process is called drying. A gel is defined as dried when the physically adsorbed water is completely evacuated, which typically occurs between 100 °C and 180 °C. The porous gel is transformed to a dense ceramic material when all pores are eliminated. This process is completed under elevated temperatures (generally above 1000 °C).

Typically, there are four components to prepare a sol solution: (1) a sol-gel precursor, usually a metal alkoxide M(OR)$_x$; (2) a solvent system; (3) a catalyst or a inhibitor; and (4) water.
Figure 2.2 Overview of the sol-gel process. Adapted from ref. [1].
2.2.1 Sol-gel precursors

In general, silica-based and/or non-silica-based metal alkoxides are commonly used as a starting material (precursor) for sol-gel process. The stability of metal alkoxide decreases as the electronegativity of the metal increases, which is from left to right direction across the periodic table [52]. Although tetrafunctional alkoxide precursors are most commonly used to incorporate into the sol solution, one or more functional group substituted alkoxides may also be used in sol-gel process. While there are many sol-gel precursors containing different metal elements, such as Si, Ti, Al, V, Zr, and Ge, silica is the most convenient from many aspects: well-known chemistry, stability of Si-O bond, well-documented sol-gel methodology [45,46,48], facility of characterizations and commercially available starting materials [53]. Some common silica- and non-silica-based sol-gel precursors are shown in Table 2.1.
Table 2.1 List of common alkoxide-based sol-gel precursors.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Name/Structure</th>
<th>MW</th>
<th>bp (˚C)</th>
<th>n\textsubscript{D} (20˚C)</th>
<th>d (g/mL) (20˚C)</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetramethoxysilane (TMOS)</td>
<td>152.2</td>
<td>121</td>
<td>1.3688</td>
<td>1.02</td>
<td>Alcohols</td>
</tr>
<tr>
<td>(\text{H}_3\text{CO} – \text{Si} – \text{OCH}_3) \quad \text{OCH}_3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetaethoxysilane (TEOS)</td>
<td>208.3</td>
<td>169</td>
<td>1.3838</td>
<td>0.93</td>
<td>Alcohols</td>
</tr>
<tr>
<td>(\text{C}_2\text{H}_3\text{O} – \text{Si} – \text{OC}_2\text{H}_5) \quad \text{OC}_2\text{H}_5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetra-(n)-propoxysilane</td>
<td>264.4</td>
<td>224</td>
<td>1.401</td>
<td>0.916</td>
<td>Alcohols</td>
</tr>
<tr>
<td>(\text{C}_3\text{H}_7\text{O} – \text{Si} – \text{OC}_3\text{H}_7) \quad \text{OC}_3\text{H}_7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[\text{Si}(\text{n-C}_3\text{H}_7\text{O})_4]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetra-(n)-butoxysilane</td>
<td>320.5</td>
<td>115</td>
<td>1.4126</td>
<td>0.899</td>
<td>Alcohols</td>
</tr>
<tr>
<td>(\text{C}_4\text{H}_9\text{O} – \text{Si} – \text{OC}_4\text{H}_9) \quad \text{OC}_4\text{H}_9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[\text{Si}(\text{n-C}_4\text{H}_9\text{O})_4]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Titanium (IV) Isopropoxide</td>
<td>284.22</td>
<td>232</td>
<td>1.464</td>
<td>0.96</td>
<td>Alcohols</td>
</tr>
<tr>
<td>(\text{CH}_3\text{O} – \text{CH}_3\text{CH}_3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zirconium (IV) (n)-Butoxide</td>
<td>383.68</td>
<td>117</td>
<td>1.466</td>
<td>1.049</td>
<td>Alcohols</td>
</tr>
<tr>
<td>(\text{O} (\text{CH}_2)_2\text{CH}_3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\*MW: Molecular weight; bp: Boiling point; n\textsubscript{D}: Refractive index; d: Density. \textsuperscript{a}Adapted from ref. [1,54,55].
2.2.2 Sol-gel solvent system

The sol solution does not just contain the sol-gel precursor(s), it may also contain polymer, catalyst(s) (or inhibitor(s)), and water. Usually the solvent is chosen depending on its ability to generate a homogeneous sol and progression of the gelation process. The solvent should also be chemically inert so it does not participate in the sol-gel reactions that could lead to the creation of undesirable side products.

The selection of the solvent may depend on the nature of the alkoxy substituents present on the central metal atom. For example, the hydrolysis by-product of TMOS is methanol; however, typically any miscible alcohol other than methanol would often be used as the solvent to prevent any liquid-liquid phase separation during the sol-gel hydrolysis reactions. Since the hydrolysis by-product of TMOS is methanol, using methanol as the solvent would suppress the hydrolysis reaction by Le Châtelier’s principle. Often more than one solvent system (e.g., mixture of methanol and methylene chloride) may be used depending on the solubility of the sol-gel precursor(s) and the copolymer. In addition, the amount of solvent(s) used for sol-gel processing may affect the speed of the sol-gel reaction. Using higher amounts of solvent(s) may dramatically increase gelation time.

A drying control chemical additive (DCCA) (e.g., formamide, glycerin, dimethyl ether, and oxalic acid) as a co-solvent may be also play another crucial role in the sol-gel reaction [56,57]. As the solvent escapes from within the gel or as the pore size changes during these processes, stress is developed in the sol-gel network, which may cause cracking. To overcome this, DCCA is incorporated in the starting mixture before gelation [1,58,59] so it may facilitate a more effective way of drying the gel. Unfortunately, the
exact way in which the DCCA improves the drying process is still unclear [59].

In addition, the water/precursor(s) ratio in the sol solution may also affect the speed of reaction as well as the physical properties of the obtained sol-gel materials. So this ratio can be carefully controlled to fine tuned porous structures of sol-gel materials. Constantin and Freitag [60] reported that there is an optimum content of water (about 200 %) that facilitates the formation of uniform and porous sol-gel products.

2.2.3 Sol-gel catalysts/inhibitors

Among all constituents used in sol-gel processes, the catalyst plays an important role in a silica-based system due to its slow reaction rate. Acid or base catalysts can accelerate both the hydrolysis and condensation reactions and influence the structure of the resulting sol-gel materials [17]. Various catalysts (e.g., acetic acid [61], hydrochloric acid [62], trifluoroacetic acid [23], ammonia [63], amines [63], and potassium hydroxide [64]) have been used to cause faster sol-gel processes for silicon alkoxides.

Under acidic conditions, the hydrolysis reaction rates of silicon alkoxide precursors are significantly faster than the condensation reaction [65]. It is likely that an alkoxide group is protonated under acidic conditions in a rapid first step followed by S_N2-type water molecule attack to form a transition state [66]. As a result, the resulting sol-gel material is weakly branched polymers with a predominant more microporous structure [67]. Unlike the acid-catalyzed reaction, under basic conditions the condensations reaction rates of silicon alkoxide precursors are higher than the hydrolysis reaction rates. It is likely that water molecules dissociate to produce nucleophilic hydroxyl anions (OH⁻) in a rapid first step followed by the formation of transition state and then the displacement of alkoxy (OR⁻) group by S_N2 reaction mechanism. As a result, the
resulting sol-gel material is highly branched polymers with more mesoporous structure [68].

Generally, the sol-gel precursor is less reactive in hydrolysis and condensation reactions if the alkoxide group attached to a central metal atom is longer and/or bulkier [69]. Obviously, changing the precursor and/or its concentration is one of the ways to control the rates of the hydrolysis and condensation reactions. In addition, a judicious selection of the sol-gel catalyst is also important in determining the morphology of the sol-gel materials in silica-based systems.

The addition of acidic catalyst decreases the gel time of silicon alkoxide systems. However, some alkoxide precursors do not require the use of catalyst in sol-gel reaction due to their higher reactivity. Sol-gel process with titanium and zirconium alkoxides, for example, requires the use of a chelating agent to inhibit sol-gel reaction. Livage and co-workers [70] and Sanchez and co-workers [71] have performed several experiments including X-ray absorption near edge spectroscopy (XANES), extended X-ray absorption fine structure spectroscopy (EXAFS), and nuclear magnetic resonance (NMR) to understand the behavior of titanium isopropoxide, Ti(OPr\(^i\))\(_4\), in glacial acetic acid (GAA).

Ti atom in monomeric Ti(OPr\(^i\))\(_4\) has a coordination number of 4 (\(N = 4\)). XANES experiment showed that \(N\) increased to 6 after the addition of glacial acetic acid. \(^{13}\)C NMR showed that alteration of the chemical environments of the carboxylic and methyl carbons in GAA after reaction with Ti(OPr\(^i\))\(_4\) was caused by bonding with titanium. It also showed that both terminal and bridging OPr\(^i\) groups were present in the NMR spectra. Livage and co-workers [70] showed that OPr\(^i\) groups were hydrolyzed on a preferential basis, whereas the bridging acetate ligands remain bonded much longer to titanium
throughout the condensation process and thereby slowed down the gelation process [72].

Because they are not hydrolyzed, bridging acetate ligands change the condensation process possibly promoting the formation of linear polymeric products.

The $^1$H and $^{13}$C NMR [71] chemical shifts studies showed that corresponding acetylacetone (acac) was not free in the equimolar mixture of acac and Ti(OPr$i$)$_4$, but was bonded to titanium. The XANES spectrum also indicated that the acac reaction caused the coordination of Ti to increase from 4 to 5. EXAFS data shows no Ti-Ti correlation. This data explains a chelated titanate precursor, Ti(OPr$i$)$_3$acac for a 1:1 mixture of acac and Ti(OPr$i$)$_4$, or an octahedrally coordinated dichelated precursor for a 2:1 mixture of acac and Ti(OPr$i$)$_4$. Figure 2.3 shows the structures of bridging and chelating ligands with titanium alkoxide.
Figure 2.3 Structures of bridging and chelating ligands, R = Pr$: (A) bidentate bridging ligand [73], (B) chelating ligand [74], and (C) two chelating agents [75].
2.3 Chemical Reactions of Transition Metal Alkoxides during the Sol-Gel Process

Metal alkoxides, M(OR)$_n$, are versatile sol-gel precursors, which are known for most of the transitional metal elements including the lanthanides [76]. For silica based systems, the sol-gel chemistry is well-known and involves hydrolysis and condensation reactions. Moreover, there is a lack of data concerning the sol-gel process of transition metal alkoxides. The main differences between silicon alkoxides and transition metal alkoxides are: (1) the lower electronegativity of transition elements leads to a much higher electrophilic character of the metal and (2) lack of fully satisfied coordination in the molecular precursors due to the several possible coordination numbers for transition metals. As a result, transition metal alkoxides are much more reactive compared with silicon alkoxides, more moisture sensitive, and they readily form precipitates rather than gels when water is added.

Typical sol-gel process produces inorganic or organic-inorganic hybrid materials through the growth of the sol-gel network incorporating condensation residues from both precursors and organic components with sol-gel-active sites. In general, two main reactions are involved in the sol-gel process: (1) hydrolysis and (2) water or alcohol condensation reactions. Typical sol-gel hydrolysis and condensation reactions of metal alkoxide precursor are illustrated in Scheme 2.1 [55].
Hydrolysis: \[ \text{RO-M-OR} + \text{H}_2\text{O} \rightarrow \text{RO-M-OH} + \text{ROH} \]

Water condensation: \[ \text{RO-M-OH} + \text{HO-M-OR} \rightarrow \text{RO-M-O-M-OR} + \text{H}_2\text{O} \]

Alcohol condensation: \[ \text{RO-M-OR} + \text{HO-M-OR} \rightarrow \text{RO-M-O-M-OR} + \text{ROH} \]

where R is an alky group, and M is a metal atom (e.g. Si, Al, Ti, Zr, Ge, etc.)

Scheme 2.1 Sol-gel hydrolysis and condensation reactions. Adapted from ref. [55].
2.3.1 Hydrolysis

Generally, hydrolysis reaction of metal alkoxides occurs upon addition of water or a mixture of water and alcohol generating reactive hydroxo group, M-OH, since electronegative alkoxo groups, OR, make the metal atom highly prone to nucleophilic attack. Scheme 2.2 shows a three-step mechanism which is typically proposed in the literature [77].

The first step (a) is a solvation of metal cations by water molecule, so-called a nucleophilic addition, which leads to a transition state (b), where the coordination number of metal atom has increased by one. The second step involves a proton transfer within (b) leading to the intermediate (c), where a proton from the water molecule is transferred to the negatively charged oxygen of an adjacent OR group making the water molecule more acidic. The third step is the departure of leaving group which should be the most positively charged species within the transition state. The entire process, (a) to (d), follows a nucleophilic substitution mechanism. [52].
Overall: $\text{M(OR)}_n + \text{nH}_2\text{O} \rightarrow \text{M(OH)}_n + \text{nROH}$

Three-step mechanism:

\[
\begin{align*}
\text{(a)} \quad \text{O} & \quad \text{H} \quad + \quad \text{M-OR} \quad \rightarrow \quad \text{H} \quad \text{OH} \quad \rightarrow \quad \text{H} \quad \text{O-MR} \quad \rightarrow \quad \text{HO-M} \quad \rightarrow \quad \text{M-OH} \quad + \quad \text{ROH} \\
\text{(b)} \quad \text{(c)} \quad \text{(d)}
\end{align*}
\]

where $\text{M}$ is a metal atom (e.g. Al, Ti, Zr, B, etc.), and $\text{R}$ is an alkyl group.

**Scheme 2.2** Hydrolysis of metal alkoxides in the sol-gel process. Adapted from ref. [77].
2.3.2 Condensation reaction

Condensation is also a complex process and can occur as soon as hydroxo groups are generated. It can proceed by either nucleophilic substitution ($S_N$) when the preferred coordination is satisfied or nucleophilic addition ($A_N$) when the preferred coordination is not satisfied. Depending on experimental conditions, three competitive mechanisms have to be considered: (1) oxolation, (2) alkoxolation, and (3) olation. Scheme 2.3 illustrates three different condensation mechanisms.

Oxolation is a reaction by which an oxo bridge ($\sim O\sim$) is formed between metal atoms through the elimination of a water molecule. Basically, the mechanism is the same as for condensation process with metal atom replacing hydrogen atom in the entering group. When the metal is unsaturated in terms of coordination number, oxolation occurs by nucleophilic addition ($A_N$) with rapid kinetics ($> 10^5 M^{-1} s^{-1}$) [78].

Alkoxolation follows pretty much the same mechanisms as oxolation except the leaving group is an alcohol molecule.

Olation forms a hydroxy bridge, and can occur by nucleophilic substitution ($S_N$) where the hydroxy group is the nucleophile and water molecule is the leaving group. In this case bridging hydroxo ($M\sim OH$) groups can be generated through the elimination of a solvent molecule (either $H_2O$ or $ROH$) depending on the water concentration in the medium.

The transformation of a sol-gel precursor into an oxide network may be governed by hydrolysis and condensation reactions. Therefore, these reactions play an important role in the structure and morphology of the resulting oxide. Their roles can be optimized if the experimental conditions are carefully adjusted, and are related to both internal
(1) Oxolation:

\[
M-O + M-OH \rightarrow M-O-M \rightarrow M-O-M + H_2O
\]

(2) Alkoxolation:

\[
M-O + M-OR \rightarrow M-O-M \rightarrow M-O-M + ROH
\]

(3) Olation:

\[
\begin{align*}
M-\delta^- + M-\delta^+ \rightarrow M-\delta^+ + M-\delta^- \\
\rightarrow M-O-M + H_2O : 2(OH)_1
\end{align*}
\]

where, \( M \) is a metal atom (e.g. Al, Ti, Zr, B, etc.), and \( R \) is an alkyl group.

**Scheme 2.3** Sol-gel condensation reactions of metal alkoxides. \( x(OH)_y \) defines the number of \( M \) atoms linked by a single OH (x) and the number of bridges between these \( x \) metal atoms (y). Reproduced from ref. [52] with permission.
(nature of the metal atom and alkyl groups, structure of the molecular precursors) and external (water/alkoxide ratio, catalyst, concentration, solvent, and temperature) parameters.

2.4 Sol-Gel Coatings for Capillary Microextraction (CME)

In capillary microextraction (CME, also called in-tube SPME), a sorptive coating on the inner surface of a fused-silica capillary serves as the extraction medium in which the analytes are preferentially sorbed and preconcentrated from various sample matrices. It will cover the development and application of sol-gel coatings and/or monolithic beds as CME.

Due to inherent flexibility of sol-gel technology, various chromatographic applications, such as gas chromatography [22,23], high-performance liquid chromatography [79-81], and capillary electrochromatography [79,80,82], have been conducted. Preparation of the sol-gel extracting phase coatings for CME involves four steps: (1) pre-treatment of the fused-silica capillary, (2) preparation of the sol solution, (3) sol-gel coating process, and (4) treatment of the coated capillary.

2.4.1 Pre-treatment of fused-silica capillary

The main purpose of fused-silica capillary pre-treatment is to activate and increase the number of silanol (-OH) groups as much as possible on the inner surface of capillary to facilitate the effective bonding of the sol-gel sorbent (coating or monoliths) materials for in situ creation of sol-gel stationary phase. Pre-treatment also cleans the surface from possible contaminants.

Hayes and Malik [83,84] described a method of hydrothermal pre-treatment of the
fused-silica capillary in their papers. For this, a homemade gas-pressure-operated
capillary filling/purging device (Figure 2.4) was used for rinsing and coating processes.
The fused-silica capillary (250 or 320 µm i.d.) was sequentially rinsed with two organic
solvents of different polarities (e.g., CH₂Cl₂ and CH₃OH) and deionized water to clean
the inner surface of the capillary from organic and inorganic contaminants. This was
followed by a pressurized helium purge for a predetermined period of time (e.g., 10 min).
This process was meant for expelling most of the water in the capillary leaving only a
thin layer of water on the capillary inner surface. Both ends of the capillary were then
sealed using an oxyacetylene torch, and the sealed capillary was further conditioned in a
GC oven by temperature programming from 40 to 250 °C at a rate of 5 °C min⁻¹ with a
final temperature hold time of 2 hours. Then, both sealed ends of the capillary were cut
open using an alumina wafer and the capillary was subjected to further thermal
conditioning using the same temperature program, but under helium purge. In the first
step hydroxyl groups are generated due to hydrolysis of the siloxane bridges (Si-O-Si),
but the second step was done to moderate the silanol concentration of the surface (i.e., to
achieve a uniform surface concentration of silanols).

Constantin and Freitag [60] described an alternative method for the pretreatment
of fused-silica capillary. For conditioning, the capillary was sequentially rinsed with 1 M
NaOH for 60 min at 5 bar, with 0.1 M of HCl for 15 min at 5 bar, and finally with
deionized water for 15 min at 5 bar. Then, the capillary was purged with argon and dry
hexane for 10 min and placed in the vacuum oven for 12 hours (35 °C, 20 mbar). This
method was used by other researchers to prepare sol-gel columns [85-87].
Figure 2.4 Schematic of a homemade capillary filling/purging device. Reproduced from ref. [82] with permission.
2.4.2 Preparation of sol solution

The selection of the ingredients and preparation of the sol solution are the most critical steps to create the desired sol-gel sorbent. Typically the sol solution ingredients include sol-gel precursor(s), a sol-gel active organic polymer, a solvent system, a catalyst (or inhibitor), and water. Since the homogeneity of the sol system is very important, careful selection of a solvent system which is compatible with the used precursor(s) and polymer is essential.

In addition to the typical ingredients in the sol solution, various additives are often used, such as a drying control chemical additive (DCCA), and/or a surface deactivating reagent. As mentioned earlier, a DCCA may be used to minimize the shrinkage and cracking during conversion of the wet gel to dry gel, and also it helps to increase the porosity of the sol-gel material.

A surface deactivating reagent may be used to deactivate residual silanol groups on the created sol-gel material to reduce possible adsorptive effects. Malik and co-workers reported the use of various deactivation reagents for sol-gel stationary phases, such as phenyldimethylsilane (PheDMS) [83] [88], 1,1,1,3,3,3-hexamethyldisilazane (HMDS) [44,46-48], and poly(methylhydrosiloxane) (PMHS) [22,23,44,46-48].

2.4.3 Sol-gel coating technology

Malik and co-workers developed the sol-gel SPME fiber coating procedure [23]. Prior to coating, the protective polyimide layer was removed from a 1 cm segment of the fused-silica fiber using a cigarette lighter, followed by cleaning with methanol and drying. Then the cleaned end of the fiber was held inside the sol solution by vertically dipping for about 20 min, so that a sol-gel coating was formed on the bare outer surface of the fiber.
end. This coating process was repeated until the desired coating thickness was achieved. The fiber was then conditioned under helium in the GC injection port. The sol-gel SPME coating technology has been used and modified by other SPME research groups [89-91].

A sol-gel in-tube SPME (capillary microextraction, CME) coating technology was first introduced by Malik and co-workers [22,92]. A hydrothermally treated fused-silica capillary (1 m x 250 or 320 µm i.d.) was installed in a homemade capillary filling/purging device (Figure 2.4), and filled using the top clear portion of a sol solution obtained after centrifugation. After filling, the sol solution was kept inside the capillary for a controlled period of time (typically 15-30 min) to facilitate the formation of a sol-gel coating on the capillary inner surface. During this process, a sol-gel organic-inorganic hybrid network was evolving within the sol solution portion of this network got chemically bonded to the inner walls of the capillary via condensation with surface silanol groups. After this, the free unbonded portion of the sol solution was expelled from the capillary under helium pressure (e.g., 50 psi) leaving behind a surface-bonded sol-gel coating within the capillary. The sol-gel coated capillary was further purged with helium for additional 30 min to facilitate the evaporation of the remaining volatile organic solvents.

2.4.4 Further treatment of sol-gel-coated CME capillary

The sol-gel coated capillary was thermally conditioned in a GC oven using temperature-programmed heating from 40 to 320 °C, for example, at a rate of 1 °C min⁻¹ with a final temperature hold time of 2 hours under helium purge, where the final temperature being determined by thermal stability of organic component used to prepare the sorbent coating. The capillary was cooled down to room temperature and sequentially
rinsed with organic solvents (e.g., methylene chloride and methanol) to clean the coated surface. Finally, the capillary was installed in the GC oven for drying and further conditioning using the same temperature-programmed heating, except that this time the capillary was held at the final temperature for 30 min. The conditioned capillary was then cut into small pieces (e.g., 10-40 cm) that were used to perform capillary microextraction.

2.5 Characterization of Sol-Gel Stationary Phase and Its Morphology

To understand the morphology of sol-gel organic-inorganic hybrid materials, various material characterization methods are performed. Scanning electron microscopy (SEM) [84,93] is a power tool and the most widely used techniques to study the morphology of sol-gel materials. SEM images show the uniformity of the sol-gel coating thickness and structural details through cross sectional and surface views of sol-gel coating. In addition to SEM, atomic force microscopy (AFM) [94] and X-ray photoelectron spectroscopy (XPS) [94-96] are also used to study the morphology of sol-gel materials. However, these techniques, except XPS, do not provide the information of chemical bonds within the sol-gel structure.

To investigate the chemical bonds in sol-gel structure, Fourier transform infrared spectroscopy (FTIR) [97-99] and nuclear magnetic resonance (NMR) [100,101] have been used. FTIR spectroscopy is a simple tool to study sol-gel process in its evolution with time and to identify specific chemical bonds within sol-gel coatings. Another powerful analytical technique is nuclear magnetic resonance (NMR) to investigate the structural features present in the sol-gel materials. The connectivity of the inorganic network has been studied by $^{29}$Si, $^{27}$Al, and $^{17}$O NMR techniques [102-105]. The latter
technique has been useful to study the existence of Si-O-Ti and Si-O-Zr bonds in liquid sol solutions [103,104] and verify the homogeneous distribution within hybrid systems. Since the properties of the sol-gel capillary are based on the species present in sol-gel coating, it is essential to understand the morphology and chemical bonds of sol-gel coating in details.

2.6 The Application of Sol-Gel-Coated Microextraction Capillary in SPME and CME

The extracting phase coating in a capillary microextraction (CME), capillary must be able to survive under harsh operating conditions, such as high temperature and pressure, wide pH ranges, and occasional elevated temperature with organic solvents. The chemical bonds between the inner surface of the fused-silica capillary and the sol-gel sorbent coating provide both thermal [91,106] and solvent stabilities [91,107] with reproducible performance of the coating. This attribute of sol-gel coatings provides an effective means to couple CME with HPLC [108,109] as well as GC [44,89] and CEC [82,88]. In addition, the thickness of the coating can be controlled using sol-gel coating technology [109,110] to enhance the detection limits by using a variety of sol-gel active organic ligands.

To perform CME-HPLC, the sol-gel coated capillary can be installed in the injection port as a sampling loop, and then on-line sample extraction [47] and separation can be performed in one place without additional instrumental modification to desorb the extracted analytes from CME capillary coating and transfer them to the separation column. This is accomplished by simply switching the injection valve from the “load” to
“inject” position. The injected analytes are then separated on the separation column by either under isocratic or gradient conditions.

Since sol-gel technology can provide tunable selectivity and efficiency, it is possible to create organic-inorganic hybrid porous materials through manipulation of the sol solution compositions. It significantly increases the surface area of the extracting phase and provides acceptable sorbent loading, sample capacity, and faster mass transfer using thinner coatings [23]. Table 2.2 summarizes organic components used to prepare sorbents reported in the literatures.
Table 2.2. Summary of sol-gel sorbent used in SPME and CME.

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>Structure</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxy-terminated poly(dimethylsiloxane)</td>
<td><img src="image" alt="Structure of Hydroxy-terminated poly(dimethylsiloxane)" /></td>
<td>[23]</td>
</tr>
<tr>
<td>(PDMS)</td>
<td><img src="image" alt="Structure of Silanol-terminated poly(dimethyldiphenylsiloxane)" /></td>
<td>[48]</td>
</tr>
<tr>
<td>Silanol-terminated poly(dimethyldiphenylsiloxane) (PDMDPS)</td>
<td><img src="image" alt="Structure of Silanol-terminated poly(dimethyldiphenylsiloxane)" /></td>
<td>[48]</td>
</tr>
<tr>
<td>Phenyl-terminated dendrimer</td>
<td><img src="image" alt="Structure of Phenyl-terminated dendrimer with a triethoxysilyl root." /></td>
<td>[45]</td>
</tr>
<tr>
<td>with a triethoxysilyl root.</td>
<td><img src="image" alt="Structure of Hydroxy-terminated dibenzo-14-crown-4 (OH-DB14C4)" /></td>
<td>[110]</td>
</tr>
</tbody>
</table>
Amide bridged-calix[4]arene

Divinyl benzene (DVB)

Poly(vinyl alcohol) (PVA)

Superox-4 (PEG)

Hydroxyfullerene (fullerol)-OH-TSO

A. Methoxypoly(ethylene glycol)-silane (PEG 1)

B. Poly(ethylene glycol)-bis-silane (PEG 2)

α,ω-Diallyltriethylene glycol

Dihydroxy-terminated benzo-15-crown-5 (DOH-B15-C5)

A. 4-Allyldibenzo-18-crown-6
B. 3-Allyldibenzo-15-crown-5
C. Allyloxyethoxymethyl-18-crown-6

Poly(methylphenylvinylsiloxane)

Allyloxy bisbenzo 16-crown-5 trimethoxysilane
For decades, chromatographers have been obtaining better efficiency by reducing the size of column packing materials in HPLC. However, the maximum obtainable number of theoretical plates by using a separation column has been steady at 10,000-25,000 in HPLC due to high pressure drop caused by small-size column packing materials under the upper limit of operating pressure (5000 psi. with current instrumentation).

Increasing the column permeability can increase the column performance. One possible way to attain higher column permeability is to use a sol-gel monolithic column. The sol-gel monolith is a single piece of continuous bed composed of chemically bonded network structures, which is often called as rod column or fritless column [19]. The main advantages of the sol-gel monolith are: (1) higher permeability and column efficiency, (2) free from back pressure problem due to the absence of retaining end frits, and (3) higher surface areas.

In late 1980s, Cortes and co-workers [19] reported the preparation of the sol-gel silica-based monolithic capillary columns for liquid chromatography for the first time. However, the most successful preparation of sol-gel based monolithic column (or silica-based rod column) was reported by Nakanishi and co-workers [79,120] in early 1990s. They prepared bimodal pore silica gel monolithic columns (macropores and mesopores) by sol-gel process of using TMOS and a water-soluble polymer. Later the usefulness of this method was reported by Tanaka and co-workers [81] and Lubda and co-workers [121], who demonstrated that the sol-gel monolithic columns possessed many advantages, such as higher efficiency, lowered the backpressure, and reduced separation time.
compared to the conventional packed columns. Merck KGaA (Darmstadt, Germany) introduced this silica monolithic column, Chromolith®, in the market, which opened the new era in column technology. Cabrera and co-workers [122] reported the use of this commercial monolithic column for fast analysis in HPLC with up to 9 mL/min flow rate (Figure 2.5). The SEM images of the commercial sol-gel monolithic column (Chromolith®) are shown in Figure 2.6 [123].

The Tanaka group [124] prepared sol-gel monolithic column in a fused-silica capillary using TMOS for CEC application. However, the efficiency of column was not as good as the polymer-based monolithic columns reported by Svec’s group [125]. Later Tanaka and co-workers [126] reported the use of a low pressure-assisted operation for fast CEC separations to achieve better column efficiency and faster analysis. Since monolithic column possesses highly porous structure and excellent permeability, it allows the use of pressure-driven flow.

Hayes and Malik [83] introduced the single-step preparation of sol-gel monolithic columns in the fused-silica capillary for CEC using a commercially available sol-gel precursor, N-octadecyldimethyl[3-(trimethoxysilyl)propyl] ammonium chloride (C_{18}-TMS) to create a C_{18} moiety in a monolithic bed. They reported highly porous structure, sufficient stationary phase for chromatographic interactions, and enhanced permeability of the monolithic column. SEM images of the sol-gel monolithic column are shown in Figure 2.7 [83].

Fujimoto [127] prepared the sol-gel monolithic CEC column. Later, Takeuchi and co-workers [128] used a modified procedure of Fujimoto to prepare sol-gel silica-based monolithic beds, and use it as a pre-column extraction loop for on-line sample
Figure 2.5 Chromatographic separation of five β-blocking drugs on a silica rod column at different flow rates: Column: silica rod column RP 18e, 50 x 4.6 mm; mobile phase: acetonitrile/0.1% TFA in water (20/80; v/v); flow rate: 1 - 9 mL/min, detection: UV 254 nm, samples: 1. atenolol, 2. pindolol, 3. metoprolol, 4. celiprolol, 5. bisoprolol. Reproduced from ref. [122] with permission.
Figure 2.6 SEM image of a cross-section from a Chromolith® structure. Reproduced from ref. [123] with permission.
Figure 2.7 SEM of a sol-gel monolithic column: (A) cross-sectional view (1800×), (B) longitudinal view (7000×), and (C) longitudinal view (15000×). Adapted from ref. [83].
preconcentration in HPLC.

Sol-gel monolithic column format is probably the one major approach used in developing new types of stationary phases for both HPLC and CEC. A single piece of porous material allows the preparation of frit-free columns, which eliminates the band-broadening and operation problems often caused by the formation of bubbles. In addition, enhanced column permeability enables to use higher flow rates, which allows faster analysis, and provides better column efficiency.

2.8 References for Chapter Two


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3.1 Introduction

Titania (TiO$_2$) is a white pigment that has found various applications in many branches of industry such as plastics, enamels, artificial fibers, electronic materials, and rubber [1]. Titania is also the most efficient photocatalyst in some cases such as water decomposition [2,3]. In contrast to silica (SiO$_2$), titania is mainly used as a support due to its excellent mechanical properties, inertness, and low price.

Typically, titania is encountered in three different crystallographic forms: anatase, rutile, and brookite [4]. Due to inherently low stability of brookite, it does not seem to offer significant practical importance. Anatase is the one which is thermodynamically stable up to 800 °C and conversion to rutile takes place if the temperature is higher than 800 °C. Both anatase and rutile form crystal structures in a tetragonal lattice. The coordination number of titanium is 6, and that of oxygen, 3. The two modifications differ in number of common edges of the TiO$_6$ octahedra: 4 for anatase and 2 for rutile. Both anatase and rutile have about 10 hydroxyl groups per nm$^2$ of surface area [5]. In general, anatase is widely used for catalytic purposes. Figure 3.1 shows crystal structures of anatase and rutile [6].
Figure 3.1 Crystal structures of two crystallographic modifications of titanium dioxide:
(A) anatase and (B) rutile. Reproduced from ref. [6] with permission.
3.1.1 Titania as a chromatographic support in separation science

Among chromatographic supports, silica (SiO$_2$) is traditionally the most commonly used in separation science due to its highly favorable properties, such as mechanical strength to withstand high pressures, ability to provide rapid mass transfer during chromatographic separations, the ease of surface modification due to the presence of highly reactive silanol groups (SiOH), and commercial availability [7-9].

Unfortunately, silica supports suffer many limitations for use in chromatography, especially in HPLC. The presence of the residual silanol groups on the silica surface leads to low chromatographic performance [8,10]. In addition, silica possesses stability only in the pH range of 2-8 [11,12]. Below pH 2, siloxane (-Si-O-Si-) linkages undergo hydrolytic attack and bonded ligands are slowly removed from the surface, which leads to deterioration in chromatographic performance [7,13]. Above pH 8, silica begins to dissolve leading to a collapse of the column bed [8,14]. Therefore, to overcome these inherent limitations of silica support, alternative inorganic support materials have been introduced such as alumina [15], zirconia [16] and titania [17,18].

Titania was introduced by Kawahara and co-workers [17] in the late 1980s and early 1990s for the chromatographic applications to overcome inherent drawbacks of silica supports. In contrast to silica, titania materials show many advantages, such as high chemical and mechanical stabilities, that make it an attractive choice to use titania as support material in separation science [17,19].

Titania also possesses excellent stability in the wide ranges of pH, which enables to separate analytes under extreme pH conditions [20-22]. In addition, titania has anion-exchange properties at acidic pH and cation-exchange properties at basic pHs [23],
whereas silica has only a cation-exchange property. Several studies have been performed on the chromatographic applications of titania material due to its good prospect as a new chromatographic support.

3.1.1.1 Titania as a chromatographic column support in HPLC

Traditionally, surface-derivatization of titania was limited to the preparation of octadecysilyl derivatives. Trudinger and co-workers [19] synthesized porous amorphous titania particles using titanyl chloride and octadecyltrimethoxysilane by a sol-gel process, and used them as reversed-phase packing materials for HPLC. Tani and co-workers [20,24] prepared titania-based reversed-phase packings with octadecyltriethoxysilane by the sol-gel method for normal-phase liquid chromatography. Pesek and co-workers [25] reported an alternative surface derivatization method for titania with triethoxysilane to prepare bonded titania-based stationary phases via silanization/hydrosilylation (Figure 3.2) [26], and the resulting surface-derivatized titania was investigated by NMR spectroscopy.

Sato and co-workers [27] reported the sol-gel preparation of spherical titania-based packing materials for HPLC using titanium isopropoxide, and demonstrated the possibility of pore size control in titania using stearic acid as a pore regulating reagent. Ikeguchi and Nakamura [28] used a titania precolumn to selectively trap organic phosphates for online preconcentration under acidic conditions. Later, Fadeev and McCarthy [29] reported the covalent reaction of hydridosilanes with titanium surfaces by Ti-O-Si bonds, and Shafi and co-workers [30] reported a fast and efficient method for coating octadecyltrihydrosilanes [CH₃(CH₂)₁₇SiH₃] on rutile surfaces by sonochemistry.
Figure 3.2 Preparation of “bonded” titania-based stationary phases for HPLC via silanization/hydrosilylation. Reproduced from ref. [25] with permission.
In 1997, a German company, Sachtleben, introduced porous titania sorbent (Sachtopore) for HPLC. Winklet and Marme [31] reported the use of “Sachtopore for the application of normal-phase liquid chromatography to separate basic molecules such as amines.

The conventional silica-based packings show degradation at temperatures under 100 °C [32]. Moreover, the solubility of silica in water increases above 100 °C, which is a problem for faster analysis and Kephart and Dasgupta [33] demonstrated the thermal stability and mechanical strength by using HPLC columns packed with C18-modified TiO$_2$, which was capable to operate at 200 °C and pressures up to 10,000 psi for high speed capillary liquid chromatography.

Miyazaki and co-workers [34] developed novel titania-coated monolithic silica columns for liquid chromatography to separate phosphorous-containing compounds. They mentioned that titania-coated monolithic silica columns possess excellent selectivity for phosphorylated substances with low pressure drop compared to the conventional packed column.

Lucy and co-workers [35] compared silica, zirconia, and titania columns for their ability to separate diesel samples by supercritical fluid chromatography (SFC), and they found that a titania column coupled in series to a silica column was found to provide the highest overall group-type resolutions.

Recently, conventional and comprehensive two-dimensional (2D) HPLC systems using the combination of packed-titania and monolithic columns were established by Ueda and co-workers [36] for the online analysis of phosphopeptides, which would be useful for online phosphoproteome analyses in future.
3.1.1.2 Titania as a chromatographic column support in CE

The use of titania as a sorbent in liquid chromatography has been described in the proceeding section. Although applications of titania in HPLC have been widely investigated, little attention has been given to the applicability in capillary electrophoresis (CE) and capillary electrochromatography (CEC). Tsai and co-workers [21] prepared sol-gel titania coatings in fused-silica capillaries to separate proteins by CE. Fujimoto [37] created titania coatings on the inner wall of the fused-silica capillaries with a solution of a titanium peroxo complex for CE and CEC applications. The titania-coated capillaries were found to possess both directions of electroosmotic flow (EOF) and low solubility in aqueous solutions between pH 3 and 12, which is indicative of long term stability of titania compared to silica-based bonded phases. Xu and co-workers [38] also reported the preparation of sol-gel titania-coated capillary with switchable EOF for nonaqueous CE separation of widely different mobilities.

3.2 Other Applications of Titania

Traditionally, titania-silica composite materials were prepared in the form of thin layer coating by several methods such as flame hydrolysis [39], chemical vapor deposition [40], electron-beam evaporation [41], and sol-gel processes [42].

Titania typically resides in its application as pigment to provide whiteness and/or opacity in paints, plastics, and paper [43] due to its excellent optical properties due to high refractive index, lack of absorption of visible light, stability, nontoxicity, and malleability in the desired size range. Titania is also used as a material in other applications such as ceramic membranes [44,45], adsorbents [46,47], and catalyst support
The use of titania in the area of catalysis is presented in Figure 3.3 [49]. The properties of the titania surface are decisive for its catalytic activity and selectivity depending on the type and concentration of different active sites. In this case, the surface affects the formation of definite structures of the active phase of titania-supported catalysts.

Recently, the fine particles of titania have found applications as an advanced semiconductor material for solar cells [50], a luminescent material [51], and a photocatalyst for photolysis of water [52] or organic compounds [53] and as a bacteriocide [54].

Kunitake and Lee [55] prepared ultrathin titania gel films via sol-gel process for molecular imprinting application, and mentioned several advantages of the metal oxide films such as thermal stability, formation of multi-functional sites, and simplicity of operation. Schubert [56] summarized the use of several chemical additives for the chemical modification of titanium alkoxide precursors using their Lewis acid properties.

Currently, titania has become the subject of intensive research efforts in view of the potential and promise of titania nanotubes in bone growth and regeneration [57], environmental applications [58], dielectrics [59], optoelectronics [60], and sensors [61].
Figure 3.3 Application of TiO$_2$ in the field of catalysis. Reproduced from ref. [48] with permission.
3.3 Sol-Gel Titania as an Extraction Sorbent in CME

Sol-gel chemistry has been recently applied to solid-phase microextraction (SPME) [62-66] and capillary microextraction (CME) [67] to create silicon-based hybrid organic-inorganic coatings. The sol-gel technique provided chemically bonded coatings on the inner surface of fused-silica capillaries, and easily solved the coating stability problems as described in the proceeding chapter.

Although sol-gel technique helped overcome some significant shortcomings of SPME or in-tube SPME techniques by providing an effective means of chemical immobilization of the sorbent coatings, an important problem inherent in silica-based material systems (commonly used in SPME or CME) still remains to be solved: silica-based materials possess a narrow window of pH stability [68]. In the context of SPME, it pertains to the stability of silica-based fibers and coatings. The development of alternative materials possessing superior pH stability and better mechanical strength should provide SPME with additional ruggedness, and versatility.

To date, very little (if any) research has been done on the development and application of titania-based coatings in analytical microextraction techniques, although titania possesses many attractive properties such as superior pH stability and mechanical strength compared with silica [20-22,31,37]. Moraes and co-workers [69] used a two-step sol-gel process to synthesize a silica-titania hybrid material. The hybrid materials were employed as sorbents for solid-phase extraction (SPE) for the investigation of carcinogenic N-containing compounds from aqueous samples followed by GC analysis.

In this chapter, the preparation of sol-gel TiO\textsubscript{2}-PDMS-coated capillaries will be presented and the possibility of on-line CME-HPLC operation using sol-gel TiO\textsubscript{2}-PDMS
microextraction capillaries to provide a significant improvement in pH and solvent stabilities, as well as enhancement in extraction sensitivity will be demonstrated.

3.4 Experimental

3.4.1 Equipment

On-line CME-HPLC experiments were carried out on a Micro-Tech Scientific (Vista, CA) Ultra Plus HPLC system with a variable wavelength UV detector (Linear UVIS 2000). A Nicolet model Avatar 320 FTIR (Thermo Nicolet, Madison, WI) was used for FTIR measurements. A reversed-phase ODS column (Supelco, 25 cm x 4.6 mm i.d., 5 µm d_p) and Betabasic 8 (Thermo Electron Co., 10 cm x 4.6 mm i.d., 5 µm d_p) were used for HPLC separation of the extracted analytes. A Fisher model G-560 Vortex Genie 2 system (Fisher Scientific) was used for thorough mixing of the sol solutions. A Microcentaur model APO 5760 centrifuge (Accurate Chemical and Scientific Corp., Westbury, NY) was used for centrifugation of sol solutions. A Barnstead model 04741 Nanopure deionized water system (Barnstead/Thermolyne, Dubuque, IA) was used to obtain 16.0 MΩ-cm water. A JEOL model JSM-35 scanning electron microscope (SEM) was used for the investigation of surface morphology of the sol-gel titania-PDMS-coated capillaries. On-line data collection and processing were done using ChromPerfect (version 3.5 for Windows) computer software (Justice Laboratory Software, Denville, NJ).

3.4.2 Chemicals and materials

Fused-silica capillary (250 and 320 µm i.d.) was purchased from Polymicro
Technologies Inc. (Phoenix, AZ). A commercial polysiloxane-based GC column (30 m x 0.25 mm i.d., 0.25 µm film thickness) was used for comparison with sol-gel titania-PDMS-based microextraction capillary in pH stability studies. Titanium (IV) isopropoxide (99.999 %), 1-butanol (99.4+ %), Poly(methylhydrosiloxane) (PMHS), 1,1,1,3,3,3-hexamethyldisilazane (HMDS), trifluoroacetic acid (TFA), polycyclic aromatic hydrocarbons (PAHs) (acenaphthylene, fluorene, phenanthrene, fluoranthene, pyrene), ketones (butyrophenone, valerophenone, hexanophenone, heptanophenone), and alkylbenzenes (toluene, ethylbenzene, cumene, n-propylbenzene, n-butylbenzene, amylbenzene) were purchased from Aldrich (Milwaukee, WI). Hydroxy-terminated poly(dimethysiloxane) (PDMS) was purchased from United Chemical Technologies, Inc. (Bristol, PA). HPLC-grade solvents (acetonitrile, methylene chloride, and methanol) were purchased from Fisher Scientific (Pittsburgh, PA).

3.4.3 Preparation of the sol solution

The sol solution was prepared by thoroughly vortexing the following reagents in a 2-mL polypropylene centrifuge tube: a sol-gel-active organic component (hydroxy-terminated PDMS, 50 mg), a sol-gel precursor [titanium(IV) isopropoxide, 50 µL], two solvents (methylene chloride and 1-butanol, 200 µL each), a mixture of two surface deactivation reagents (HMDS, 8 µL and PMHS, 2 µL), and a sol-gel chelating agent (27 % TFA in H₂O, 18 µL). The content of the tube was then centrifuged for 5 min (at 13000 rpm; 15682 x g). Finally the top clear solution was transferred to another clean vial by decantation, and was further used for coating the fused-silica microextraction capillary. The chemical ingredients used in the sol-gel coating solutions are represented in Table 3.1.
**Table 3.1** Names, functions, and chemical structures of the coating solution ingredients used to prepare sol-gel TiO$_2$-PDMS-coated microextraction capillaries.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Function</th>
<th>Chemical structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titanium (IV) isopropoxide</td>
<td>Sol-gel precursor</td>
<td>$\text{Ti} \text{OCH(CH}_3\text{)}_2$ \text{OCH(CH}_3\text{)}_2$</td>
</tr>
<tr>
<td>Poly(dimethylsiloxane), hydroxy terminated (PDMS)</td>
<td>Sol-gel-active polymer</td>
<td>$\text{HO} \left{ \text{Si} \text{O} \right}_n \text{H}$</td>
</tr>
<tr>
<td>Trifluoroacetic acid (TFA)</td>
<td>Bridging (chelating) ligand</td>
<td>$\text{HO} \quad \text{O}$ \quad \text{CF}_3$</td>
</tr>
<tr>
<td>1,1,1,3,3,3-Hexamethyl-disilazane (HMDS)</td>
<td>Deactivating reagent</td>
<td>$\text{H}_3\text{C} - \text{Si} \text{N} - \text{Si} - \text{CH}_3$</td>
</tr>
<tr>
<td>Poly(methylhydrosiloxane) (PMHS)</td>
<td>Deactivating reagent</td>
<td>$\text{H}_3\text{C} - \text{Si} - \left{ \text{Si} \text{O} \right}_m \text{Si} - \text{CH}_3$</td>
</tr>
</tbody>
</table>


3.4.4 Preparation of sol-gel TiO$_2$-PDMS-coated microextraction capillary

A 1-m long hydrothermal treated [70] fused-silica capillary (250 or 320 μm i.d.) was installed on an in-house built gas pressure-operated capillary filling/purging device [71] shown in Figure 2.4, and the capillary was filled with the prepared sol solution under 10 psi helium pressure. After filling, the sol solution was kept inside the capillary for 15 min to facilitate the creation of a surface-bonded coating through sol-gel reactions taking place in the coating solution inside the capillary. Following this, the unbonded portion of the sol solution was expelled from the capillary under helium pressure (20 psi), and the capillary was further purged with helium for 30 min. The coated capillary was then conditioned in a GC oven by programming the temperature from 40 °C to 320 °C at 1 °C/min under helium purge. The capillary was held at 320 °C for 180 min. Finally, the capillary was cooled down to room temperature and rinsed with methylene chloride and methanol (3 mL each). Following this, the capillary was installed in the GC oven for drying and further thermal conditioning under temperature-programmed heating as described above, except that this time the capillary was held at the final temperature for 30 min.

3.4.5 Sol-gel titania coatings in capillary microextraction (CME) for on-line CME-HPLC analysis

A schematic of the CME-HPLC setup for on-line capillary microextraction and HPLC analysis is presented in Figure 3.4. An ODS column (25 cm × 4.6 mm i.d., 5 μm d$_p$) was previously installed in the HPLC system and pre-equilibrated with the mobile phase consisting of an acetonitrile/water mixture (80:20, v/v). A 40-cm segment of the
sol-gel TiO$_2$-PDMS-coated microextraction capillary was mounted on the HPLC injection port as an external sampling loop. Analytes were preconcentrated in the sol-gel TiO$_2$-PDMS coating by passing the aqueous sample from a gravity-fed dispenser [67] through this sol-gel titania-PDMS-coated microextraction capillary for 40 min. Using a syringe, the sampling loop was flushed out with deionized water to remove the sample matrix. The analytes extracted in the sol-gel TiO$_2$-PDMS coating of the sampling loop were then transferred into the HPLC column by desorbing with 100 % acetonitrile for 30 seconds. This was accomplished by simply switching the injection valve from the “load” to “inject” position. The injected analytes were then separated on the ODS column under gradient elution conditions by programming acetonitrile composition in the organo-aqueous mobile phase from 80 % (v/v) to 100 % in 15 min.
Figure 3.4 Schematic diagram of the on-line CME-HPLC setup.
3.4.5.1 Treatment of sol-gel titania-PDMS-coated capillaries with 0.1 M NaOH solution

A 40-cm segment of the sol-gel TiO$_2$-PDMS-coated microextraction capillary was directly installed on the gravity-fed sample dispenser, and continuously rinsed with 0.1 M NaOH solution (pH = 13) for 12 hours. The capillary was then flushed out with deionized water for 30 minutes, and mounted back on the HPLC injection port. The target analytes (PAHs) were extracted on-line for 40 min, followed by their HPLC analysis as described in section 3.4.5.

Using the same procedure, a 40-cm segment of the commercial GC capillary was treated with a 0.1 M NaOH solution. CME performances of the used capillaries were evaluated both before and after the alkaline treatment to explore pH stability of the used coatings.

3.4.5.2 Treatment of sol-gel titania-PDMS-coated capillaries with 0.1 M HCl solution

A new 40-cm segment of the sol-gel TiO$_2$-PDMS-coated microextraction capillary was directly installed on the gravity-fed sample dispenser, and continuously rinsed with 0.1 M HCl solution (pH = 1) for 12 hours. The capillary was then treated as described in 3.4.5.1, and used to extract the target analytes (PAHs and ketones) on-line for 40 min, followed by their HPLC analysis.

3.4.5.3 Treatment of sol-gel titania-PDMS-coated capillaries with HPLC solvents at high temperature

A 40-cm segment of the sol-gel TiO$_2$-PDMS-coated microextraction capillary was filled with a mixture of acetonitrile and deionized water (50:50, v/v), and both ends were
sealed by a mini union connector. The sealed capillary was subjected to high temperature (150 °C) in the GC oven for 12 hours. The capillary was then washed with DI water, purged with helium gas, and mounted back on the HPLC injection port as an external sampling loop. The target analytes (PAHs and ketones) were extracted on-line for 40 min, followed by their HPLC analysis. In similar fashion, the sol-gel TiO$_2$-PDMS coating stability was also studied using either 100% acetonitrile or 100% methanol.

**3.4.5.4 Safety precautions**

The presented work involved the use of various chemicals (organic and inorganic) and solvents that might be environmentally hazardous with adverse health effects. In accordance with material safety data sheet (MSDS) proper safety measures were taken in handling strong acids, bases and organic solvents such as methanol, methylene chloride, and acetonitrile. All used chemicals were disposed in the proper waste containers to ensure personnel and environmental safety.

**3.5 Results and Discussion**

The goal of this research was to develop high pH-resistant, surface-bonded sol-gel titania coatings for capillary microextraction to facilitate effective hyphenation of CME with HPLC. Judicious utilization of unique attributes of sol-gel chemistry allowed us to create a surface-bonded hybrid organic-inorganic titania coating on the inner walls of a fused-silica capillary providing an opportunity to exploit advanced material properties of titania-based sorbents [17,19] in capillary microextraction. The sol-gel coating technique was fast, straightforward, and highly effective. Unlike the conventional multi-step coating technology [73-76], the sol-gel approach involved a single-step procedure to
accomplish the sorbent coating, its chemical immobilization, and deactivation [77].

3.5.1 Sol-gel reactions for the preparation of sol-gel TiO₂-PDMS coating

In sol-gel chemistry, alkoxides are commonly used as sol-gel precursors. Titanium alkoxides differ significantly from silicon alkoxides in terms of their chemical reactivity and complex-forming ability. These differences dictate the adoption of different strategies for the creation of titania-based sol-gel sorbents compared with those for silica-based analogs. While sol-gel reactions in a silica-based system are rather slow and often require the use of catalysts to accelerate the process [78], titania-based (transition metal oxide-based in general) sol-gel reactions are very fast. This is explained by the fact that titanium alkoxides are very reactive toward such nucleophilic reagents as water [79]. They readily undergo hydrolysis which results in a very fast sol-gel process. Because of this, titania-based sol-gel reactions need to be decelerated by a suitable means to allow for the sol-gel process to be conducted in a controlled manner. This is usually accomplished through the use of suitable chelating agents that form complexes with the sol-gel precursors (or replace the reactive alkoxy group with a less reactive group), thus hindering their participation in the sol-gel reactions. Without such a chelating agent, the gelation takes place instantaneously as the sol-gel solution ingredients are mixed with water. Chelating agents such as acetic acid [80,81], trifluoroacetic acid [82], or metal β-diketonates [83] are often used for this purpose.

In the present work, sol-gel TiO₂-PDMS-coated capillaries were prepared through hydrolytic polycondensation reactions performed within fused-silica capillaries followed by thermal conditioning of the created coatings to achieve porous coating structures of enhanced surface area. In this work, TFA served as a bridging (or chelating) agent [82],
and decelerated the gelation process for the creation of TiO₂-PDMS coating. It has been shown by infra-red spectroscopy [80,82,84] that the acetate ion can serve as a bidentate ligand (chelating and bridging) to the transition metal alkoxides, such as Ti(OR)₄ or Zr(OR)₄.

The sol-gel process for the generation and chemical immobilization of the hybrid organic-inorganic surface coating involved: (A) hydrolysis of the titanium alkoxide precursor [85] (Scheme 3.1A), (B) polycondensation of the hydrolysis products into a three-dimensional sol-gel network [86,87] (Scheme 3.1B), chemical incorporation of hydroxy-terminated PDMS in the sol-gel network [88,89] (Scheme 3.2), and chemical anchoring of the sol-gel hybrid polymer to the inner walls of the capillary [86,87] (Scheme 3.3). Finally, Scheme 3.4 represents chemical reactions involving HMDS [90] and PMHS [29,91] to deactivate the sol-gel TiO₂-PDMS coating.

There exists a marked distinction between the silica-based and transition metal oxide-based (including titania-based) sol-gel systems in terms of reaction kinetics. While sol-gel reactions in a silica-based system are rather slow, and require the use of catalysts [92], titania-based (transition metal oxide-based in general) sol-gel reactions are very fast [79]. Here the sol-gel reactions need to be slowed down to achieve controllable rates. This is usually accomplished through the use of suitable chelating agents that form complexes with the sol-gel precursors (or replace the reactive alkoxy group with a less reactive group), thus hindering their participation in the sol-gel reactions. Without such a chelating agent, the gelation takes place instantaneously as the sol-gel precursor comes in contact with water during preparation of the sol solution by mixing the ingredients.
Scheme 3.1 (A) Hydrolysis of titanium (IV) isopropoxide, and (B) polycondensation of hydrolysis product, titanium hydroxide. Composition of the sol solution: a sol-gel precursor (titanium (IV) isopropoxide, 50 µL), two solvents (methylene chloride and 1-butanol, 200 µL each), and a sol-gel chelating agent (27 % TFA in H_2O, 18 µL) (at room temperature).
Scheme 3.2 (C) Polycondensation of hydroxyl-terminated PDMS with the evolving sol-gel network. Composition of the sol solution: a sol-gel precursor (titanium (IV) isopropoxide, 50 µL), two solvents (methylene chloride and 1-butanol, 200 µL each), a sol-gel chelating agent (27 % TFA in H₂O, 18 µL), and a sol-gel-active organic component (hydroxy-terminated PDMS, 50 mg) (at room temperature).
Scheme 3.3 (D) Chemical anchoring of the sol-gel polymer to the inner walls of the capillary. Composition of the sol solution: a sol-gel precursor (titanium (IV) isopropoxide, 50 µL), two solvents (methylene chloride and 1-butanol, 200 µL each), a sol-gel chelating agent (27 % TFA in H₂O, 18 µL), and a sol-gel-active organic component (hydroxy-terminated PDMS, 50 mg) (at room temperature).
Scheme 3.4 Deactivation of surface-bonded sol-gel TiO$_2$-PDMS coating with HMDS and PMHS taking place during thermal treatment of the coated microextraction capillary at 150 °C.
3.5.2 Scanning electron microscopy (SEM) of surface-bonded sol-gel TiO\textsubscript{2}-PDMS coating

Figure 3.5 represents two scanning electron micrographs showing the fine structural features of a 320-\textmu m i.d. fused-silica capillary with sol-gel TiO\textsubscript{2}-PDMS coating on the inner surface. As is evident from these images, the sol-gel TiO\textsubscript{2}-PDMS coating in the microextraction capillary acquires a porous structure, providing enhanced surface area and sorption ability. Based on the SEM data, the thickness of the sol-gel TiO\textsubscript{2}-PDMS coating was estimated at ~0.5 \textmu m. These images also show remarkable coating thickness uniformity in the sol-gel TiO\textsubscript{2}-PDMS-coated microextraction capillaries.
Figure 3.5 Scanning electron microscopic (SEM) images of a 320-µm i.d. fused-silica capillary with sol-gel TiO₂-PDMS coating: (A) cross-sectional view (500×) and (B) surface view (10000×).
3.5.3 Deactivation of the sol-gel TiO₂-PDMS coating

Deactivation of the sol–gel coatings can be expected to take place mainly during thermal conditioning of the capillary, through derivatization of the free hydroxyl groups in the coating structure with HMDS [90] and PMHS [29,91] get incorporated in the sol-gel titania hybrid coating during its formation from the solution. To control the gelation time and to obtain a transparent gel, it was essential to find an optimum ratio (v/v) of HMDS and PMHS. In the present study, this ratio was found to be 4:1 (HMDS:PMHS, v/v).

3.5.4 Fourier transform infrared (FTIR) spectroscopic investigation of the created sol-gel titania sorbent

The formation of Ti-O-Si bonds in the prepared sol-gel sorbent was examined by using a FTIR. The FTIR experiments were performed by passing IR radiation through a thin layer of sol-gel TiO₂-PDMS coating material that was used in the fused-silica capillary. This was done in separate experiments outside the fused-silica capillary. It has been reported [93,94] that a characteristic IR band representing Si-O-Ti bonds is located at 940-960 cm⁻¹. Figure 3.6 shows FTIR spectra of the sol-gel Ti-PDMS coating with a specific band at 952.63 cm⁻¹. This is indicative of the presence of Si-O-Ti bonds in the sol-gel sorbent used in the fused-silica microextraction capillaries to perform on-line CME-HPLC analysis.
Figure 3.6 FTIR spectra of the sol-gel TiO$_2$-PDMS coating.
3.5.5 Applications of sol-gel TiO₂-PDMS-coated microextraction capillary

Sol-gel technology is quite versatile, and allows for the control of coating thickness either by manipulating the reaction time or composition of the sol solution. Zeng and co-workers [65] has recently reported the preparation of 70 µm thick silica-based sol-gel coating on conventional SPME fiber. It should be possible to create such thick coatings (either silica-based or transition metal oxide-based) on the inner surface of fused-silica capillaries as well. Use of thicker coatings should enhance the sample capacity and extraction sensitivity in CME with titania-based sol-gel coatings.

Figure 3.7 presents a chromatogram illustrating CME-HPLC analysis of polycyclic aromatic hydrocarbons (PAHs) (acenaphthylene, fluorene, phenanthrene, and fluoranthene) from an aqueous sample using a sol-gel TiO₂-PDMS-coated microextraction capillary. The extraction was performed on a 40 cm x 0.32 mm i.d. fused-silica microextraction capillary for 40 min using a gravity-fed sample delivery system at room temperature. The concentrations of PAHs were in 20-500 ng/mL range. The extracted PAHs are listed in Table 3.2. The nonpolar nature of the sol-gel TiO₂-PDMS coating showed high affinity and detection limits for these low polar analytes. In this case, the run-to-run peak area repeatability was less than 9.74 % RSD. Detection limits for the extracted PAHs ranged between 0.15 ng/mL for phenanthrene to 3.07 ng/mL for acenaphthylene in conjunction with UV detection.

Figure 3.8 presents a chromatogram illustrating CME-HPLC analysis of moderately polar aromatic ketones extracted from an aqueous sample using a 0.32 mm i.d. sol-gel TiO₂-PDMS-coated microextraction capillary. Compared to PAHs samples, ketones sample with higher analyte concentrations (300 ng/mL – 1 µg/mL) were to be
Figure 3.7 CME-HPLC analysis of PAHs. Extraction conditions: 40 cm × 0.32 mm i.d. x 0.5 µm sol-gel TiO$_2$-PDMS-coated microextraction capillary; extraction time, 40 min (gravity-fed at room temperature). Other conditions: 25 cm x 4.6 mm i.d. ODS column (5 µm d$_p$); gradient elution with mobile phase composition programmed from 80:20 (v/v) ACN/water to 100 % ACN for 20 min; 1 mL/min flow rate; UV detection at 254 nm; ambient temperature. Peaks: (1) acenaphthylene (500 ng/mL), (2) fluorene (100 ng/mL), (3) phenanthrene (20 ng/mL), and (4) fluoranthene (100 ng/mL).
Table 3.2 Physical properties and chemical structures of PAHs used to prepare aqueous samples for CME-HPLC analysis employing a sol-gel TiO$_2$-PDMS-coated microextraction capillary. Data obtained from www.sigmaaldrich.com. Adapted from ref. [95].

<table>
<thead>
<tr>
<th>Name of Compound</th>
<th>MW</th>
<th>mp (°C)</th>
<th>bp (°C)</th>
<th>d (g/mL) at 25 °C</th>
<th>Structure of Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acenaphthylene</td>
<td>154.21</td>
<td>95</td>
<td>279</td>
<td>1.02</td>
<td><img src="image" alt="Acenaphthylene Structure" /></td>
</tr>
<tr>
<td>Fluorene</td>
<td>166.21</td>
<td>116.5</td>
<td>295</td>
<td>1.202</td>
<td><img src="image" alt="Fluorene Structure" /></td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>178.22</td>
<td>101</td>
<td>340</td>
<td>1.179</td>
<td><img src="image" alt="Phenanthrene Structure" /></td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>202.26</td>
<td>105</td>
<td>380</td>
<td>1.252</td>
<td><img src="image" alt="Fluoranthene Structure" /></td>
</tr>
</tbody>
</table>

*MW: Molecular weight; mp: Melting point; bp: Boiling point; d: Density.*
used for CME-HPLC analysis. This may be explained by the nonpolar nature of the sol-gel TiO$_2$-PDMS coating, higher solubility of ketones in water due to higher polarity compared with PAHS, and the working principles of UV detection. In this case, the run-to-run peak area repeatability was less than 7.90 % RSD. Detection limits for the extracted ketones ranged between 2.47 ng/mL for heptanophenone to 11.60 ng/mL for valerophenone in conjunction with UV detection. From the presented results it is evident that sol-gel TiO$_2$-PDMS coating is able to extract both nonpolar and moderately polar analytes with good extraction sensitivity. Such an ability of the used sol-gel coating may be due to the presence of two different types of domains (a nonpolar organic domain based on PDMS and a more polar inorganic domain based on sol-gel titania material) in such coatings [96]. The extracted ketones are listed in Table 3.3.

Figure 3.9 illustrates on-line CME-HPLC analysis of alkylbenzenes using a sol-gel TiO$_2$-PDMS-coated microextraction capillary. Excellent detection limits were also achieved for these analytes (0.65 – 5.45 ng/mL), using UV detection. Like PAHs, alkylbenzenes are less polar analytes than aromatic ketones, and they are well extracted by a sol-gel TiO$_2$-PDMS coating in a microextraction capillary providing low ng/mL and sub-ng/mL level detection limits. The alkylbenzenes extracted from an aqueous sample are listed in Table 3.4.

Table 3.5 summarizes the peak area repeatability and detection limit data for PAHs, ketones, and alkylbenzenes.
Figure 3.8 CME-HPLC analysis of ketones. Extraction conditions: 40 cm × 0.32 mm i.d. x 0.5 μm sol-gel TiO₂-PDMS-coated microextraction capillary; extraction time, 40 min (gravity-fed at room temperature). Other conditions: 25 cm x 4.6 mm i.d. ODS column (5 μm dₚ); gradient elution with mobile phase composition programmed from 80:20 (v/v) ACN/water to 100 % ACN in 15 min; 1 mL/min flow rate; UV detection at 254 nm; ambient temperature. Peaks: (1) butyrophenone (1 μg/mL), (2) valerophenone (1 μg/mL), (3) hexanophenone (500 ng/mL), and (4) heptanophenone (300 ng/mL).
Table 3.3 Physical properties and chemical structures of ketones used to prepare aqueous samples for CME-HPLC analysis employing a sol-gel TiO₂-PDMS-coated microextraction capillary. Data obtained from www.sigmaaldrich.com. Adapted from ref. [95].

<table>
<thead>
<tr>
<th>Name of Compound</th>
<th>MW</th>
<th>mp (°C)</th>
<th>bp (°C)</th>
<th>d (g/mL) at 25 °C</th>
<th>Structure of Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrophenone</td>
<td>148.2</td>
<td>12</td>
<td>221</td>
<td>0.988</td>
<td><img src="structure1.png" alt="Structure" /></td>
</tr>
<tr>
<td>Valerophenone</td>
<td>162.23</td>
<td>-9</td>
<td>106</td>
<td>0.988</td>
<td><img src="structure2.png" alt="Structure" /></td>
</tr>
<tr>
<td>Hexanophenone</td>
<td>176.26</td>
<td>28</td>
<td>265</td>
<td>0.957</td>
<td><img src="structure3.png" alt="Structure" /></td>
</tr>
<tr>
<td>Heptanophenone</td>
<td>190.29</td>
<td>17</td>
<td>155</td>
<td>0.946</td>
<td><img src="structure4.png" alt="Structure" /></td>
</tr>
</tbody>
</table>

*MW: Molecular weight; mp: Melting point; bp: Boiling point; d: Density.
Figure 3.9 CME-HPLC analysis of alkylbenzenes. Extraction conditions: 40 cm × 0.32 mm i.d. x 0.5 μm sol-gel TiO₂-PDMS-coated microextraction capillary; extraction time, 40 min (gravity-fed at room temperature). Other conditions: 25 cm x 4.6 mm i.d. ODS column (5 μm dₚ); gradient elution with mobile phase composition programmed from 80:20 ACN/water to 100 % ACN in 15 min; 1 mL/min flow rate; UV detection at 205 nm; ambient temperature. Peaks: (1) toluene (600 ng/mL), (2) ethylbenzene (200 ng/mL), (3) cumene (50 ng/mL), (4) n-propylbenzene (50 ng/mL), (5) n-butylbenzene (50 ng/mL), and (6) amylbenzene (50 ng/mL).
Table 3.4 Physical properties and chemical structures of alkylbenzenes used to prepare aqueous samples for CME-HPLC analysis employing a sol-gel TiO$_2$-PDMS-coated microextraction capillary. Data obtained from www.sigmaaldrich.com.

<table>
<thead>
<tr>
<th>Name of Compound</th>
<th>MW</th>
<th>mp (°C)</th>
<th>bp (°C)</th>
<th>d (g/mL) at 25 °C</th>
<th>Structure of Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene</td>
<td>92.13</td>
<td>-95</td>
<td>111</td>
<td>0.86</td>
<td><img src="image" alt="Structure of Toluene" /></td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>106.16</td>
<td>-95</td>
<td>136</td>
<td>0.867</td>
<td><img src="image" alt="Structure of Ethylbenzene" /></td>
</tr>
<tr>
<td>Cumene (isopropyl benzene)</td>
<td>120.19</td>
<td>-96</td>
<td>151</td>
<td>0.86</td>
<td><img src="image" alt="Structure of Cumene" /></td>
</tr>
<tr>
<td>n-Propylbenzene</td>
<td>120.19</td>
<td>-99</td>
<td>159</td>
<td>0.862</td>
<td><img src="image" alt="Structure of n-Propylbenzene" /></td>
</tr>
<tr>
<td>n-Butylbenzene</td>
<td>134.22</td>
<td>-88</td>
<td>183</td>
<td>0.86</td>
<td><img src="image" alt="Structure of n-Butylbenzene" /></td>
</tr>
<tr>
<td>Amylbenzene</td>
<td>148.25</td>
<td>-75</td>
<td>205</td>
<td>0.86</td>
<td><img src="image" alt="Structure of Amylbenzene" /></td>
</tr>
</tbody>
</table>

*MW: Molecular weight; mp: Melting point; bp: Boiling point; d: Density.*
Table 3.5 Peak area repeatability and limits of detection (LOD) data for PAHs, ketones, and alkylbenzene obtained in CME-HPLC experiments using sol-gel TiO$_2$-PDMS-coated microextraction capillaries.$^a$

<table>
<thead>
<tr>
<th>Chemical class</th>
<th>Name</th>
<th>Mean peak area (Arbitrary Unit)</th>
<th>RSD (%)</th>
<th>LOD (ng/mL) (S/N = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAH</td>
<td>Acenaphthylene</td>
<td>23.52</td>
<td>9.50</td>
<td>3.07</td>
</tr>
<tr>
<td></td>
<td>Fluorene</td>
<td>12.17</td>
<td>8.87</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td>Phenanthrene</td>
<td>19.91</td>
<td>8.78</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Fluoranthen</td>
<td>21.42</td>
<td>9.74</td>
<td>0.84</td>
</tr>
<tr>
<td>Ketone</td>
<td>Butyrophenone</td>
<td>48.64</td>
<td>3.92</td>
<td>9.62</td>
</tr>
<tr>
<td></td>
<td>Valerophenone</td>
<td>27.72</td>
<td>4.64</td>
<td>11.60</td>
</tr>
<tr>
<td></td>
<td>Hexanophenone</td>
<td>27.91</td>
<td>3.51</td>
<td>4.35</td>
</tr>
<tr>
<td></td>
<td>Heptanophenone</td>
<td>21.60</td>
<td>7.90</td>
<td>2.47</td>
</tr>
<tr>
<td>Alkylbenzene</td>
<td>Toluene</td>
<td>20.28</td>
<td>1.93</td>
<td>5.45</td>
</tr>
<tr>
<td></td>
<td>Ethylbenzene</td>
<td>23.93</td>
<td>1.64</td>
<td>1.24</td>
</tr>
<tr>
<td></td>
<td>Cumene</td>
<td>12.28</td>
<td>6.09</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>n-Propylbenzene</td>
<td>13.62</td>
<td>4.52</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>n-Butylbenzene</td>
<td>14.41</td>
<td>9.93</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>Amylbenzene</td>
<td>9.44</td>
<td>7.43</td>
<td>1.07</td>
</tr>
</tbody>
</table>

$^a$Extraction conditions: 40 cm x 0.32 mm i.d. x 0.5 μm sol-gel TiO$_2$-PDMS-coated microextraction capillary; extraction time: 40 min (gravity-fed at room temperature); HPLC conditions: 25 cm x 4.6 mm i.d. ODS column (5 μm d$_p$); gradient elution from 80:20 (v/v) ACN/water to 100% ACN in 15 min (20 min for PAHs); 1 mL/min flow rate; UV detection at 254 nm (at 205 nm for alkylbenzenes); ambient temperature.
3.5.6 Extraction kinetic profile for sol-gel TiO$_2$-PDMS-coated microextraction capillary

Figure 3.10 illustrates the extraction kinetic profile for: (A) fluorene (nonpolar analyte) and (B) hexanophenone (moderately polar analyte) on a sol-gel TiO$_2$-PDMS-coated microextraction capillary. The microextraction experiments were performed using aqueous samples containing 100 ng/mL and 300 ng/mL concentrations of fluorene and hexanophenone, respectively. Experimental data for the two kinetic profile curves extraction were obtained by individually performing capillary microextraction for each of the solutes. A series of capillary microextraction experiments were conducted to vary the extraction time for each of the two analytes that were extracted from their standard solutions. Three replicate extractions of each analyte were performed for 1, 5, 10, 20, 30, 40, 50, and 60 min. The average peak area was then plotted against the extraction time to obtain Figure 3.10. For both fluorene and hexanophenone, extraction equilibrium was reached within 40 min as is evidenced by the plateau on the extraction curve. Since PDMS has nonpolar characteristics, the TiO$_2$-PDMS coating tends to extract a nonpolar analyte, in this case fluorene, better than a more polar analyte, hexanophenone, which has higher affinity for the aqueous medium.
Figure 3.10 Illustration of the extraction kinetic profile of fluorene (♦), and hexanophenone (●) obtained on a 40 cm × 0.32 mm i.d. x 0.5 μm sol-gel TiO₂-PDMS-coated microextraction capillary using 100 and 300 ng/mL aqueous solutions, respectively. Extraction conditions: 40 cm × 0.32 mm i.d. x 0.5 μm sol-gel TiO₂-PDMS-coated microextraction capillary; extraction time, 40 min (gravity-fed at room temperature). Other conditions: 25 cm x 4.6 mm i.d. ODS column (5 μm dₚ); Isocratic elution 85:15 (v/v), and 90:10 (v/v) acetonitrile/water, respectively; 1 mL/min flow rate; UV detection at 254 nm; ambient temperature.
3.5.7 High pH stability of sol-gel TiO$_2$-PDMS coating

The sol-gel titania-PDMS coatings demonstrated excellent pH stabilities compared with conventionally created coatings like those used in commercial GC capillary columns. Figure 3.11 illustrates the CME performance of a TiO$_2$-PDMS-coated microextraction capillary (250 µm i.d.) in CME-HPLC analysis of PAHs before (Figure 3.11A) and after (Figure 3.11B) rinsing the capillary with a 0.1 M NaOH solution (pH=13) for 12h. Analogously obtained data for a piece of commercial GC column are presented in Figures 3.12A and 3.12B, respectively. Chromatogram 3.11B was obtained on the sol-gel TiO$_2$-PDMS-coated microextraction capillary after it was thoroughly rinsed with deionized water following the NaOH treatment. The extraction of PAHs was performed under the same set of conditions as in the Figure 3.7, except the extraction capillary with 0.25 mm i.d was used instead. From the comparison of peak profiles and peak heights in Figures 3.11A and 3.11B, it is evident that the sol-gel TiO$_2$-PDMS coating in the microextraction capillary remained unaffected even after the prolonged rinsing with 0.1 M NaOH solution at pH 13.

On the other hand, the stationary phase coating in the commercial GC capillary showed significantly less extraction sensitivity as is evident from the comparison of peak heights in Figure 3.12 (A vs. B). It also failed to survive the harsh conditions of rinsing with 0.1 M NaOH solution, which is evidenced by a dramatic decrease in the extraction sensitivity after the NaOH treatment (compare Figures 3.12A obtained before NaOH treatment and 3.12B obtained after NaOH treatment). These results show that a sol-gel TiO$_2$-PDMS-coated microextraction capillary possesses excellent stability under high pH conditions and retains its extraction ability even after being subjected to extreme pH
Figure 3.11 Chromatograms representing CME-HPLC analysis of PAHs using a sol-gel TiO$_2$-PDMS-coated microextraction capillary before (A) and after (B) rinsing the microextraction capillary with a 0.1 M NaOH solution (pH=13) for 12h. Extraction conditions: 40 cm $\times$ 0.25 mm i.d. x 0.25 $\mu$m sol-gel TiO$_2$-PDMS-coated microextraction capillary; extraction time, 40 min (gravity-fed at room temperature). Other conditions: 25 cm x 4.6 mm i.d. ODS column (5 $\mu$m d$_p$); gradient elution with mobile phase composition programmed from 80:20 (v/v) ACN/water to 100 % ACN in 20 min; 1 mL/min flow rate; UV detection at 254 nm; ambient temperature. Peaks: (1) acenaphthylene (500 ng/mL), (2) unknown, (3) phenanthrene (20 ng/mL), and (4) fluoranthene (100 ng/mL).
conditions, while conventionally prepared GC coatings were found to be unstable under such extreme pH conditions [97,98].

Table 3.6 shows repeatability and detection limit data for CME-HPLC analysis using sol-gel TiO$_2$-PDMS-coated microextraction capillaries. For a 0.25 mm i.d. sol-gel TiO$_2$-PDMS microextraction capillary, the RSD value in peak area remained within 9.22 %, and using a UV-detector, detection limits in the range of 0.28 - 5.37 ng/mL were achieved.
Figure 3.12 Chromatograms representing CME-HPLC analysis of PAHs using a segment of commercial PDMS-based GC column as the microextraction capillary: (A) before and (B) after rinsing the microextraction capillary with a 0.1 M NaOH solution (pH=13) for 12h. Extraction conditions: 40 cm × 0.25 mm i.d. × 0.25 µm commercial GC capillary; extraction time, 40 min (gravity-fed at room temperature). Other conditions: 25 cm × 4.6 mm i.d. ODS column (5 µm dₚ); gradient elution with mobile phase composition programmed from 80:20 (v/v) ACN/water to 100 % ACN in 20 min; 1 mL/min flow rate; UV detection at 254 nm; ambient temperature. Peaks: (1) acenaphthylene (500 ng/mL), (2) unknown, (3) phenanthrene (20 ng/mL), and (4) fluoranthene (100 ng/mL).
Table 3.6 Peak area repeatability and limits of detection (LOD) data for PAHs obtained on a sol-gel TiO$_2$-PDMS-coated microextraction capillary before and after treatment with 0.1 M NaOH for 12 h.$^a$

| Extracted PAHs  | Before Rinsing | After Rinsing | % change in peak area (|$A_2$-$A_1$|/$A_1$ x 100%) | LOD (ng/mL) (S/N = 3) |
|-----------------|----------------|---------------|--------------------------------------------------|------------------------|
|                 | Mean Peak Area$^*$ ($A_1$) | RSD (%)       | Mean Peak Area$^*$ ($A_2$) | RSD (%)               |
| Acenaphthylene  | 17.65          | 1.71          | 16.24          | 3.55                  | 5.14                  | 5.37 | 4.39 |
| Phenanthrene    | 13.94          | 6.20          | 14.20          | 1.03                  | 1.44                  | 0.28 | 0.24 |
| Fluoranthene    | 16.52          | 9.22          | 16.20          | 2.29                  | 1.23                  | 1.32 | 1.00 |

*n=3, arbitrary unit, $^a$Extraction conditions: 40 cm x 0.25 mm i.d. x 0.25 µm sol-gel TiO$_2$-PDMS-coated microextraction capillary; extraction time: 40 min (gravity-fed at room temperature); HPLC conditions: 25 cm x 4.6 mm i.d. ODS column (5 µm d$_p$); gradient elution from 80:20 (v/v) ACN/water to 100% ACN in 20 min; 1 mL/min flow rate; UV detection at 254 nm; ambient temperature.
3.5.8 Stability of sol-gel TiO$_2$-PDMS coating under highly acidic conditions

As demonstrated in the previous section, sol-gel titania-PDMS coatings possess excellent stabilities under highly alkaline conditions compared with the PDMS coatings in commercial GC capillary columns. In this section, stability of a TiO$_2$-PDMS-coated microextraction capillary under highly acidic conditions is illustrated. Figures 3.13 shows the CME performance of a sol-gel TiO$_2$-PDMS-coated microextraction capillary (250 µm i.d.) in CME-HPLC analysis of four compounds (butyrophenone, valerophenone, phenanthrene, and pyrene) before (Figure 3.13A) and after (Figure 3.13B) rinsing the capillary with a 0.1 M HCl solution (pH = 1) for 12h. Chromatogram 3.13B was obtained on the sol-gel TiO$_2$-PDMS-coated microextraction capillary after it was thoroughly rinsed with deionized water following the HCl treatment. The extraction of the mixture of ketones and PAHs was performed under the same set of conditions as in the Figure 3.11A. From the comparison of peak profiles and peak heights in Figures 3.13A and 3.13B, it is also evident that the sol-gel TiO$_2$-PDMS coating in the microextraction capillary remained unaffected even after the prolonged rinsing with 0.1 M HCl solution of pH 1. These results again show that a sol-gel TiO$_2$-PDMS-coated microextraction capillary possesses excellent stability under highly acidic conditions and retains its extraction ability under low pH conditions.

Table 3.7 shows peak area repeatability and detection limit data obtained in CME-HPLC analysis using a sol-gel TiO$_2$-PDMS-coated microextraction capillary before and after rinsing with 0.1 M HCl for 12 h. For a 0.25 mm i.d. sol-gel TiO$_2$-PDMS microextraction capillary, the RSD value in peak area remained within 4.09 %, and using UV-detection, detection limits in the range of 0.18-10.45 ng/mL were achieved.
Figure 3.13 Chromatograms representing CME-HPLC analysis of ketones and PAHs using a sol-gel TiO$_2$-PDMS-coated microextraction capillary before (A) and after (B) rinsing the microextraction capillary with a 0.1 M HCl solution (pH=1) for 12h. Extraction conditions: 40 cm $\times$ 0.25 mm i.d. $\times$ 0.25 $\mu$m sol-gel TiO$_2$-PDMS-coated microextraction capillary; extraction time, 40 min (gravity-fed at room temperature). Other conditions: 10 cm x 4.6 mm i.d. Betabasic 8 column (5 $\mu$m d$_p$); isocratic elution with mobile phase composition of 70:30 (v/v) ACN/water; 1 mL/min flow rate; UV detection at 254 nm; ambient temperature. Peaks: (1) butyrophenone (500 ng/mL), (2) valerophenone (500 ng/mL), (3) phenanthrene (10 ng/mL), and (4) pyrene (50 ng/mL).
Table 3.7 CME-HPLC peak area repeatability and limits of detection (LOD) data for a mixture of ketones and PAHs obtained on a sol-gel TiO$_2$-PDMS-coated microextraction capillary before and after treatment with 0.1 M HCl for 12 h.$^a$

<table>
<thead>
<tr>
<th>Extracted ketones and PAHs</th>
<th>Before Rinsing</th>
<th>After Rinsing</th>
<th>% change in peak area</th>
<th>LOD (ng/mL) (S/N = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Peak Area’ ($A_1$)</td>
<td>RSD (%)</td>
<td>Mean Peak Area’ ($A_2$)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>Butyrophenone</td>
<td>6.82</td>
<td>3.32</td>
<td>6.72</td>
<td>3.62</td>
</tr>
<tr>
<td>Valerophenone</td>
<td>4.91</td>
<td>3.87</td>
<td>4.61</td>
<td>4.01</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>7.68</td>
<td>1.47</td>
<td>7.59</td>
<td>1.03</td>
</tr>
<tr>
<td>Pyrene</td>
<td>8.80</td>
<td>2.14</td>
<td>9.03</td>
<td>2.29</td>
</tr>
</tbody>
</table>

$n=3$, arbitrary unit. $^a$Extraction conditions: 40 cm x 0.25 mm i.d. x 0.25 µm sol-gel TiO$_2$-PDMS-coated microextraction capillary; extraction time: 40 min; HPLC conditions: 10 cm x 4.6 mm i.d. Betabasic 8 column (5 µm d$_p$); isocratic elution with mobile phase composition of 70:30 (v/v) ACN/water; 1 mL/min flow rate; UV detection at 254 nm.
3.5.9 Stability of sol-gel TiO₂-PDMS coating in HPLC solvents under elevated temperatures

There are some distinct efficiency advantages in chromatographic separations if high temperature of the eluent is used, and is becoming increasingly accepted as a separation parameter. The effect of temperature has been discussed in the 1970s [99]. HPLC operation under elevated column temperatures increases the mass transfer rates and decreases column back-pressure due to decrease in mobile phase viscosity with increase in temperature, which markedly reduces total analysis time [100]. Antia and Horvath [101] theoretically showed that the optimum velocity of the mobile phase is shifted to higher flow rates as a consequence. Since the diffusion coefficient is proportional to the absolute temperature and inversely proportional to the viscosity according to Stokes-Einstein relationship [102-104], HPLC separations under elevated temperatures allow HPLC operation at higher flow rates and using a longer column for faster analysis and better separation efficiency [105,106]. The Stokes-Einstein relation as the simple hydrodynamic diffusion model can be expressed as following:

\[
D = \frac{k_B T}{6\pi \eta r}
\]  

(3-1)

where,

\(D\): the translational diffusion coefficient for a solute,
\(r\): radius of a solute in a solvent,
\(\eta\): viscosity of a solvent,
\(k_B\): Boltzmann's constant,
\(T\): temperature.

Although the advantages using high temperature in HPLC have been widely discussed [107-109], using high temperature HPLC is limited due to the lack of
temperature-resistant stationary phases. Generally, silica-based stationary phases are not recommended to use at temperatures above 80 °C for a prolonged period [110,111], especially if the aggressive additives are used in the mobile phase such as phosphate buffers, or the column is run at very high or low pH.

Recently, zirconia-based stationary phase [112] was introduced to use as an alternative column packing materials, and it has been shown to be stable in long-term use up to 200 °C [113,114]. The sol-gel zirconia-PDMDPS-coated microextraction capillary [115] was developed by to show excellent pH and temperature stabilities for CME applications.

Chemically bonded sol-gel TiO$_2$-PDMS-coated capillaries demonstrated excellent stabilities under operational conditions involving high temperature HPLC solvent environment. Figure 3.14 shows HPLC chromatograms of CME-HPLC analysis of a mixture of ketones and PAHs before (Figure 3.14A) and after (Figure 3.14B) the microextraction capillary was treated with a mixture of acetonitrile/water (50:50, v/v) at 150 °C for 12h. These results show that the newly developed sol-gel titania and zirconia hybrid CME sorbents may also serve as stable stationary phases in high temperature HPLC operations.

Table 3.8 shows repeatability and detection limit data obtained in CME-HPLC analysis using a sol-gel TiO$_2$-PDMS-coated microextraction capillary. For a 40 cm x 0.25 mm i.d. sol-gel TiO$_2$-PDMS microextraction capillary, the RSD value in peak area remained within 5.01 %, and using a UV-detector, detection limits in the range of 0.10 - 8.62 ng/mL were achieved. In addition, % change in peak area remained within 6.43 %, which demonstrates excellent solvent and temperature stabilities and extraction capability
Figure 3.14 Chromatograms representing CME-HPLC analysis of ketones and PAHs using a sol-gel TiO$_2$-PDMS-coated microextraction capillary before (A) and after (B) the microextraction capillary was filled with the mixture of ACN/water (50/50, v/v), sealed using a mini union connector, and heated at 150 °C for 12h. Extraction conditions: 40 cm $\times$ 0.25 mm i.d. $\times$ 0.25 µm sol-gel TiO$_2$-PDMS-coated microextraction capillary; extraction time, 40 min (gravity-fed at room temperature). Other conditions: 10 cm $\times$ 4.6 mm i.d. Betabasic 8 column (5 µm d$_p$); isocratic elution with mobile phase composition of 70:30 (v/v) ACN/water; 1 mL/min flow rate; UV detection at 254 nm; ambient temperature. Peaks: (1) butyrophenone (500 ng/mL), (2) valerophenone (500 ng/mL), (3) phenanthrene (10 ng/mL), and (4) pyrene (50 ng/mL).
of the sol-gel TiO$_2$-PDMS-coated microextraction capillary.

Sol-gel TiO$_2$-PDMS coating stability at elevated temperature was also studied using 100% of acetonitrile and 100% of methanol. Tables 3.9 (with 100% acetonitrile) and 3.10 (with 100% methanol) show the high temperature solvent stability results using sol-gel TiO$_2$-PDMS-coated capillaries.
Table 3.8 Peak area repeatability and limits of detection (LOD) data for a mixture of ketones and PAHs using a sol-gel TiO$_2$-PDMS-coated microextraction capillary before and after the extraction capillary was filled with the mixture of ACN/water (50/50, v/v) and heated at 150 °C for 12 h.$^a$

| Extracted ketones and PAHs | Before Heating | After Heating | % change in peak area ($|A_2 - A_1|/A_1 \times 100\%$) | LOD (ng/mL) (S/N = 3) |
|----------------------------|---------------|---------------|---------------------------------|-----------------------|
|                            | Mean Peak Area $^*$ ($A_1$) | RSD (%) | Mean Peak Area $^*$ ($A_2$) | RSD (%) | Before Heating | After Heating |
| Butyrophenone | 5.91 | 4.52 | 5.53 | 5.01 | 6.43 | 7.61 | 8.08 |
| Valerophenone | 5.12 | 4.91 | 5.43 | 4.28 | 6.05 | 8.62 | 8.12 |
| Phenanthrene | 7.08 | 2.40 | 6.88 | 2.79 | 2.80 | 0.10 | 0.19 |
| Pyrene | 9.03 | 2.86 | 8.70 | 3.12 | 3.65 | 0.82 | 0.93 |

*$^3$ arbitrary unit, $^a$Extraction conditions: 40 cm x 0.25 mm i.d. x 0.25 µm sol-gel TiO$_2$-PDMS-coated microextraction capillary; extraction time: 40 min (gravity-fed at room temperature); HPLC conditions: 10 cm x 4.6 mm i.d. Betabasic 8 column (5 µm d$_p$); isocratic elution with a mobile phase composition of 70:30 (v/v) ACN/water; 1 mL/min flow rate; UV detection at 254 nm; ambient temperature.
Table 3.9 Peak area repeatability and limits of detection (LOD) data for a mixture of ketones and PAHs using a sol-gel TiO$_2$-PDMS-coated microextraction capillary before and after the extraction capillary was filled with 100% ACN and heated at 150 °C for 12 h.$^a$

<table>
<thead>
<tr>
<th>Extracted ketones and PAHs</th>
<th>Before Heating</th>
<th>After Heating</th>
<th>% change in peak area</th>
<th>LOD (ng/mL) (S/N = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Peak Area$^*$ ($A_1$)</td>
<td>RSD (%)</td>
<td>Mean Peak Area$^*$ ($A_2$)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>Butyrophenone</td>
<td>5.81</td>
<td>5.10</td>
<td>5.32</td>
<td>6.41</td>
</tr>
<tr>
<td>Valerophenone</td>
<td>5.03</td>
<td>5.54</td>
<td>4.73</td>
<td>7.12</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>7.29</td>
<td>2.13</td>
<td>6.84</td>
<td>3.20</td>
</tr>
<tr>
<td>Pyrene</td>
<td>9.11</td>
<td>3.21</td>
<td>8.60</td>
<td>4.04</td>
</tr>
</tbody>
</table>

*n=3, arbitrary unit, $^a$Extraction conditions: 40 cm x 0.25 mm i.d. x 0.25 µm sol-gel TiO$_2$-PDMS-coated microextraction capillary; extraction time: 40 min (gravity-fed at room temperature); HPLC conditions: 10 cm x 4.6 mm i.d. Betabasic 8 column (5 µm d$_p$); isocratic elution with mobile phase composition of 70:30 (v/v) ACN/water; 1 mL/min flow rate; UV detection at 254 nm; ambient temperature.
Table 3.10 Peak area repeatability and limits of detection (LOD) data for a mixture of ketones and PAHs using a sol-gel TiO$_2$-PDMS-coated microextraction capillary before and after the extraction capillary was filled with 100% MeOH and heated at 150 °C for 12 h.$^a$

<table>
<thead>
<tr>
<th>Extracted ketones and PAHs</th>
<th>Before Heating</th>
<th>After Heating</th>
<th>% change in peak area</th>
<th>LOD (ng/mL) (S/N = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Peak Area$^*$ ($A_1$)</td>
<td>Mean Peak Area$^*$ ($A_2$)</td>
<td>$(</td>
<td>A_2-A_1</td>
</tr>
<tr>
<td>Butyrophenone</td>
<td>5.71</td>
<td>5.42</td>
<td>6.93</td>
<td>7.31</td>
</tr>
<tr>
<td>Valerophenone</td>
<td>5.20</td>
<td>4.70</td>
<td>8.00</td>
<td>8.76</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>7.23</td>
<td>6.62</td>
<td>4.91</td>
<td>0.10</td>
</tr>
<tr>
<td>Pyrene</td>
<td>8.92</td>
<td>8.41</td>
<td>5.63</td>
<td>0.81</td>
</tr>
</tbody>
</table>

$n=3$, arbitrary unit, $^a$Extraction conditions: 40 cm x 0.25 mm i.d. x 0.25 µm sol-gel TiO$_2$-PDMS-coated microextraction capillary; extraction time: 40 min (gravity-fed at room temperature); HPLC conditions: 10 cm x 4.6 mm i.d. Betabasic 8 column (5 µm d$_p$); isocratic elution with mobile phase composition of 70:30 (v/v) ACN/water; 1 mL/min flow rate; UV detection at 254 nm; ambient temperature.
3.6 Conclusion

To the best of our knowledge, this is the first report on the creation and use of a sol-gel TiO$_2$-PDMS coating in solid-phase microextraction. Sol-gel TiO$_2$-PDMS-coated microextraction capillaries possess excellent pH stabilities and retain their extraction characteristics intact even after prolonged treatment with highly alkaline (pH=13) NaOH or highly acidic (pH=1) HCl solutions. In addition, sol-gel TiO$_2$-PDMS-coated microextraction capillaries demonstrated excellent temperature and solvent stabilities. Direct chemical bonding of the sol-gel coatings to capillary inner walls provides these coatings with excellent solvent resistance, and makes sol-gel TiO$_2$-PDMS-coated microextraction capillaries very much suitable for on-line sample preconcentration in CME-HPLC analysis. The newly developed sol-gel TiO$_2$-PDMS coating was effectively used for the extraction of different classes of analytes with good extraction sensitivity, and run-to-run repeatability. Low ng/mL and sub-ng/mL level (0.15 ng/mL – 11.60 ng/mL) detection limits were achieved for PAHs, ketones, and alkylbenzenes in CME-HPLC analysis using the newly constructed sol-gel TiO$_2$-PDMS-coated microextraction capillary in conjunction with UV detection. Through proper optimization of experimental conditions for sol-gel coating and the capillary microextraction processes it should be possible to further enhance the extraction sensitivity. For volatile and thermally stable analytes, use of sol-gel TiO$_2$-PDMS-coated capillaries in CME-GC should provide significant enhancement in sensitivity.

3.6 References for Chapter Three


4.1 Introduction

In general, polar analytes such as alcohols, phenols, and carboxylic acids are hydrophilic and show pronounced affinity toward aqueous media. This makes their isolation and preconcentration from an aqueous environment by conventional solid-phase microextraction (SPME) a different analytical problem. For efficient extraction of such hydrophilic analytes from aqueous samples, the SPME coating must possess high polarity. However, it is not an easy task to immobilize polar stationary phases (or sorbent coatings) on silica substrates using conventional techniques [1]. If the SPME coating and the fused-silica fiber are not chemically bonded, as is generally the case in conventionally prepared SPME coatings, they often show low thermal and/or solvent stabilities [2]. Use of such coatings for the extraction of polar analytes from the aqueous sample matrices commonly leads to desorption and sample carryover problems.

In 1995, Pan and co-workers [3] demonstrated the extraction of underivatized short chain volatile fatty acids from aqueous media using headspace SPME (HS-SPME) with in-fiber derivatization on a poly(acrylate) (PA)-coated fiber. Lopes and Augusto [4] prepared the sol-gel-based polydimethylsiloxane/divinylbenzene (PDMS/DVB) sorbent, and reported the improved thermal stability of the composite phase compared to sol-gel
PDMS sorbent. Other types of polymer blends such as Carboxen/polydimethylsiloxane/(CAR/PDMS) [5,6], Carbowax/divinylbenzene (CW/DVB) [6-8], polydimethylsiloxane/Carboxen/divinylbenzene (PDMS/CAR/DVB) [6] have been also used for extraction of highly polar compounds. Yun [9] reported the use of the open crown ether \(\alpha,\omega\)-diallyltriethylene glycol/hydroxyl-terminated silicon oil (DATEG/OH-TSO) as extracting phases in SPME using sol-gel coating technology and cross-linking reaction and showed enhanced extraction efficiency for both polar and nonpolar analytes compared to PDMS and CW/DVB-coated fibers. Liu and co-workers [10] reported the use of hydroxyl-terminated silicon oil-butyl methacrylate-divinylbenzene (OH-TSO-BMA-DVB) copolymers for the extraction of polar alcohols and fatty acids, and nonpolar esters. Recently, Campins-Falco and co-workers [11] reported the use of SPME fiber with a Carbowax-templated resin for on-fiber derivatization of trimethylamine in water and air samples for HPLC analysis. Kulkarni and co-workers [12] developed a sol-gel cyanopropyl-PDMS (CN-PDMS) coating containing highly polar cyanopropyl (CN) and nonpolar poly(dimethylsiloxane) (PDMS) for extraction and preconcentration of both nonpolar and highly polar analytes from aqueous sample media without additional derivatization process.

4.1.1 Polyethylene glycols (PEGs) as sorbent in solid-phase microextraction (SPME)

Extraction of polar compounds from aqueous sample matrices is typically challenging due to the hydrophilic affinity of analytes towards water. Since the hydrophilic nature of PEGs may be a suitable choice for the extraction of polar analytes, high molecular weight PEGs such as Carbowax 20M, were used as composite coatings in SPME [5,7]. However, in SPME-GC applications low thermal stability of PEG fiber
coating was a major shortcoming responsible for incomplete desorption and sample carryover problems.

Recently, Wang and co-workers [13] reported on utilizing Superox-4 (polyethylene glycol, PEG) in sol-gel technology for SPME-GC, and demonstrated many advantageous features over the conventional SPME fibers, including high thermal stability, long life time, and faster mass transfer rate. Malik and co-workers [14,15] reported an effective immobilization of sol-gel PEGs on inner walls of fused-silica capillaries and used these sol-gel PEG coatings as GC stationary phase in separation columns and as extracting media for capillary microextraction (CME also called in-tube SPME). Silva and Augusto [16] prepared SPME fibers with Carbowax 20M-modified Ormosil (organically modified silica) using sol-gel process, and demonstrated superior extraction efficiencies compared with commercial SPME fibers coated with PDMS and CW/DVB. They also mentioned in their paper that keeping the PEG fiber at high temperature for a long period (at 230 °C, 50 hr) improved the precision of analysis evidenced by lower RSD values. This phenomenon was pointed out by Sato and co-workers [17] that the presence of PEGs in sol-gel matrix contributes in controlling pore size distribution to give a porous structure which drastically increases the surface area of the extracting phase.

4.1.2 Applications of low molecular weight PEG, \( N-(\text{triethoxysilylpropyl})-\text{O-} \)polyethylene oxide urethane (TESP-PEO)

Successful immobilization of comparatively high molecular weights polyethylene glycols (PEGs) by sol-gel process has been reported in several papers [13-15]. However, high molecular weight PEGs have lower polarities than their low molecular weight
counterparts, which may limit their suitability for extracting highly polar analytes from aqueous matrices. Recently, \(N\)-(triethoxysilylpropyl)-O-polyethylene oxide urethane (TESP-PEO) was used on silica surfaces to achieve effective surface passivation of microfluidic devices in order to avoid cell adhesion and adsorption to the silicon and glass surfaces via self-assembled monolayer (SAM) [18,19].

For sol-gel CME applications, the presence of sol-gel-active trialkoxysilane groups at one end in TESP-PEO will allow for the creation of sol-gel organic-inorganic hybrid titania coatings on inner walls of fused-silica capillaries. In addition, hydrophilic nature of PEO chains in the sol-gel TiO\(_2\)-SiO\(_2\)-TESP-PEO coatings will allow extracting moderately polar and highly polar analytes from aqueous samples for capillary microextraction hyphenated with GC and HPLC.

4.2 Experimental

4.2.1 Equipment

On-line CME-HPLC experiments with sol-gel TiO\(_2\)-SiO\(_2\)-TESP-PEO-coated capillaries were carried out on a Micro-Tech Scientific (Vista, CA) Ultra Plus HPLC system with a variable wavelength UV detector (Linear UVIS 2000). Off-line CME-GC experiments were performed on a Shimadzu Model 17A GC (Shimadzu, Kyoto, Japan) equipped with flame ionization detection (FID) system and a split-splitless injector. On-line data collection and processing were done using Chrom-Perfect (version 3.5 for Windows) computer software (Justice Laboratory Software, Denville, NJ). An in-house built gas pressure-operated capillary filling/purging device [20] was used to rinse the fused-silica capillary with solvents, fill the extraction capillary with sol solution, expel
the sol solution from the capillary at the end of sol-gel coating process, and purge the capillary with helium. An in-house-designed liquid sample dispenser [14] was used to facilitate gravity-fed flow of aqueous samples through the sol-gel microextraction capillary. A Barnstead model 04741 Nanopure deionized water system (Barnstead/Thermolyne, Dubuque, IA) was used to obtain 16.0 MΩ·cm water. A Fisher model G-560 Vortex Genie 2 system (Fisher Scientific) was used for thorough mixing of the sol-gel ingredient in the coating solution. A Microcentaur model APO 5760 centrifuge (Accurate Chemical and Scientific Corp., Westbury, NY) was used for centrifugation of sol solutions. A Chemcadet model 5984-50 pH meter (Cole-Palmer Instrument Co., Chicago, IL) equipped with a TRIS-specific pH electrode (Sigma-Aldrich, St. Louis, MO) was used to measure the buffer pH. A Nicolet model Avatar 320 FTIR (Thermo Nicolet, Madison, WI) was used for FTIR measurements. A JEOL model JSM-35 scanning electron microscope (SEM) was used for the investigation of surface morphology of the sol-gel TiO₂-SiO₂-TESP-PEO-coated capillaries. A reversed-phase Betasil-C8 (Thermo Electron Co., 12.5 cm x 4.0 mm i.d., 5 µm dₚ) and Betabasic 8 (Thermo Electron Co., 10 cm x 4.6 mm i.d., 5 µm dₚ) columns were used for HPLC separation of the extracted analytes.

4.2.2 Chemicals and materials

Fused-silica capillary (250 µm i.d.) was purchased from Polymicro Technologies Inc. (Phoenix, AZ). HPLC-grade solvents (acetonitrile, dichloromethane, and methanol), Kimwipes, polypropylene microcentrifuge tubes (2.0 mL), and 7.0 mL borosilicate vials (used to store standard solutions) were purchased from Fisher Scientific (Pittsburgh, PA).
Titanium (IV) isopropoxide (99.999 %), 1-butanol (99.4+ %), methyltrimethoxysilane (MTMOS, 98%), trifluoroacetic acid (TFA, 99%), potassium phosphate monobasic (KH$_2$PO$_4$, 99.99%), aromatic aldehydes (p-anisaldehyde, benzaldehyde, 4-isopropylbenzaldehyde), aniline derivatives (benzanilide, acridine, N,N-dimethylaniline, N-butylaniline), substituted phenols (2-chlorophenol, 2-methoxy-4-methylphenol, 4-chloro-3-methylphenol, 4-tert-butylphenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, pentachlorophenol), and free aliphatic fatty acids (octanoic acid, nonanoic acid, decanoic acid, and undecanoic acid), were purchased from Aldrich (Milwaukee, WI). N-(triethoxysilylpropyl)-O-polyethylene oxide urethane (TESP-PEO, 95%) was purchased from Gelest (Morrisville, PA).

4.2.3 Preparation of the sol solution

The sol solution was prepared by thoroughly vortexing the following reagents in a 2-mL polypropylene centrifuge tube: a sol-gel-active organic polymer (N-(triethoxysilylpropyl)-O-polyethylene oxide urethane (TESP-PEO), 50 mg), two sol-gel precursors (titanium (IV) isopropoxide, 25 µL and methyltrimethoxysilane (MTMOS), 50 µL), two solvents (methylene chloride and 1-butanol, 200 µL each), and a sol-gel catalyst/chelating agent (TFA containing 10% H$_2$O, 100 µL). The content of the tube was then centrifuged for 4 min (at 13000 rpm; 15682 x g). Finally the top clear solution was transferred to another clean vial by decantation, and was further used for coating the fused-silica microextraction capillary (250 µm i.d.). The chemical ingredients used in the sol-gel coating solutions are represented in Table 4.1.
Table 4.1 Names, functions, and chemical structures of the coating solution ingredients used to prepare sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillaries.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Function</th>
<th>Chemical structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titanium (IV) isopropoxide</td>
<td>Sol-gel precursor</td>
<td>$\text{Ti} \xrightarrow{(H_3C)_2HCO - OCH(CH_3)_2 - OCH(CH_3)_2}$</td>
</tr>
<tr>
<td>Methyltrimethoxysilane (MTMOS)</td>
<td>Sol-gel precursor</td>
<td>$\text{Si} \xrightarrow{\text{OCH}_3 - \text{OCH}_3}$</td>
</tr>
<tr>
<td>$N$-(triethoxysilylpropyl)-O-polyethylene oxide urethane (TESP-PEO)</td>
<td>Sol-gel-active polymer</td>
<td>$\text{NH} \xrightarrow{\text{O}} \text{C} \xrightarrow{\text{O}} \text{O} \xrightarrow{(\text{CH}_2\text{CH}<em>2\text{O})</em>{4-6}\text{H}} \text{Si} \xrightarrow{\text{O}} \text{C}_2\text{H}_5 \xrightarrow{\text{OC}_2\text{H}_5}$</td>
</tr>
<tr>
<td>Trifluoroacetic acid (TFA)</td>
<td>Catalyst/Bridging (chelating) ligand</td>
<td>$\text{HO} \xrightarrow{\text{CF}_3}$</td>
</tr>
</tbody>
</table>
4.2.4 Preparation of sol-gel TiO₂-SiO₂-TESP-PEO-coated microextraction capillary

A 60-cm long hydrothermal treated [15,21] fused-silica capillary (250 µm i.d.) was installed on an in-house built gas pressure-operated capillary filling/purging device [20] (Figure 2.4 in Chapter 2), and the capillary was filled with the prepared sol solution under 10 psi helium pressure. After filling, the sol solution was kept inside the capillary for 15 min to facilitate the creation of a surface-bonded coating due to sol-gel reactions taking place in the coating and on the capillary inner surface. Following this, the unbonded portion of the sol solution was expelled from the capillary under helium pressure (20 psi), leaving behind a surface-bonded sol-gel coating on the inner surface of the fused-silica capillary. The capillary was further purged and dried with helium for 30 min under the same pressure. The coated microextraction capillary was then installed and conditioned in a GC oven by temperature-programmed heating from 40 °C to 300 °C at 1 °C/min under helium purge. The capillary was held at 300 °C for 60 min. Finally, the capillary was cooled down to room temperature and rinsed with methylene chloride and methanol (3 mL each). Following this, the capillary was installed in the GC oven for drying and further thermal conditioning under temperature-programmed heating as described above, with exception that this time the capillary was held at the final temperature for 30 min. The conditioned capillary was then cut into 40-cm and 12-cm long pieces that were further used in CME-HPLC and CME-GC analyses, respectively.

4.2.5 Capillary microextraction (CME) and on-line CME-HPLC analysis

Selected analytes were prepared in methanol (10 mg/mL) and stored in surface-deactivated amber glass vials for use as stock solutions. Each stock solution was further
diluted 100 times (100 µg/mL) in methanol, and stored in separate surface-deactivated amber glass vials before to prepare the aqueous sample solutions. For extraction, fresh aqueous samples were prepared by further diluting these stock solutions in deionized water to 1 µg/mL or lower concentrations. A schematic of the CME-HPLC setup for on-line capillary microextraction and HPLC analysis is presented in Figure 3.4 in Chapter 3. The HPLC column was pre-equilibrated with selected mobile phase consisting of a mixture of acetonitrile and water (or buffer solution), for example. A 40-cm segment of the sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillary was mounted on the HPLC injection port as an external sampling loop. Analytes were preconcentrated in the sol-gel TiO$_2$-SiO$_2$-TESP-PEO coating by passing the aqueous sample from a gravity-fed dispenser [14] through this sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillary for 40 min. The analytes extracted in the sol-gel TiO$_2$-SiO$_2$-TESP-PEO coating of the sampling loop were then transferred into the HPLC column by desorbing with the mobile phase. This was accomplished by simply switching the injection valve from the “load” to “inject” position. The injected analytes were then separated on the HPLC analysis column under isocratic elution conditions until all peaks representing each analyte were eluted, typically in 10 or 15 minutes.

4.2.6 Off-line CME-GC analysis

Selected free fatty acid samples and stock solutions were prepared as described earlier. For extraction, fresh aqueous samples were prepared by further diluting the stock solutions in deionized water to 1 µg/mL or lower concentrations. A Chromaflex AQ column (Knotes Glass, Vineland, NJ) was modified as described in Figure 4.1 and used for gravity-fed sample delivery in capillary microextraction. A 12-cm long piece of
thermally conditioned sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillary (250 µm i.d.) was vertically connected to the lower end of the sample dispenser. The aqueous sample containing trace amounts of fatty acids was poured into the dispenser from its top end and allowed to flow through the microextraction capillary under gravity. The extraction was carried out for 40 min for equilibrium to be established. The capillary was then detached from the dispenser and the residual sample droplets were removed by touching one of the ends of microextraction capillary with Kimwipe tissue. After this, the capillary was installed in the GC injection port, keeping ~3 cm of its lower end protruding into the GC oven. This end was then interfaced with the inlet of a GC capillary column using a deactivated two-way press–fit quartz connector. Under splitless conditions, the extracted analytes were then thermally desorbed from the capillary by rapidly raising the temperature of the injection port (from 30 °C to 300 °C at 60 °C/min), while keeping the GC oven temperature at 35 °C. Such a rapid temperature program of the injection port facilitated effective desorption of the extracted analytes from the sol-gel TiO$_2$-SiO$_2$-TESP-PEO microextraction capillary and their focusing at the inlet of the GC analysis column. Following this, the GC oven was temperature programmed from 35 °C to 300 °C at rate of 20 °C/min to achieve separation of the focused analytes on the GC column. A flame ionization detector (FID) maintained at 350 °C was used for analyte detection.
Figure 4.1 Gravity-fed sample delivery system (1.0 x 60 cm, Glass barrel only) for capillary microextraction. Adapted from ref. [22].
4.2.7 Safety precautions

The presented work involved the use of various chemicals (organic and inorganic precursors) and solvent that might be environmentally hazardous with adverse health effects. Proper safety measures were taken in handling organic solvents such as methanol, methylene chloride, and acetonitrile. All chemicals once used, were disposed of in the proper waste containers to ensure personnel and environmental safety.

4.3 Results and Discussion

The main purpose of this research was to develop surface-bonded sol-gel titania-based polar coatings to facilitate effective extraction of polar analytes in aqueous sample matrices as well as hyphenation of capillary microextraction (CME) with HPLC. Sol-gel chemistry allowed us to create a surface-bonded hybrid organic-inorganic titania coating of desired properties on the inner walls of a fused-silica capillary in CME. As a versatile tool, sol-gel technology has been effectively utilized to create surface-bonded coatings on the outer surface of conventional SPME fibers [23] as well as on the inner walls of fused-silica capillary for use in CME (or in-tube SPME) [14,24-26]. In the present work, sol-gel chemistry was utilized to create a hybrid coating based on titania and a low molecular weight TESP-PEO on the inner surface of fused-silica capillary for efficient extraction and CME-HPLC analysis of trace amounts of polar analytes from aqueous samples.

4.3.1 Sol-gel reactions for the preparation of sol-gel TiO$_2$-SiO$_2$-TESP-PEO coating

As described earlier in Chapter 3, chemical reactivity and complex-forming ability of titanium alkoxides differ from silicon alkoxides. It is well-known that silicon alkoxides are capable of undergoing hydrolytic polycondensation reactions in the
presence of a sol-gel catalyst [27]. However, compared with silica-based systems, titania-based sol-gel reactions are much faster [28] and often require a chelating (or bridging) agent to control and decelerate the sol-gel process [29]. Because of this, developing titania-based organic-inorganic hybrid materials usually requires the use of the optimum ratio of catalyst (or inhibitor) and water. Otherwise, the gelation or precipitation often takes place instantaneously as the sol-gel solution ingredients are mixed together, especially in hybrid systems. In this research, trifluoroacetic acid [30] was used as chelating (or bridging) agents for titanium alkoxide, but catalyst for silicon alkoxides.

In the present work, sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated capillaries were prepared through hydrolytic polycondensation reactions performed within fused-silica capillaries followed by thermal conditioning of the created coatings to achieve fine surface structures. The N-(triethoxysilylpropyl)-O-polyethylene oxide urethane (TESP-PEO) ligand possesses polar polyethylene oxide repeating units at one end and sol-gel-active triethoxysilane groups at the other. Via sol-gel active groups, TESP-PEO was chemically bonded to the other sol-gel precursors as well as to the inner surface of the fused-silica capillary. The sol-gel process for the generation and chemical immobilization of the coating involves: (A) hydrolysis of the titanium alkoxide precursor [31] and the alkoxy silane compounds, MTMOS and TESP-PEO (Scheme 4.1), (B) polycondensation and chemical incorporation of the hydrolysis products into a three-dimensional sol-gel network [32-35] (Scheme 4.2), and (C) chemical anchoring of the sol-gel hybrid polymer to the inner walls of the fused-silica capillary [32,33] (Scheme 4.3).
Scheme 4.1 (A) Hydrolysis of titanium (IV) isopropoxide and the alkoxy silane compounds.
Scheme 4.2 (B) Polycondensation and chemical incorporation of the hydrolysis products with the evolving sol-gel network.
Scheme 4.3 (C) Chemical anchoring of the sol-gel TiO$_2$-SiO$_2$ hybrid polymer to the inner walls of the capillary.
4.3.2 Scanning electron microscopy (SEM) of sol-gel titania-silica-TESP-PEO coatings bonded to the inner surface of a fused-silica capillary

Figure 4.2 represents the scanning electron micrograph showing the side view of a 250-µm i.d. fused-silica capillary with sol-gel TiO$_2$-SiO$_2$-TESP-PEO coating with 10000x magnification. As is evident from these images, the sol-gel TiO$_2$-SiO$_2$-TESP-PEO coating in the microextraction capillary acquires a fine and thin coating structure, providing remarkable capability of sorption with such a thin coating. Based on the SEM data, the thickness of the sol-gel TiO$_2$-SiO$_2$-TESP-PEO coating was estimated at 0.2 µm. This image also shows uniformity of coating thickness in a consistent way in the sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillaries.
Figure 4.2 Scanning electron microscopic image of a 250-µm i.d. fused-silica capillary with sol-gel TiO$_2$-SiO$_2$-TESP-PEO coating: side view (10000×).
4.3.3 Fourier transform infrared (FTIR) spectroscopy of the sol-gel titania-silica TESP-PEO surface

The formation of Ti-O-Si and Si-O-Si bonds in the prepared sol-gel sorbent was examined by FTIR by performing separate experiments outside the fused-silica capillary. The FTIR experiments were performed by passing IR radiation through a thin layer of sol-gel TiO₂-SiO₂-TESP-PEO coating material that was used in the fused-silica capillary. It has been reported that the characteristic IR band representing Si-O-Ti bonds is located at 940-960 cm⁻¹ [36,37], and Si-O-Si bonds is located at 1000-1200 cm⁻¹ [37-40]. Figure 4.3 shows a FTIR spectrum of the sol-gel Ti-TESP-PEO coating with a specific band at 946.61 cm⁻¹ for Si-O-Ti bonds and 1070 cm⁻¹ for Si-O-Si bonds. A band for Si-O-Si may overlap with existing C-O-C band which is also located at 1000-1300 cm⁻¹ [41,42]. The FTIR result is indicative of the presence of both Si-O-Ti and Si-O-Si bonds in the sol-gel sorbent used in the fused-silica microextraction capillaries to perform on-line CME-HPLC analysis.
Figure 4.3 FTIR spectrum of the sol-gel TiO$_2$-SiO$_2$-TESP-PEO coating.
4.3.4 Applications of sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillary

Sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated capillaries showed excellent affinity for the polar analytes in the aqueous phase, thus enabled the extraction of analytes belonging to moderately or highly polar chemical classes, such as aldehydes, anilines, phenols, and fatty acids.

Due to the environmental and toxicological significance, analysis of aldehydes is important in general, and in aquatic and atmospheric oxidation processes, in particular. More recently, low molecular mass of aldehydes have been found to be major organic by-products in disinfection, oxidation, and ozonation of natural waters to produce drinking water [43,44]. In addition, aromatic aldehyde products are important intermediate of pharmaceuticals [45], pesticides [46], and dyestuffs [47].

Figure 4.4 illustrates a chromatogram illustrating CME-HPLC analysis of aromatic aldehydes (p-anisaldehyde, benzaldehyde, 4-isopropylbenzaldehyde) using a sol-gel coated TiO$_2$-SiO$_2$-TESP-PEO microextraction capillary. The extraction was performed on a 40 cm x 0.25 mm i.d. fused-silica microextraction capillary for 40 min using a gravity-fed sample delivery system at room temperature. The concentrations of aldehydes were in 50-500 ng/mL range. The polar nature of the sol-gel TiO$_2$-SiO$_2$-TESP-PEO coating showed good affinity and detection limits for moderately polar analytes. In this case, the run-to-run peak area repeatability was less than 6.91 % RSD. Detection limits for the extracted aldehydes ranged between 0.98 ng/mL for 4-isopropylbenzaldehyde to 11.92 ng/mL for p-anisaldehyde in conjunction with UV detection. The aldehydes extracted from an aqueous sample are listed in Table 4.2.

Aromatic amines are commonly used as intermediates in the photographic,
pesticide, dye, and pharmaceutical industries [48-50]. Aromatic amines are also found in air, water and soil [51,52], and many of them have been classified as mutagenic and carcinogenic [53]. Therefore, detection of trace-level contents of aromatic amines in the environment and in drinking water is very important.

Figure 4.5 presents a chromatogram illustrating CME-HPLC analysis of aniline derivatives extracted from an aqueous sample using a 0.25 mm i.d. sol-gel coated TiO$_2$-SiO$_2$-TESP-PEO capillary. Compared to moderately polar aldehyde samples, aniline derivatives needed lower analyte concentrations (50 ng/mL - 300 ng/mL) for CME-HPLC analysis. For aromatic amine compounds, the run-to-run peak area repeatability was less than 6.08 % RSD. Detection limits for the extracted anilines ranged from 0.53 ng/mL for acridine to 3.48 ng/mL for $N,N$-dimethylaniline in conjunction with UV detection. From the presented results it is evident that sol-gel TiO$_2$-SiO$_2$-TESP-PEO coating is able to extract moderately polar analytes with good extraction sensitivity. The extracted ketones from an aqueous sample are listed in Table 4.3.

Chlorophenols are present as important group of highly toxic pollutants [54]. Chlorophenols have been widely used in various applications such as preservatives, pesticides, antiseptics, and disinfectants [55]. They are often found in waters [56,57], soils, and sediments [58] and are formed as a result of hydrolysis, oxidation, and microbiological degradation of chlorinated pesticides. Chlorophenols are environmental important compounds.
Figure 4.4 CME-HPLC analysis of aldehydes. Extraction conditions: 40-cm × 0.25 mm i.d. x 0.2 µm sol-gel TiO₂-SiO₂-TEPS-PEO-coated microextraction capillary; extraction time, 40 min (gravity-fed at room temperature). Other conditions: 10 cm x 4.6 mm i.d. Betabasic 8 column (5 µm dₚ); isocratic elution with mobile phase composition of 70:30 (v/v) ACN/water; 1 mL/min flow rate; UV detection at 214 nm; ambient temperature. Peaks: (1) p-anisaldehyde (500 ng/mL), (2) benzaldehyde (500 ng/mL), and (3) 4-isopropylbenzaldehyde (50 ng/mL).
Table 4.2 Physical properties and chemical structures of aldehydes extracted from an aqueous sample using a sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillary.

Data obtained from www.sigmaaldrich.com.

<table>
<thead>
<tr>
<th>Name of Compound</th>
<th>MW</th>
<th>mp (°C)</th>
<th>bp (°C)</th>
<th>d (g/mL) at 25 °C</th>
<th>Structure of Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p$-Anisaldehyde</td>
<td>136.15</td>
<td>-1</td>
<td>248</td>
<td>1.12</td>
<td><img src="image" alt="p-Anisaldehyde Structure" /></td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>106.12</td>
<td>-26</td>
<td>178.1</td>
<td>1.0415</td>
<td><img src="image" alt="Benzaldehyde Structure" /></td>
</tr>
<tr>
<td>4-Isopropyl-benzaldehyde</td>
<td>148.21</td>
<td>N/A</td>
<td>234</td>
<td>0.977</td>
<td><img src="image" alt="4-Isopropyl-Benzaldehyde Structure" /></td>
</tr>
</tbody>
</table>

*MW: Molecular weight; mp: Melting point; bp: Boiling point; d: Density.*
Figure 4.5 CME-HPLC analysis of aniline derivatives. Extraction conditions: 40-cm × 0.25 mm i.d. x 0.2 µm sol-gel TiO₂-TEPS-PEO-coated microextraction capillary; extraction time, 40 min (gravity-fed at room temperature). Other conditions: 10 cm x 4.6 mm i.d. Betabasic 8 column (5 µm dₚ); isocratic elution with mobile phase composition of 60:40 (v/v) ACN/water; 1 mL/min flow rate; UV detection at 254 nm; ambient temperature. Peaks: (1) benzanilide (70 ng/mL), (2) acridine (50 ng/mL), (3) N,N-dimethylaniline (300 ng/mL), and (4) N-butylaniline (70 ng/mL).
**Table 4.3** Physical properties and chemical structures of aniline derivatives extracted from an aqueous sample using a sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillary. Data obtained from www.sigmaaldrich.com.

<table>
<thead>
<tr>
<th>Name of Compound</th>
<th>MW</th>
<th>mp (°C)</th>
<th>bp (°C)</th>
<th>pK$_a$</th>
<th>Structure of Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzanalide</td>
<td>197.24</td>
<td>162</td>
<td>117</td>
<td>13.52</td>
<td><img src="image1" alt="Structure of Compound" /></td>
</tr>
<tr>
<td>Acridine</td>
<td>179.22</td>
<td>108</td>
<td>346</td>
<td>5.60</td>
<td><img src="image2" alt="Structure of Compound" /></td>
</tr>
<tr>
<td>N,N-Dimethylaniline</td>
<td>121.18</td>
<td>2</td>
<td>193</td>
<td>4.70</td>
<td><img src="image3" alt="Structure of Compound" /></td>
</tr>
<tr>
<td>N-Butylaniline</td>
<td>149.24</td>
<td>-14.4</td>
<td>239</td>
<td>5.05</td>
<td><img src="image4" alt="Structure of Compound" /></td>
</tr>
</tbody>
</table>

*MW: Molecular weight; mp: Melting point; bp: Boiling point.*
Figure 4.6 illustrates on-line CME-HPLC analysis of substituted phenols using a TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillary. A 50 mM of potassium biphosphate buffer (UV cutoff: < 200 nm (0.1 %)) solution was prepared, and then pH was adjusted to 7 by adding a concentrated NaOH solution to control the pH of the mobile phase based on $pK_a$ of each phenolic sample. Low ng/mL level detection limits were also achieved for these analytes (11.61 – 22.34 ng/mL), using UV detection. Unlike moderately polar analytes, such as aldehydes and anilines, substituted phenols are highly polar compounds and they favor the aqueous media. However, a sol-gel TiO$_2$-SiO$_2$-TESP-PEO extraction capillary can successfully compete with the aqueous media towards polar analytes and extract the phenols. The extracted substituted phenols from an aqueous sample are listed in Table 4.4.

Figure 4.7 illustrates other example of on-line CME-HPLC analysis of substituted phenols using a TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillary. Acceptable detection limits were also achieved for these analytes (23.20 – 50.18 ng/mL) using UV detection. The extracted substituted phenols from an aqueous sample are listed in Table 4.5.

Fatty acids are carboxylic acids with a long hydrocarbon chain, generally straight, which are important key metabolites and intermediates in biological processes [59]. Especially a high level of saturated fatty acids in the diet raises blood cholesterol levels. Due to the hydrophilic characteristics of underivatized short-chain fatty acids, extraction of fatty acids, especially in aqueous sample matrices, is extremely challenging. Here, sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillaries demonstrated effective extraction of underivatized fatty acids in aqueous sample media. Due to the lack of
Figure 4.6 CME-HPLC analysis of substituted phenols. Extraction conditions: 40-cm × 0.25 mm i.d. x 0.2 µm sol-gel TiO₂-TEPS-PEO-coated microextraction capillary; extraction time, 40 min (gravity-fed at room temperature). Other conditions: 12.5 cm x 4.0 mm i.d. Betasil-C8 column (5 µm dₚ); isocratic elution with mobile phase composition of 60:40 ACN/KH₂PO₄ (50 mM, pH=7); 1 mL/min flow rate; UV detection at 280 nm; ambient temperature. Peaks: (1) pentachlorophenol (600 ng/mL), (2) 2,4,6-trichlorophenol (600 ng/mL), (3) 2-chlorophenol (1.0 µg/mL), and (4) 2,4-dichlorophenol (1.5 µg/mL).
Table 4.4 Physical properties and chemical structures of substituted phenols extracted from an aqueous sample using a sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillary. Data obtained from www.sigmaaldrich.com.

<table>
<thead>
<tr>
<th>Name of Compound</th>
<th>MW</th>
<th>mp ($^\circ$C)</th>
<th>bp ($^\circ$C)</th>
<th>pK$_a$</th>
<th>Structure of Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentachlorophenol</td>
<td>266.34</td>
<td>175</td>
<td>310</td>
<td>4.92</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>197.45</td>
<td>65</td>
<td>246</td>
<td>6.23</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>128.56</td>
<td>8</td>
<td>175</td>
<td>8.52</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>163.00</td>
<td>42</td>
<td>209</td>
<td>7.85</td>
<td><img src="image" alt="Structure" /></td>
</tr>
</tbody>
</table>

*MW: Molecular weight; mp: Melting point; bp: Boiling point; d: Density.*
Figure 4.7 CME-HPLC analysis of other substituted phenols. Extraction conditions: 40-cm × 0.25 mm i.d. x 0.2 µm sol-gel TiO₂-TEPS-PEO-coated microextraction capillary; extraction time, 40 min (gravity-fed at room temperature). Other conditions: 12.5 cm x 4.0 mm i.d. Betasil-C8 column (5 µm dₚ); isocratic elution with mobile phase composition of 65:35 ACN/KH₂PO₄ (25 mM, pH=8); 1 mL/min flow rate; UV detection at 280 nm; ambient temperature. Peaks: (1) 2-methoxy-4-methylphenol (5.0 µg/mL), (2) 4-chloro-3-methylphenol (3.0 µg/mL), and (3) 4-tert-butylphenol (3.0 µg/mL).
Table 4.5 Physical properties and chemical structures of substituted phenols extracted from an aqueous sample using a sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillary. Data obtained from www.sigmaaldrich.com.

<table>
<thead>
<tr>
<th>Name of Compound</th>
<th>MW</th>
<th>mp (°C)</th>
<th>bp (°C)</th>
<th>pK$_a$</th>
<th>Structure of Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-methoxy-4-methylphenol</td>
<td>138.17</td>
<td>5</td>
<td>221</td>
<td>10.27</td>
<td></td>
</tr>
<tr>
<td>4-chloro-3-methylphenol</td>
<td>142.59</td>
<td>64</td>
<td>235</td>
<td>9.549</td>
<td></td>
</tr>
<tr>
<td>4-tert-butylphenol</td>
<td>150.22</td>
<td>98</td>
<td>237</td>
<td>10.43</td>
<td></td>
</tr>
</tbody>
</table>

*MW: Molecular weight; mp: Melting point; bp: Boiling point; d: Density.
sensitive chromophores in aliphatic free fatty acids, CME-GC experiment was performed instead.

Figure 4.8 illustrates on-line CME-GC analysis of fatty acids using. Using a TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillary for CME-GC with FID detection enabled to achieve excellent detection limits for these analytes (2.26 – 6.76 ng/mL). Free fatty acids are highly polar analytes and not easy to extract if they are in aqueous media, however they were well extracted by using a sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated extraction capillary with low ng/mL level detection limits. The free fatty acids extracted from an aqueous sample are listed in Table 4.6.

Table 4.7 summarizes the peak area repeatability and detection limit data for aldehydes, substituted phenols, aromatic amines, and free fatty acids.
Figure 4.8 CME-GC analysis of fatty acids. Extraction conditions: 12-cm × 0.25 mm i.d. x 0.2 μm sol-gel TiO₂-TEPS-PEO-coated microextraction capillary; extraction time, 40 min (gravity-fed at ambient temperature). Other conditions: 5 m × 250 μm i.d. sol–gel PDMS column; splitless injection; injector temperature: initial 30 °C, final 300 °C, programmed at a rate of 60 °C/min; GC oven temperature programmed from 35 °C to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) octanoic acid (500 ng/mL), (2) nonanoic acid (200 ng/mL), (3) decanoic acid (150 ng/mL), and (4) undecanoic acid (150 ng/mL).
Table 4.6 Physical properties and chemical structures of fatty acids extracted from an aqueous sample using a sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillary.

Data obtained from www.sigmaaldrich.com.

<table>
<thead>
<tr>
<th>Name of Compound</th>
<th>MW</th>
<th>mp (°C)</th>
<th>bp (°C)</th>
<th>d (g/mL)</th>
<th>Structure of Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octanoic acid</td>
<td>144.21</td>
<td>16</td>
<td>237</td>
<td>0.91</td>
<td>CH$_3$(CH$_2$)$_5$CH$_2$COOH</td>
</tr>
<tr>
<td>Nonanoic acid</td>
<td>158.24</td>
<td>9</td>
<td>268</td>
<td>0.906</td>
<td>CH$_3$(CH$_2$)$_6$CH$_2$COOH</td>
</tr>
<tr>
<td>Decanoic acid</td>
<td>172.26</td>
<td>30</td>
<td>270</td>
<td>0.893</td>
<td>CH$_3$(CH$_2$)$_7$CH$_2$COOH</td>
</tr>
<tr>
<td>Undecanoic acid</td>
<td>186.29</td>
<td>29</td>
<td>249</td>
<td>0.86</td>
<td>CH$_3$(CH$_2$)$_8$CH$_2$COOH</td>
</tr>
</tbody>
</table>

*MW: Molecular weight; mp: Melting point; bp: Boiling point; d: Density.
Table 4.7 Peak area repeatability and limits of detection (LOD) data for aldehydes, aniline derivatives, and substituted phenols in CME-HPLC, and fatty acids in CME-GC using a sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillary.

<table>
<thead>
<tr>
<th>Chemical class</th>
<th>Name</th>
<th>Peak area repeatability (n = 3)</th>
<th>LOD (ng/mL) (S/N = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean peak area (Arbitrary Unit)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>Aldehydes</td>
<td>$p$-Anisaldehyde</td>
<td>11.32</td>
<td>6.91</td>
</tr>
<tr>
<td></td>
<td>Benzaldehyde</td>
<td>14.31</td>
<td>5.92</td>
</tr>
<tr>
<td></td>
<td>4-Isopropylbenzaldehyde</td>
<td>13.69</td>
<td>4.84</td>
</tr>
<tr>
<td>Anilines</td>
<td>Benzanilide</td>
<td>22.54</td>
<td>5.70</td>
</tr>
<tr>
<td></td>
<td>Acridine</td>
<td>25.30</td>
<td>4.22</td>
</tr>
<tr>
<td></td>
<td>$N,N$-Dimethylaniline</td>
<td>23.22</td>
<td>4.78</td>
</tr>
<tr>
<td></td>
<td>$N$-Butylaniline</td>
<td>15.43</td>
<td>6.08</td>
</tr>
<tr>
<td>Phenols</td>
<td>Pentachlorophenol</td>
<td>13.90</td>
<td>7.62</td>
</tr>
<tr>
<td></td>
<td>2,4,6-Trichlorophenol</td>
<td>13.82</td>
<td>7.14</td>
</tr>
<tr>
<td></td>
<td>2-Chlorophenol</td>
<td>16.89</td>
<td>6.86</td>
</tr>
<tr>
<td></td>
<td>2,4-Dichlorophenol</td>
<td>18.11</td>
<td>5.60</td>
</tr>
<tr>
<td></td>
<td>2-Methoxy-4-methylphenol</td>
<td>26.87</td>
<td>4.11</td>
</tr>
<tr>
<td></td>
<td>4-Chloro-3-methylphenol</td>
<td>34.91</td>
<td>3.40</td>
</tr>
<tr>
<td></td>
<td>4-tert-Butylphenol</td>
<td>34.11</td>
<td>3.02</td>
</tr>
<tr>
<td>Fatty acids$^*$</td>
<td>Octanoic acid</td>
<td>20.03</td>
<td>1.78</td>
</tr>
<tr>
<td></td>
<td>Nonanoic acid</td>
<td>18.37</td>
<td>1.93</td>
</tr>
<tr>
<td></td>
<td>Decanoic acid</td>
<td>15.72</td>
<td>2.44</td>
</tr>
<tr>
<td></td>
<td>Undecanoic acid</td>
<td>17.88</td>
<td>1.76</td>
</tr>
</tbody>
</table>

$^a$Extraction conditions: 40-cm (12-cm for fatty acids$^*$) x 0.25 mm i.d. x 0.2 µm sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillary; extraction time, 40 min (gravity-fed at ambient temperature).
4.3.5 Extraction kinetic profile of sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillary

Figure 4.9 illustrates the extraction kinetic profile for: (A) acridine and (B) 2-chlorophenol on a sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillary. Experimental data for these curves representing extraction kinetic profiles were obtained by individually performing capillary microextraction for each of the solutes. The microextraction experiments were performed using aqueous samples containing 25 ng/mL and 500 ng/mL concentrations of acridine and 2-chlorophenol, respectively. A series of capillary microextraction experiments were conducted to vary the extraction time for each of the two analytes that were extracted from their standard solutions. Three replicate extractions of each analyte were performed for 1, 5, 10, 20, 30, 40, 50, and 60 min. The average HPLC peak area was then plotted against the extraction time to obtain Figure 4.8. For both acridine and 2-chlorophenol, extraction equilibrium was reached within 40 min as is evidenced by the plateau on the extraction curve.
Figure 4.9 Illustration of the extraction kinetic profile of acridine (♦), and 2-chlorophenol (●) obtained on a 40 cm x 0.25 mm i.d. x 0.2 µm sol-gel TiO₂-SiO₂-TESP-PEO-coated microextraction capillary using 25 and 500 ng/mL aqueous solutions, respectively. Extraction conditions are the same as in the Figure 4.4. Other conditions: 10 cm x 4.6 mm i.d. Betabasic 8 column (5 µm dₚ); isocratic elution with mobile phase composition of 60:40 (v/v) ACN/water for acridine: 12.5 cm x 4.0 mm i.d. Betasil-C8 column (5 µm dₚ); isocratic elution with mobile phase composition of 60:40 ACN/KH₂PO₄ (50 mM, pH=7) for 2-chlorophenol; 1 mL/min flow rate; UV detection at 254 nm; ambient temperature.
4.4 Conclusion

This is the first report on the creation and use of a sol-gel TiO$_2$-SiO$_2$-TESP-PEO coating in solid-phase microextraction to extract various classes of polar compounds. Sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillaries possess excellent extraction capability for highly polar analytes. Direct chemical bonding of the coating to capillary inner walls provides these coatings with excellent solvent resistance, and makes sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated capillaries very much suitable for on-line sample preconcentration in CME-HPLC analysis. The newly developed sol-gel TiO$_2$-SiO$_2$-TESP-PEO coating was effectively used for the extraction of different classes of analytes with good extraction sensitivity, and run-to-run repeatability in CME-HPLC and CME-GC analyses. Low ng/mL level detection limits were achieved for aldehydes, aniline derivatives, and substituted phenols in CME-HPLC-UV, and free fatty acids in CME-GC-FID analyses using the newly constructed sol-gel TiO$_2$-SiO$_2$-TESP-PEO-coated microextraction capillaries. Through optimization of experimental conditions for sol-gel coating procedure and the capillary microextraction process it should be possible to further enhance the extraction sensitivity.

4.5 References for Chapter Four


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APPENDICES
Appendix A

High pH-resistant, surface-bonded sol–gel titania hybrid organic–inorganic coating for effective on-line hyphenation of capillary microextraction (in-tube solid-phase microextraction) with high-performance liquid chromatography

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Available online at www.sciencedirect.com


Abstract

Sol–gel titania–poly(dimethylsiloxane) (TiO\textsubscript{2}–PDMS) coating was developed for capillary microextraction (CME) to perform on-line preconcentration and HPLC analysis of trace impurities in aqueous samples. A method is presented describing in situ preparation of the titania-based sol–gel PDMS coating and its immobilization on the inner surface of a fused silica microextraction capillary. To perform CME/HPLC, the sol–gel TiO\textsubscript{2}–PDMS capillary was installed in the HPLC injection port as an external sampling loop, and a conventional ODS column was used for the liquid chromatographic separation. The target analytes were extracted on-line by passing the aqueous sample through this sampling loop. The sol–gel titania–PDMS coated capillaries were used for on-line extraction and HPLC analysis of polycyclic aromatic hydrocarbons, ketones, and aldehydes. The extracted analytes were then transferred to the HPLC column using an organic-rich mobile phase followed by HPLC separation via gradient elution. To our knowledge, this is the first report on the use of sol–gel titania-based organic–inorganic material as a sorbent in capillary microextraction. The newly developed sol–gel titania–PDMS capillary remained practically unchanged after continuous running with a 0.1 M NaOH solution (pH 13) for 12 h.

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Keywords: Sol–gel; Capillary microextraction; In-tube solid-phase microextraction; Gradient elution; Titania–poly(dimethylsiloxane); Polycyclic aromatic hydrocarbons; Ketones; Aldehydes

1. Introduction

Solid-phase microextraction (SPME), a solvent-free sample preparation technique, was developed by Pawliszyn and co-workers\footnote{Corresponding author. Tel.: +1 813 974 0688; fax: +1 813 974 3252. E-mail address: malik@usfca.usf.edu (A. Malik).} using a fused-silica fiber externally coated with a polymeric sorbent covering a small segment of it at one of the ends. Analytes present in the sample medium were directly extracted and preconcentrated by the coated sorbent in the process of reaching an extraction equilibrium with the sample matrix. The preconcentrated analytes were then desorbed into a GC instrument for analysis.

In conventional fiber-based SPME, still there exist a number of shortcomings that need to be overcome. These include inadequate thermal and solvent stability of conventionally prepared sorbent coatings\footnote{DOI: 10.1016/j.chroma.2004.06.079}, low sample capacity, difficulties associated with the immobilization of thick coatings, susceptibility of the fiber (especially the coated end) to mechanical damage\footnote{0021-9673/04 - see front matter © 2004 Elsevier B.V. All rights reserved. doi:10.1016/j.chroma.2004.06.079}, and technical difficulties...
associated with the hyphenation of fiber-based SPME with liquid-phase separation techniques [7, 8].

Capillary microextraction (CME) [9] (also called m-tube SPME [10]) presents a convenient format for coupling SPME to HPLC and for automated operation of SPME-HPLC. Hyphenation of CME to HPLC is especially important for the analysis of a wide range of less volatile or thermally labile compounds [12] that are not amenable to GC separation. In the open tubular format of CME, a sorbent coating is applied to the inner surface of a capillary. This alternative format provides an effective solution to the problem associated with the mechanical damage of sorbent coating frequently encountered in conventional fiber-based SPME where the coating is applied on the outer surface of the fiber. In this new format of SPME, a segment of wall-coated capillary GC column is commonly used [10–12] for the direct extraction of organic analytes from an aqueous medium. To perform HPLC analysis, the extracted analytes are transferred to the HPLC column by desorbing them with an appropriate mobile phase.

Capillary microextraction has great prospects in liquid-phase trace analysis. However, to achieve its full analytical potential, the technology needs further improvements in a number of areas. First, segments of GC columns that are commonly used for sample preconcentration have thin coatings that limit the sorption capacity, and hence, the extraction sensitivity of m-tube SPME. Second, the sorbent coatings in such microextraction capillaries usually are not chemically bonded to capillary inner walls, which limits their thermal and solvent resistances. Third, conventionally prepared GC coatings that are used in m-tube SPME capillaries are inherently possess poor pH stability. This places serious limitations on the range of applications amenable to CME-HPLC analysis. Low pH stability of m-tube SPME coatings generally excludes the applicability of the technique to high-pH samples or analyses that require high-pH solvent systems for desorption from the microextraction capillary. Therefore, development of methodologies for the creation of high-pH- and solvent-resistant sorbent coatings is an important area in the future development of m-tube SPME, and is expected to play a major role in effective hyphenation of this sample preconcentration technique with liquid-phase separation techniques that commonly use organic-aqueous mobile phases with a wide range of pH conditions [13].

Sol-gel chemistry has been recently applied to solid-phase microextraction [4,14–17] and capillary microextraction [9] to create silica-based hybrid organic-inorganic coatings. The sol-gel technique provided chemically bonded coatings on the inner surface of fused-silica capillaries, and easily solved the coating stability problems described above.

Although sol-gel technique helped overcome some significant shortcomings of SPME or m-tube SPME techniques by providing an effective means of chemical immobilization for sorbent coatings, an important problem inherent in silica-based material systems (commonly used in SPME or CME) still remains to be solved: silica-based materials possess a narrow window of pH stability [18]. In the context of SPME, it pertains to the stability of silica-based fibers and coatings. The development of alternative materials possessing superior pH stability and better mechanical strength should provide SPME with additional ruggedness and versatility.

Recently, titania has attracted interest in separation science due to its superior pH stability and mechanical strength compared with silica [19–21]. Several studies have been conducted on the application of titania in chromatographic separations. Tanaka and Suzuki [21] reported the preparation of titania-based packing materials for HPLC by sol–gel method, and investigated their properties. Tanaka et al. [22] prepared silica capillaries coated with titania or alumina for capillary electrophoresis (CE) separation of proteins. Fujimoto et al. [23] used a thermal decomposition technique to create titania coatings on the inner surface of fused-silica capillaries for capillary zone electrophoresis (CZE) and capillary electrophoresis/mass spectrometry (CE/MS) applications. The titania-coated capillaries were found to possess a bi-directional electrophoretic migration (EOF) and low solubility in aqueous solutions within a pH range of 3–12. Pesek et al. [24] reported the surface derivatization of titania with ethylene oxide to prepare titania-based stationary phases via silanization/hydroxylation. Some other groups [25,26] reported preparations of silica-coated titania monolayers. Further study of titania-based coatings is important for further development of nanocomposites.

To date, very little (if any) research has been done on the development and application of titania-based coatings in analytical microextraction techniques. In this paper, we report the preparation of sol–gel TiO₂–PDMS coated capillaries and the possibility of on-line CME-HPLC operation using sol–gel TiO₂–PDMS microextraction capillaries to provide significant improvement in pH stability and extraction sensitivity.

2. Experimental

2.1. Equipment

On-line CME-HPLC experiments were carried out on a Micro-Tec Scientific (Vista, CA) Ultrasil HPLC system with a variable wavelength UV detector (Linear UVIS 200). A Nicolet model Avatar 320 FT-IR (Thermo Nicolet, Madison, WI) was used for FT-IR measurements. A reversed-phase ODS column (25 cm × 4.6 mm i.d., 5 μm dp) was used for HPLC separation of the extracted analytes. A Fisher model G-500 Vortex Genie 2 system (Fisher Scientific, Pittsburgh, PA) was used for thorough mixing of the sol solutions. A Microcentrifuge model A2-5800 centrifuge (Accurate Chemical and Scientific Corp., Westbury, NY) was used for centrifugation of sol solutions. A Barnstead model 04741 nanopure deionized water system (Hemstreet/Thermadyne, Dubuque, IA) was used to obtain 16.0 MΩcm water. On-line data collection and processing were done using
Appendix A (Continued)


2.2. Chemicals and materials

Fused silica capillary (250 and 220 μm i.d.) was purchased from Polymicro Technologies Inc. (Phoenix, AZ). A commercial poly(dimethylsiloxane)-based GC column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used for comparison with sol-gel titania–PDMS-based microextraction capillary in pH stability studies. Titanium (IV) isopropoxide (99.998%), 1-butanol (99.4%), poly(methylhydroxiloxane) (PMHS), 1,1,1,3,3,3-hexamethyldisilazane (HMDS), trifluoroacetic acid (TFA), polycyclic aromatic hydrocarbons (PAHs) (acenaphthylene, fluorene, phenanthrene, fluoranthene), kerosene (dubro, vaporphene, hexanephenone, heptanophene), and allylbenzenes (allylene, allylbenezene, cumene, propylbenzenes, butylbenzenes, amylbenzenes) were purchased from Aldrich (Milwaukee, WI). Hydroxy terminated poly(dimethylsiloxane) (PDMS) was purchased from United Chemical Technologies Inc. (Bristol, PA). HPLC-grade solvents (acetone, methanol, chloroform, and methanol) were purchased from Fisher Scientific.

2.3. Preparation of the sol solution

The sol solution was prepared by thoroughly vortexing the following reagents in a 2 mL polypropylene centrifuge tube: a sol–gel-active organic component (hydroxy-terminated PDMS, 50 mg), a sol–gel precursor (titanium (IV) isopropoxide, 50 μL), two solvents (methanol and 1-butanol, 300 μL each); a mixture of two surface desorption agents (HMDS, 8 μL and PMHS, 2 μL); and a sol–gel catalyst agent (27% TFA in H2O, 18 μL). The content of the tube was then centrifuged for 5 min (at 13,000 rpm; 15,682 × g). Finally, the top clear solution was transferred to another clean vial by decantation, and was further used for coating the fused silica microextraction capillary.

2.4. Preparation of sol–gel TiO2–PDMS coated microextraction capillaries

A 1 m long hydrothermally treated [27] fused silica capillary (250 or 220 μm i.d.) was installed in an in-house built gas pressure-operated capillary filling/purge/pump device [28], and the capillary was filled with the prepared sol solution under 10 psi helium pressure. After filling, the sol solution was kept inside the capillary for 15 min to facilitate the creation of a surface-bonded coating due to sol–gel reactions taking place in the coating solution inside the capillary. Following this, the unbonded portion of the sol solution was expelled from the capillary under helium pressure (20 psi), and the capillary was further purged with helium for 30 min. The coated capillary was then conditioned in a GC oven by programming the temperature from 40 °C to 320 °C at 1 °C/min under helium purge. The capillary was held at 320 °C for 180 min. Finally, the capillary was cooled down to room temperature and rinsed with methylene chloride and methanol (3 mL each). Following this, the capillary was installed in the GC oven for drying and further thermal conditioning under temperature-programmed heating as described above, except that this time the capillary was held at the final temperature for 30 min.

2.5. Capillary microextraction (CME) and on-line CME-HPLC analysis

A schematic of the CME–HPLC setup for on-line capillary microextraction and HPLC analysis is presented in Fig. 1. An ODS column (25 cm × 4.6 mm i.d., 5 μm φ) was previously installed in the HPLC system and pre-equilibrated with the mobile phase consisting of a mixture of acetoni- trile and water (80:20, v/v). A 40 cm segment of the sol–gel TiO2–PDMS coated microextraction capillary was mounted on the injection port as an external sampling loop. Samples were pressurized inside the sol–gel TiO2–PDMS coating by passing the aqueous sample from a gravity-fed dispenser [9] through this sol–gel titania–PDMS coated microextraction capillary for 40 min. Using a syringe, the sampling loop was flushed with deionized water to remove the sample matrix. The analytes extracted in the sol–gel TiO2–PDMS coating of the sampling loop were then transferred into the HPLC column by subdividing with 100% acetonitrile for 30 s. This was accomplished by simply switching the injection valve from the “load to inject” position. The injected analytes were then separated on the ODS column under gradient elution conditions by programming acetonitrile composition in the aqueous–organic mobile phase from 80% (v/v) to 100% in 15 min.

2.5.1 Treatment of coated capillaries with 0.1 M NaOH solution

A 40 cm segment of the sol–gel TiO2–PDMS coated capillary was directly inserted on the gravity-fed sample dispenser, and continuously rinsed with 0.1 M NaOH solution (pH 13) for 12 h. The capillary was then flushed out with deionized water for 30 min, and mounted back on the HPLC injection port. The target analytes (PAHs) were extracted on-line for 40 min, followed by their HPLC analysis as described in Section 2.5.

Using the same procedure, a 40 cm segment of the commercial PDMS-based GC capillary was treated with a 0.1 M NaOH solution. CME performances of the used capillaries were evaluated both before and after the alkaline treatment to explore pH stability of the used coatings.

2.6. Safety precautions

The presented work involved the use of various chemicals (organic and inorganic) and solvents that might be environmentally hazardous with adverse health effects. Proper safety measures should be taken in handling strong bases
and organic solvents such as methanol, methylene chloride, and acetonitrile. All used chemicals must be disposed in the proper waste containers to ensure personnel and environmental safety.

3. Results and discussion

The goal of this research was to develop high pH-resistant, surface-bonded sol-gel titania coatings for capillary microextraction to facilitate effective bypassing of CME with HPLC. Judicious utilization of unique attributes of sol-gel chemistry allowed us to create a surface-bonded hybrid organic-inorganic titania coating on the inner walls of a fused silica capillary providing an opportunity to exploit advanced material properties of titania-based sorbent in capillary microextraction. Unlike the conventional multi-step coating technologies [31–34], the sol-gel approach involves a single-step procedure to accomplish the sorbent coating, its chemical immobilization, and deactivation [35].

As sol-gel precursors, titanium alkoxides differ significantly from silicon alkoxides in terms of their chemical reactivity and complex-forming ability. These differences dictate the adoption of different strategies for the creation of titania-based sol-gel sorbents compared with those for silica-based analogs. While sol-gel reactions in a silica-based system are rather slow and often require the use of catalysts to accelerate the process [36], titania-based (transition metal oxide-based in general) sol-gel reactions are very fast. This is explained by the fact that titanium alkoxides are very reactive toward nucleophilic reagents like water [37]. They readily undergo hydrolysis, which results in a very fast sol-gel process. Because of this, titania-based sol-gel reactions need to be decelerated by a suitable means to allow for the sol-gel process to be conducted in a controlled manner. This is usually accomplished through the use of suitable chelating agents that form complexes with the sol-gel precursors (or replace the reactive alkoxy group with a less reactive group), thus hindering their participation in the sol-gel reactions. Without such a chelating agent, the gelation takes place instantaneously as the sol-gel solution ingredients are mixed together. Chelating agents such as acetic acid [38,39], trifluoroacetic acid [40], or metal β-diketonates [41] are often used for this purpose.

In the present work, sol-gel TiO₂-PDMS coated capillaries were prepared through hydrolytic polycondensation reactions performed within fused silica capillaries followed by thermal conditioning of the created coatings to achieve fine porous structures. Here, TFA served as a chelating agent [40] and decelerated the gelation process for the creation of TiO₂-PDMS coating. It has been shown by infrared spectra that the acetate ion can serve as a bidentate ligand (chelating and bridging) to the transition metal alkoxides, such as Ti(OR)₄ or Zr(OR)₄ [38,40,42].

Fig. 2 represents two scanning electron micrographs (SEM) showing the fine structural features of a 320 μm i.d. fused silica capillary with sol-gel TiO₂-PDMS coating on the inner surface. As is evident from these images, the sol-gel TiO₂-PDMS coating in the microextraction capillary acquires a porous structure, providing enhanced surface area and sorption ability. Based on the SEM data, the
Appendix A (Continued)


Fig. 2. Scanning electron microscopic images of a 120 μm i.d. fused silica capillary with sol–gel TiO₂–PDMS coating. (A) cross-sectional view (5000×) and (B) surface view (10,000×).

The sol–gel process for the generation and chemical immobilization of the coating involves: (A) hydrolysis of the titanium alkoxide precursor [43], (B) polycondensation of the hydrolysis products into a three-dimensional sol–gel network [44,45], (C) chemical incorporation of hydroxy-terminated PDMS in the sol–gel network [46,47], and (D) chemical anchoring of the sol–gel hybrid polymer to the inner walls of the capillary [44,45]. Scheme 1 illustrates the hydrolysis and polycondensation reactions of the sol–gel precursor, titanium (IV) isopropoxide, and Scheme 2 represents the final structure of the sol–gel TiO₂–PDMS coating on the inner surface of a fused silica capillary.

The formation of Si–O–Si bonds in the prepared sol–gel sorbent was examined by FT-IR. The FT-IR experiments were performed by passing IR radiation through a thin layer of sol–gel titania coating material that was used in the fused silica capillary. This was done in separate experiments outside the fused silica capillary. It has been reported [48,49] that a characteristic IR band representing Si–O–Si bonds located at 940–960 cm⁻¹. Fig. 3 shows FT-IR spectra of the sol–gel TiO₂–PDMS coating with a specific band at 952.68 cm⁻¹. This is indicative of the presence of Si–O–Si bonds in the sol–gel sorbent used in the fused silica microextraction capillaries to perform on-line CME-HPLC analysis.

Deactivation of the sol–gel coatings can be expected to take place mainly during thermal conditioning of the capillary, through derivatization of the free hydroxyl groups in the coating structure with HMDS [50] and PMHS [29,51] incorporated in the sol solution. To control the gelation time and to obtain a transparent gel, it was essential to find an optimum ratio (v/v) of HMDS and PMHS. In the present study, this ratio was found to be 4:1 (HMDS:PMHS, v/v).

Sol–gel technology is quite versatile, and allows for the control of coating thickness either by manipulating the reaction time or composition of the sol solution. Zeng et al. [16] has recently reported the preparation of 70 μm thick silica-based sol–gel coating on conventional SPME fiber. It should be possible to create such thick coatings (either silica-based or transition metal oxide-based) on the inner surface of fused silica capillaries as well. Use of thicker coatings should enhance the sample capacity and extraction sensitivity in CME with titania-based sol–gel coatings.
Appendix A (Continued)

Table 1
Peak area repeatability and detection limit data for PAHs using a sol-gel TiO₂–PDMS-coated capillary treated with 0.1 M NaOH for 12 h.

<table>
<thead>
<tr>
<th>Extracted PAHs</th>
<th>Peak area repeatability (n = 3)</th>
<th>Percent change in peak area ([I/\text{area} - A_1]/A_1) × 100%</th>
<th>Detection limits (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before rinsing</td>
<td>After rinsing</td>
<td>Before rinsing</td>
</tr>
<tr>
<td></td>
<td>Mean peak area (arbitrary unit)</td>
<td>R.S.D. (%)</td>
<td>Mean peak area (arbitrary unit)</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>17.7</td>
<td>1.7</td>
<td>16.2</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>13.9</td>
<td>6.2</td>
<td>14.2</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>16.5</td>
<td>9.2</td>
<td>16.3</td>
</tr>
</tbody>
</table>

* Extraction conditions: 40 cm × 0.25 mm i.d. × 0.25 μm sol-gel TiO₂–PDMS-coated capillary, extraction time 40 min, HPLC conditions: 25 cm × 4.6 mm i.d. ODS column (5 μm dₚ), gradient elution 80:20 (v/v) ACN/water to 100% ACN for 20 min; 1 mL/min flow rate; UV detection at 254 nm.

The sol–gel titania–PDMS coatings demonstrated excellent pH stabilities over conventionally created coatings like those used in commercial GC capillary columns. Fig. 4 illustrates the CME performance of a TiO₂–PDMS coated microextraction capillary (250 μm i.d.) in CME-HPLC analysis of PAHs before (Fig. 4a) and after (Fig. 4b) rinsing the capillary with a 0.1 M NaOH solution (pH 13) for 12 h. Analogously obtained data for a piece of commercial PDMS-based GC column are presented in Fig. 4c and 4d, respectively. Chromatogram of Fig. 4b was obtained on the sol–gel TiO₂–PDMS coated microextraction capillary after it was thoroughly rinsed with deionized water. The extraction of PAHs was performed under the same set of conditions as in Fig. 4a. From the comparison of peak profiles and peak heights in Fig. 4a and 4b, it is evident that the sol–gel TiO₂–PDMS coating in the microextraction capillary remained unaffected even after the prolonged rinsing with 0.1 M NaOH solution of pH 13.

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Fig. 5. Capillary microextraction-HPLC analysis of benzenes. Extraction conditions: 40 cm × 0.252 mm i.d. × 5 μm sol–gel TiO₂–PDMS-coated capillary; extraction time, 40 min (gravity-fed at room temperature). Other conditions: 25 cm × 4.6 mm i.d. ODS column (5 μm dₚ), gradient elution with mobile phase composition programmed from 80:20 acetonitrile/water to 100% acetonitrile for 15 min; 1 mL/min flow rate; UV detection at 254 nm; ambient temperature. Peaks: (1) toluene (1 ppm), (2) ethylbenzene (500 ppb), (3) propylbenzene (50 ppb), (4) propylbenzene (50 ppb), (5) butylbenzene (50 ppb), and (6) amylbenzene (50 ppb).

Fig. 6. Capillary microextraction-HPLC analysis of allylbenzenes. Extraction conditions are the same as in Fig. 5. Other conditions: 25 cm × 4.6 mm i.d. ODS column (5 μm dₚ), gradient elution with mobile phase composition programmed from 80:20 acetonitrile/water to 100% acetonitrile for 15 min; 1 mL/min flow rate; UV detection at 254 nm; ambient temperature. Peaks: (1) toluene (500 ppb), (2) ethylbenzene (200 ppb), (3) propylbenzene (50 ppb), (4) propylbenzene (50 ppb), (5) butylbenzene (50 ppb), and (6) amylbenzene (50 ppb).
On the other hand, the PDMS-based stationary phase coating in the commercial GC capillary showed significantly less extraction sensitivity as is evident from peak heights in Fig. 4c. It also failed to survive the harsh conditions of rinsing with 0.1 M NaOH solution, which is evidenced by a dramatic decrease in the extraction sensitivity after the NaOH treatment (compare Fig. 4c and 4d). These results show that a sol–gel TiO$_2$–PDMS coated capillary possesses excellent pH stability and retains its extraction ability under extreme pH conditions, while conventionally prepared PDMS-based GC coatings were found to be unstable under such extreme pH conditions [52,53].

Table 1 shows repeatability and detection limit data for CME-HPLC analysis using sol–gel TiO$_2$–PDMS coated microextraction capillaries. For a 0.25 mm i.d. sol–gel titania–PDMS microextraction capillary, the R.S.D. value in peak area remained within 9.3%, and detection limits in the range of 0.25–5.37 ppb were achieved using UV-detection.

Fig. 5 presents a chromatogram illustrating CME-HPLC analysis of moderately polar aromatic ketones extracted from an aqueous sample using a 0.32 mm i.d. sol–gel coated TiO$_2$–PDMS capillary.

Compared to PAHs samples, ketones needed higher analyte concentrations (300 ppb–1 ppm) for CME-HPLC analysis. This may be explained by the nonpolar nature of the sol–gel TiO$_2$–PDMS coating, higher solubility of ketones in water due to higher polarity, and the working principles of UV detection. In this case, the run-to-run peak area repeatability was less than 8% R.S.D. Detection limits for the extracted ketones ranged between 2.47 ppb for hexanophenone to 11.60 ppb for valerophenone in conjunction with UV detection. From the presented results it is evident that sol–gel TiO$_2$–PDMS coating is able to extract both nonpolar and moderately polar analytes with good extraction sensitivity. Such an ability of the used sol–gel coating may be due to the presence of two different types of domains (a nonpolar organic domain based on an PDMS and a more polar inorganic domain based on sol–gel titania materials) in such coatings [54].

Fig. 6 illustrates on-line CME-HPLC analysis of alkylbenzenes using a TiO$_2$–PDMS coated capillary. Excellent detection limits were also achieved for these analytes (0.65–5.45 ppb), using UV detection. Like PAHs, alkylbenzenes are less polar analytes than aromatic ketones, and they are well extracted by a sol–gel TiO$_2$–PDMS extraction capillary with low-ppb and sub-ppb level detection limits. Table 2 summarizes the peak area repeatability and detection limit data for PAHs, ketones, and alkylbenzenes.
Fig. 7 illustrates the extraction kinetic profile for: (A) fluorine (nonpolar analyte) and (B) benzenophenone (moderately polar analyte) on a sol–gel TiO$_2$–PDMS coated microextraction capillary. Experimental data for these curves representing extraction kinetic profiles were obtained by individually performing capillary microextraction for each of the solutes. The microextraction experiments were performed using aqueous samples containing 100 and 300 ppb concentrations of fluorine and benzenophenone, respectively. A series of capillary microextraction experiments were conducted to vary the extraction time for each of the two analytes that were extracted from their standard solutions. Three replicate extractions of each analyte were performed for 1, 5, 10, 20, 30, 40, 50, and 60 min. The average peak area was then plotted against the extraction time to obtain Fig. 7. For both fluorine and benzenophenone, extraction equilibrium was reached within 40 min as is evidenced by the plateau on the extraction curve. Since PDMS has nonpolar characteristics, the TiO$_2$–PDMS coating tends to extract a nonpolar analyte, in this case fluorine, better than a more polar analyte, benzenophenone, which has higher affinity for the aqueous medium. Further optimization of capillary preparation method and operation conditions may be necessary to exploit full analytical potential of the sol–gel titania coated extraction capillaries. It will be also interesting to use TiO$_2$–PDMS extraction capillary in CME-GC to achieve better detection limits, since CME-GC will allow for the use of highly sensitive flame ionization detector. Such an assumption stems from the fact that sol–gel TiO$_2$–PDMS coatings have already shown good extraction capabilities for CME-HPLC equipped with a UV detector, which is much less sensitive than the FID. The use of wider bore capillaries with thicker sol–gel coatings or monolithic extraction beds [55] should further enhance the extraction sensitivity.

4. Conclusion:

To the best of our knowledge, this is the first report on the creation and use of a sol–gel TiO$_2$–PDMS coating in solid-phase microextraction. Sol–gel TiO$_2$–PDMS coated microextraction capillaries possess excellent pH stability and retain their extraction characteristics intact even after prolonged treatment with highly alkaline (pH 13) NaOH solution. Direct chemical bonding of the coating to capillary inner walls provides these coatings with excellent solvent resistance, and make sol–gel TiO$_2$–PDMS coated capillaries very much suitable for on-line sample preconcentration in CME-HPLC analysis. The newly developed sol–gel TiO$_2$–PDMS coating was effectively used for the extraction of different classes of analytes with good extraction sensitivity, and run-to-run repeatability. Low ppb and sub-ppb level (0.15–11.60 ppb) detection limits were achieved for PAHs, ketones, and alkylbenzenes in CME-HPLC analysis using the newly constructed sol–gel TiO$_2$–PDMS coated microextraction capillary in conjunction with UV detection. Through proper optimization of experimental conditions for sol–gel coating and the capillary microextraction processes it should be possible to further enhance the extraction sensitivity. For volatile and thermally stable analytes, use of sol–gel TiO$_2$–PDMS coated capillaries in CME-GC should provide significant enhancement in sensitivity.

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References

Sol–gel approach to in situ creation of high pH-resistant surface-bonded organic–inorganic hybrid zirconia coating for capillary microextraction (in-tube SPME)

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Abstract

A novel zirconia-based hybrid organic–inorganic sol–gel coating was developed for capillary microextraction (CME) (in-tube SPME). High degree of chemical inertness inherent in zirconia makes it very difficult to covalently bind a suitable organic ligand to its surface. In the present work, this problem was addressed from a sol–gel chemistry point of view. Principles of sol–gel chemistry were employed to chemically bind a hydroxy-terminated silicone polymer (poly(dimethylphenylsiloxane), PDMS) to a sol–gel zirconia network in the course of its evolution from a highly reactive silicate precursor undergoing controlled hydrolytic polycondensation reactions. A fused silica capillary was filled with a properly designed sol solution to allow for the sol–gel reactions to take place within the capillary for a predetermined period of time (typically 15–30 min). In the course of this process, a layer of the evolving hybrid organic–inorganic sol–gel polymer got chemically anchored to the silanol groups on the capillary inner walls via condensation reaction. At the end of this in-capillary residence time, the unbound part of the sol solution was expelled from the capillary under helium pressure, leaving behind a chemically bonded sol–gel zirconia–PDMS coating on the inner walls. Polycyclic aromatic hydrocarbons, ketones, and aldehydes were efficiently extracted and preconcentrated from dilute aqueous samples using sol–gel zirconia–PDMS coated capillaries followed by thermal desorption and GC analysis of the extracted solutes. The newly developed sol–gel hybrid zirconia coatings demonstrated excellent pH stability, and retained the extraction characteristics intact even after continuous rinsing with a 0.1 M NaOH solution for 24 h. To our knowledge, this is the first report on the use of a sol–gel zirconia-based hybrid organic–inorganic coating as an extraction medium in solid phase microextraction (SPME).

Keywords: Capillary microextraction; In-tube SPME; Sol–gel extraction media; Sol–gel technology; Sol–gel zirconia poly(dimethylphenylsiloxane) coating; pH stability; Sample preconcentration; Gas chromatography; Hyphenated techniques; Polycyclic aromatic hydrocarbons; Aldehydes; Ketones

1. Introduction

Solid phase microextraction (SPME) was developed in 1989 by Belardi and Pawliszyn [1] to facilitate rapid sample preparation for both laboratory and field analyses. It provided a simple and efficient solvent-free method for the extraction and preconcentration of analytes from various sample matrices.

In SPME, a sorptive coating (either on the outer surface of a fused silica fiber or on the inner surface of a fused silica capillary) serves as the extraction medium in which the analytes get preferentially sorbed and preconcentrated. Polymeric surface coatings are predominantly used in conventional fiber-based SPME [1–4] as well as in the more recently materialized in-tube SPME [5–8] also referred to as capillary microextraction (CME) [9]. A number of new polymeric coatings have recently been developed [10]. Besides polymeric coatings, SPME fibers have also been prepared by using nanopolymeric materials [11] or by gluing reversed-phase high-performance liquid chromatography (HPLC) particles
onto SPME fiber surface [12]. The sorbent coating plays a fundamentally important role in the SPME analysis, and further development and growth of SPME will greatly depend on new breakthroughs in the areas of sorbent development and coating technology [13].

Sol-gel chemistry offers an effective methodology for the synthesis of macromolecular materials under extraordinarily mild thermal conditions (typically at room temperature). The room temperature operation, inherent in sol–gel chemistry, facilitates the material synthesis process by easing the operational requirements on equipment specification and laboratory safety. This greatly simplifies the job to carry out and control sol–gel reactions within small-diameter fused silica capillaries. The sol–gel approach provides a viable mechanism to chemically bond an in situ created sol–gel coating to the inner walls of the capillary made out of an appropriate sol–gel-active material. Thanks to this chemical bonding, sol–gel coatings possess significantly higher thermal and solvent stabilities [14] compared with their conventional counterparts. The sol–gel approach can be applied to create silica-based as well as the newly emerging transition metal oxide-based sorbents. Furthermore, sol–gel chemistry provides an opportunity to create advanced material systems to achieve enhanced performance and selectivity in analytical separations and sample preconcentration [10,15].

Sol-gel organic–inorganic hybrid materials provide desirable sorptive properties that are difficult to achieve by using either purely organic or purely inorganic materials. Because of this unique opportunity to achieve enhanced selectivity, hybrid sol–gel materials have created a great deal of interest in the field of microcolumn separations and sample preparation. In the recent past, silica-based organic–inorganic hybrid stationary phases have been developed in the form of surface coatings [16–18] and monolithic beds [19]. In 1993, Dubito and coworkers [20] developed a procedure for the preparation of a thin layer of silica gel with chemically bonded C18 moieties on the inner wall of fused-silica capillaries for use as open tubular column in reversed-phase high-performance liquid chromatography. Colon and Guo [21] used sol–gel technology to prepare stationary phase coatings for open-tubular liquid chromatography and electrochromatography. Malik and coworkers introduced sol–gel coated columns for capillary GC [22] and sol–gel coated fibers for solid-phase microextraction [13,23]. Subsequently, other groups also get involved in sol–gel research aiming at developing novel sorbents for solid-phase microextraction [24–28] and solid-phase extraction [29,30]. Compared with conventional fibers, sol–gel SPME fibers demonstrated superior performance by exhibiting high thermal stability (up to 380 °C) [24] and solvent stability [25]. This enhanced stability of sol–gel coated fibers is attributed to the chemical bonding between the sol–gel coating and the fiber surface. Compared with the conventionally prepared fibers, in many instances, sol–gel SPME fibers showed better selectivity and extraction sensitivity, [26] less extraction time, [27] and extended lifetime [26]. Recently, sol–gel capillary microextraction was reported by Malik and co-workers [9]. In this format, also known as in-tube SPME, sample extraction was accomplished using a sol–gel coating created on the inner surface of a fused silica capillary.

The sol–gel microextraction sorbents reported to date are predominantly silica-based. In spite of many attractive material properties (e.g., mechanical strength, surface characteristics, catalytic inertness, surface derivitization possibilities, etc.), silica-based materials have some inherent shortcomings. The main drawback of silica-based sorbents is the narrow range of pH stability. Under extreme pH conditions, silica-based materials become chemically unstable, and their sorptive properties may be compromised. For example, silica dissolves under alkaline conditions, and their dissolution process starts at a pH value of about 8 [31]. Under highly acidic pH conditions, silica-based bonded phases become hydrolytically unstable [32]. Therefore, developing sorbents with a wide range of pH stability is an important research area in contemporary separation and sample preparation technologies. Transition metal oxides (zirconia, titania, etc.) are well known for their pH stability [31], and appear to be logical candidates for exploration to overcome the above-mentioned drawbacks inherent in silica-based materials.

Zirconia possesses much better alkali resistance than other metal oxides, such as alumina, silica, and titania. It is practically insoluble within a wide pH range (1–14) [36–39]. Zirconia also shows outstanding resistance to dissolution at high temperatures [40,41]. Besides the extraordinary pH stability, excellent chemical inertness and high mechanical strength are two other attractive features that add value to zirconia for being used as a support material in chromatography [34] and membrane-based separations [35].

Extensive research work has been done on zirconia particles and their surface modifications for use as HPLC stationary phases [42,43]. A number of reports have also recently appeared in the literature on the use of zirconia-modified fused silica capillaries in capillary electrophoresis (CE) [44–48]. However, the excessive chemical inertness of zirconia particles remains a difficult hurdle to creating surface-bonded stationary phases.

We approached this problem from sol–gel chemistry point of view. We took into consideration the fact that contrary to the high inertness of zirconia particles that have already been formed and attained highly stable structural characteristics, zirconium alkoxides are highly reactive sol–gel precursors for zirconia. By using appropriate conditions, it should be possible to utilize the reactivity of such zirconia precursors to create organic–inorganic zirconia materials with covalently bonded organic ligands. In this paper, we report the preparation of zirconia–based hybrid organic–inorganic sol–gel sorbents from a highly reactive precursor, zirconium butoxide, and a sol–gel-active organic polymer (hydroxyl-terminated PDMAPS). The covalent bonding of the organic ligand to the sol–gel zirconia network structure was accomplished via condensation reaction in the course of controlled hydrolytic polycondensation reactions taking place in the sol
solution. Here, we demonstrate the outstanding performance of the in situ created sol–gel zirconia–PDMDPS coating in capillary microextraction in hyphenation with open-tubular gas chromatography (CME-GC).

2. Experimental

2.1. Equipment

All CME-GC experiments were performed on a Shimadzu Model 14A capillary GC system equipped with a flame ionization detector (FID) and a split-splitless injector. On-line data collection and processing were done using ChromPerfect (version 3.5) computer software (Justice Laboratory Software, Demville, NJ). A Fisher Model G-560 Vortex Genie 2 system (Fisher Scientific, Pittsburgh, PA) was used for thorough mixing of various sol–gel solution ingredients. A Microcentaur model APO 5760 microcentrifuge (Accurate Chemical and Scientific Corp., Westbury, NY) was used to separate the sol solution from the precipitate (if any) at 13,000 rpm (15,682 × g). A Nicolet model Avatar 320 FTIR instrument (Thermo Nicolet, Madison, WI) was used to acquire infrared spectra of the prepared sol–gel materials. A Barnstead Model 04741 Nanopure deionized water system (Barnstead Thermodyne, Dubuque, IA) was used to obtain ~16.0 MΩ water. Stainless steel mini-units (SGE Inc., Austin, TX) were used to connect the fused-silica capillary GC column with the microextraction capillary, also made of fused silica. An in-house-designed liquid sample dispenser (Fig. 1) was used to facilitate gravity-fed flow of the aqueous sample through the sol–gel microextraction capillary. A home-built, gas pressure-operated capillary filling purging device [49] was used to perform a number of operations: (a) rinse the fused-silica capillary with solvent; (b) fill the extraction capillary with the sol solution; (c) expel the sol solution from the capillary at the end of sol–gel coating process; and (d) purge the capillary with helium after treatments like rinsing, coating, and sample extraction.

2.2. Chemicals and materials

Fused-silica capillary (320 and 250 µm, i.d.) with a protective polyamide coating was purchased from Polymicro Technologies Inc. (Phoenix, AZ). Naphthalene and HPLC-grade solvents (methylene chloride, methanol) were purchased from Fisher Scientific (Pittsburgh, PA). Hexamethyldisilazane (HMDS), poly(methyl-hydroxiloxane) (PMHS), ketones (valerophenone, hexanophenone, heptanophenone, and decanophenone), aldehydes (nonylaldehyde, nonyldecahydride, undecylic aldehyde, and dodecanal), polycyclic aromatic hydrocarbons (PAHs) (naphthalene, acenaphthene, fluorene, phenanthrene, pyrene, and naphthalene), were purchased from Aldrich (Milwaukee, WI). Two types of silanol-terminated poly(dimethylphenylsiloxane) (PDMS) copolymers (with 2–3% and 14–18% contents of the diphenyl-containing component) were purchased from United Chemical Technologies Inc. (Bristol, PA).

2.3. Preparation of sol–gel zirconia–PDMDPS coating

A carefully designed sol solution was used to create the coating. The key ingredients of the sol solution used are listed in Table 1. The sol solution was prepared in a clean polypropylene centrifuge tube by dissolving the following ingredients: in mixed solvent system consisting of methylene chloride and butanol (250 µL each), 10–15 µL of zirconium(IV) butoxide (80% solution in 1-butanol), 85 mg of silanol-terminated poly(dimethylphenylsiloxane) copolymer, 70 µg of poly(methylhydroxiloxane), 10 µL of 1,1,1,3,3,3-hexamethyldisilazane, and 2–4 µL of glacial acetic acid. The dissolution process was aided by thorough vortexing. The sol solution was then centrifuged at 13,000 rpm (15,682 × g) to remove the precipitate (if any). The top clear sol solution was transferred to a clean vial and was further used in the coating process. A hydrothermally treated fused silica capillary (2 m) was filled with the clear sol solution, using pressurized helium (50 psi) in the filling/purging device [49]. The sol solution was allowed to stay inside the capillary for a controlled period of time (typically 15–30 min) to facilitate the formation of a sol–gel coating, and its chemical bonding to the capillary inner walls. After that, the free portion of the solution was expelled from the capillary, leaving behind a surface-bonded sol–gel coating.

Fig. 1. Gravity-fed extraction system for capillary microextraction.
within the capillary. The sol–gel coating was then dried by purging with helium. The coated capillary was further conditioned by temperature programming from 40 to 150 °C at 1 °C/min and held at 150 °C for 300 min. Following this, the conditioning temperature was raised from 150 to 320 °C at 1 °C/min and held at 320 °C for 120 min. The extraction capillary was further cleaned by rinsing with 3 mL of methylene chloride and conditioned again from 40 to 320 °C at 4 °C/min. While conditioning, the capillary was constantly purged with helium at 1 mL/min. The conditioned capillary was then cut into 10 cm long pieces that were further used to perform capillary microextraction.

2.4. Preparation of the samples

PAHs, ketones, and aldehydes were dissolved in methanol or tetrahydrofuran to prepare 0.1 mg/L stock solutions in silanized glass vials. For extraction, fresh samples with ppb level concentrations were prepared by diluting the stock solutions with deionized water.

2.5. Gravity-fed sample dispenser for capillary microextraction

The gravity-fed sample dispenser for capillary microextraction (Fig. 1) was constructed by in-house modification of a Chromatex AQ column (Kontes Glass Co., Vineland, NJ) consisting of a thick-walled glass cylinder coaxially placed inside an acrylic jacket. The inner surface of the thick-walled cylindrical glass column was deactivated by treating with a 5% (v/v) solution of HMDS in methylene chloride followed by overnight heating at 100 °C. The column was then cooled to ambient temperature, thoroughly rinsed with methanol and liberal amounts of deionized water, and dried in a helium flow. The entire Chromatex AQ column was subsequently reassembled.

2.6. Sol–gel capillary microextraction-GC analysis

To perform capillary microextraction, a previously conditioned sol–gel zirconia-FDMDFS coated microextraction capillary (10 cm × 320 µm i.d. or 10 cm × 250 µm i.d.) was vertically connected to the bottom end of the empty sample dispenser (Fig. 1). The aqueous sample (50 mL) was then placed in the dispenser from the top, and allowed to flow through the microextraction capillary under gravity. While passing through the extraction capillary, the analyte molecules were sorbed by the sol–gel zirconia-FDMDFS coating residing on the inner walls of the capillary. The sample flow through the capillary was allowed to continue for 30–40 min for an extraction equilibrium to be established. After this, the microextraction capillary was purged with helium at 25 kPa for 1 min and connected to the top end of a vertically placed two-way mini-union connecting the microextraction capillary with the inlet end of the GC column. Approximately, 6.5 mm of the extraction capillary remained tightly inserted into the connector, as did the same length of GC column from the opposite side of the mini-union facing each other within the connector. The installation of the capillary was completed by providing a leak-free connection at the bottom end of the GC injection port so that top 9 cm of the extraction capillary remained inside the injection port. The extracted
3. Result and discussion

Capillary microextraction [9] uses a sorbent coating on the inner surface of a capillary, and thereby overcomes a number of deficiencies inherent in conventional fiber-based SPME such as susceptibility of the sorbent coating to mechanical damage due to scraping during operation, fiber breakage, and possible sample contamination. In CME, the sorbent coating is protected by the fused silica tubing against mechanical damage. The capillary format of SPME also provides operational flexibility and convenience during the microextraction process since the protective polysiloxane coating on the outer surface of fused silica capillary remains intact. Inner surface-coated capillaries provide a simple way to perform extraction in conjunction with a gravity-fed sample dispenser (Fig. 1), and thus avoid typical drawbacks of fiber-based SPME, including the need for sample agitation during extraction as well as the sample loss and contamination problems associated with this.

The sol–gel process is a straightforward route to obtaining homogeneous gels of desired compositions. In recent years, it has received increased attention in analytical separations and sample preparations due to its outstanding versatility and excellent control over properties of the created sol–gel materials that proved to be promising for use as stationary phases and extraction media.

A general procedure for the creation of sol–gel stationary phase coating on the inner walls of fused silica capillary GC columns was first described by Malik and co-workers [22]. In the present work, a judiciously designed sol solution ingredients (Table 1) was used to create the sol–gel zirconia-PDMPS coating on the fused silica capillary inner surface. Zirconium(IV) butoxide (80% solution in 1-butanol) was used as a sol–gel precursor and served as a source for the inorganic component of the sol–gel organic–inorganic hybrid coating.

The sol–gel Zirconia-PDMPS coating presented here was generated via two major reactions: (1) hydrolysis of a sol–gel precursor, zirconium(IV) butoxide; and (2) polycondensation of the precursor and its hydrolysis products between themselves and with other sol–gel-active ingredients in the coating solution, including silanol-terminated PDMPS. The hydrolysis of the zirconium(IV) butoxide precursor is represented by Scheme 1 [50].

Condensation of the sol–gel polymer growing in close vicinity of the capillary walls with silanol group on the capillary surface led to the formation of an organic–inorganic coating chemically anchored to the capillary inner walls (Scheme 2A).

A major obstacle to preparing zirconia-based sol–gel materials using zirconium alkoxide precursors (e.g., zirconium butoxide) is the very rapid sol–gel reaction rates for these precursors. Even if the solution of zirconium alkoxide is stirred vigorously, the rates of these reactions are so high that large agglomerated zirconia particles precipitate out immediately when water is added [51]. Such fast precipitation makes it difficult to reproducibly prepare zirconium sol–gel materials.

Ganji and Kusdii [52] addressed the fast precipitation problem by dissolving zirconium propoxide in a non-polar dry solvent like cyclohexane. The hydrolysis was performed by exposing the coatings prepared from the solution to atmospheric moisture. Heating to 450 °C was necessary to obtain transparent films. The hydrolysis rates of zirconium alkoxides can also be controlled by chelating with ligand-exchange reagents. Acetic acid [53–54], valeric acid [55], β-diketones [56–58], triethanolamine [59], and 1,5-diaminopentane [56] have been used as chelating reagents for zirconia sol–gel reactions. In general, chelation occurs when the added reagent replaces one or more alkoxyl groups forming a strong bond. The formation of this bond reduces the hydrolysis rate by decreasing the number of available alkoxyl groups [60].

In the present work, we controlled the hydrolysis rate of zirconium butoxide by using glacial acetic acid [61] as a chelating agent as well as a source of water released slowly through the esterification with 1-butanol [62,63]. Two Silanol-terminated poly(dimethylsiloxane) copolymers (with 2–3% and 14–18% diphenyl-containing blocks) were used as sol–gel-active organic components to be chemically incorporated in the sol–gel network through polycondensation reactions with the zirconium butoxide.

\[
\begin{align*}
\text{Zirconium(IV) butoxide} & \quad \xrightarrow{\text{H}_{2}\text{O}} \quad \text{Zr} \quad \text{OCH}_{2}\text{CH}_{2} \text{CH}_{3} + 4 \text{H}_{2}\text{O} \\
\text{OCH}_{2}\text{CH}_{2} \text{CH}_{3} & \quad \text{OCH}_{2}\text{CH}_{2} \text{CH}_{3} \\
\text{H}_{2}\text{O} & \quad \text{H}_{2}\text{O} \\
\text{Zirconium(IV) butoxide} & \quad \text{Tetrahydroxyzirconia}
\end{align*}
\]

Scheme 1. Hydrolysis of zirconium(IV) butoxide precursor.
Scheme 2. Deactivation and shielding of sol–gel zirconia–PDMDPS coated surface using PMHS and FMDS.

This advantageous chemical incorporation of an organic component into the sol–gel network is responsible for the formation of an organic–inorganic hybrid material system that can be conveniently used for in situ creation of surface coating on a substrate like the inner wall of a fused silica capillary. Besides, the organic groups help to reduce the shrinkage and cracking of the sol–gel coating [64, 65]. Furthermore, sol–gel process can be used to control the porosity and thickness of the coating to improve its mechanical properties [66]. Polymethylhydroxiloxane and 1,1,1,3,3,3-hexamethyldisilazane that were used in the sol solution, served as deactivation reagents to perform chemical derivatization of the strongly adsorptive residual hydroxyl groups on the resulting sol–gel material. The purpose of these reactions was to minimize the strong adsorptive interactions between polar solutes and the sol–gel sorbent that may lead to sample loss, peak tailing, sample carry-over and other deleterious effects. To provide a detailed method for the preparation of the sol–gel zirconia coated microextraction capillary, the deactivation reactions were designed to take place mainly during thermal conditioning of the capillary following the sol–gel coating procedure.

Hydrolytic polycondensation reactions for sol–gel-active reagents are well established in sol–gel chemistry [67–70], and constitute the fundamental mechanism in sol–gel synthesis. The condensation between sol–gel-active zirconia and silicon compounds is also well documented [71–73]. According to published literature data, [74, 75] the characteristic IR band for Zr–O–Si bond is located in the vicinity of 945–980 cm⁻¹. Fig. 2B shows an IR spectrum of sol–gel zirconia PDMDPS material prepared by using a PDMDPS polymer containing...
Appendix B (Continued)

approximately eight time higher amounts of the phenyl group than that presented in Fig. 2A. The presence of the stretching at 954 cm⁻¹ indicates the presence of Zr-O-Si bonds in the prepared sol–gel material [74].

Metal-bound hydroxyl groups on the created sol–gel coating represent strong adsorptive sites for polar solutes. In the context of analytical microextraction or separation, presence of such groups is undesirable, and may lead to a number of deleterious effects including sample loss, reproducibility problems, sample carryover problems, and peak distortion and tailing. Therefore, appropriate measures need to be taken to deactivate these adsorptive sites. This may be accomplished by chemically reacting the hydroxyl groups with suitable derivatization reagents. Like silica-based sol–gel coatings, the surface hydroxyl groups of sol–gel zirconia coating can be deactivated using reactive silicon hydride compounds such as alkyl hydrosilanes [76,77] and hexamethyldisilazane [78]. In this work, we used a mixture of polymethylhydrosiloxane and hexamethyldisilazane for this purpose: the underlying chemical reactions are

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Peak area and retention time repeatability data for RAHs, aldehydes, and ketones extracted from aqueous samples using four replicate measurements by CME-GC using sol–gel zirconia–PDMDFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyte</td>
<td>Peak area repeatability (r = 4)</td>
</tr>
<tr>
<td>Chemical class</td>
<td>Name</td>
</tr>
<tr>
<td>RAHs</td>
<td>Naphthalene</td>
</tr>
<tr>
<td></td>
<td>Acenaphthene</td>
</tr>
<tr>
<td></td>
<td>Fluorene</td>
</tr>
<tr>
<td></td>
<td>Phenanthrene</td>
</tr>
<tr>
<td></td>
<td>Pyrene</td>
</tr>
<tr>
<td></td>
<td>Naphthacene</td>
</tr>
<tr>
<td>Aldehydes</td>
<td>1-Nonanal</td>
</tr>
<tr>
<td></td>
<td>1-Decanal</td>
</tr>
<tr>
<td></td>
<td>Undecanal</td>
</tr>
<tr>
<td></td>
<td>Dodecanal</td>
</tr>
<tr>
<td>Ketones</td>
<td>Valeronitrile</td>
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<tr>
<td></td>
<td>Hexamethylen</td>
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<td></td>
<td>Heptamethylen</td>
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<tr>
<td></td>
<td>Decamethylen</td>
</tr>
<tr>
<td></td>
<td>Trans-chalcone</td>
</tr>
</tbody>
</table>
Appendix B (Continued)

Fig. 4. CME-GC analysis of PAHs using a sol–gel zirconia–PDMS coated extraction capillary. Extraction parameters: 10 cm × 0.32 mm i.d. microextraction capillary; extraction time, 30 min (gravity fed at room temperature). Other conditions: 10 m × 0.25 mm i.d. Sol–gel PDMS GC column; splitless desorption: injector temperature rose from 30 to 300 °C; column temperature program from 30 to 300 °C at rate of 20 °C/min; helium carrier gas: FID 350 °C. Peaks: (1) naphthalene, (2) acenaphthene, (3) fluorene, (4) phenanthrene; (5) pyrene; and (6) anthracene.

Schematically represented in Scheme 2 (A and B illustrate the chemical structure of the sol–gel zirconia surface coating before and after deactivation respectively).

One of the most important undertakings in CME is the creation of a stable, surface-bonded sorbent coating on the inner walls of a fused silica capillary. Fig. 3 represents scanning electron microscopic images of a sol–gel Zirconia–PDMS coated fused silica capillary prepared in the present work. The SEM images A and B were obtained at a magnification

Table 3
Capillary-to-capillary and run-to-run peak area repeatability for mixture of PAHs, aldehydes, and ketones in four replicate measurements by CME-GC using sol–gel zirconia–PDMS coated extraction capillaries

<table>
<thead>
<tr>
<th>None of the analyte</th>
<th>Peak area repeatability (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Capillary-to-capillary</td>
</tr>
<tr>
<td></td>
<td>Mean peak area (arbitrary unit)</td>
</tr>
<tr>
<td>Undecanal</td>
<td>47940.9</td>
</tr>
<tr>
<td>Hexafluorobenzene</td>
<td>27528.4</td>
</tr>
<tr>
<td>Fluorene</td>
<td>52250.7</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>51399.9</td>
</tr>
</tbody>
</table>

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Fig. 6. CME-GC analysis of ketone using a oil–gel zinc oxide–PCMDPS coated extraction capillary. Extraction parameters: 10 cm × 0.25 mm i.d. microextraction capillary; extraction time, 40 min (gravity fed at room temperature). Other conditions: 10 m × 0.25 mm i.d. 50–500°C column; n-dodecane desorption: injector temperature rose from 50 to 300°C; column temperature program from 36 to 300°C at rate of 30°C/min; helium carrier gas; FID 350°C. Peaks: (1) valerophenone, (2) hexanophenone, (3) heptanophenone, (4) decanophenone, and (5) trim-chalcone.
of 1000 and 10,000×, respectively. The microstructural details revealed in these images clearly show that the created sol–gel zirconia coating possesses a porous make-up which substantially differs from that obtained by us for sol–gel titania coating [79].

Sol–gel zirconia–PDMDS-coated capillaries allowed the extraction of analytes belonging to various chemical classes. Experimental data highlighting CME-GC analysis of polycyclic aromatic hydrocarbons using a sol–gel zirconia–PDMDS coated capillary is shown in Fig. 4.

CME-GC experiments were performed on an aqueous sample with low ppb level analyte concentrations. Experimental data presented in Table 2 shows that CME-GC with a sol–gel zirconia–PDMDS coating provides excellent run-to-run repeatability in solute peak areas (3–7%) and the used sol–gel GC column provided excellent repeatability in retention times (less than 0.2%). It should be pointed out that the column used for GC analyses was also prepared in-house using a sol–gel method described by us in a previous publication [22].

The reproducibility of the newly developed method for the preparation of sol–gel hybrid organic–inorganic zirconia coated capillaries was evaluated by preparing three sol–gel zirconia PDMDS-coated capillaries in accordance with the new procedure and following their performance in CME-GC analysis of different classes of analytes extracted from aqueous samples. The GC peak area obtained for an extracted analyte was used as the criterion for capillary-to-capillary reproducibility which ultimately characterizes the capillary preparation method reproducibility. The results are presented in Table 3. For each analyte, four replicate extractions were made on each capillary and the mean of the four measured peak areas was used in Table 3 for the purpose of capillary-to-capillary reproducibility. The presented data shows that the capillary-to-capillary reproducibility is characterized by an RSD value of less than 5.5% for all three classes of compounds used for this evaluation. For a sample preparation method, a less than 5.5% R.S.D. is indicative of excellent reproducibility.

Fig. 5 illustrates a gas chromatogram of several free aldehydes extracted from an aqueous sample using a sol–gel zirconia–PDMDS coated capillary. Here, the concentrations of the used aldehydes were in 80–500 ppb range. The extraction was carried out on a 10 cm × 0.32 mm i.d. Sol–gel zirconia–PDMDS coated microextraction capillary for 30–40 min. The extraction of the analytes was performed at room temperature. Aldehydes are known to have toxic and carcinogenic properties, and therefore, their presence in the environment is of great concern because of their adverse effects on public health and vegetation [80]. Aldehydes are major disinfection products formed as a result of chemical reaction between disinfectant (ozone or chlorine) and organic compounds in drinking water [81]. Therefore, accurate analysis of trace-level contents of aldehyde in the environment and in drinking water is important [82]. Aldehydes are polar compounds that are often derivatized [83] for GC analysis to avoid undesirable adsorption that causes peak tailing. Sol–gel zirconia–PDMDS coated capillary provided highly efficient extraction of the aldehydes, and the used sol–gel GC column provided excellent peak shapes which is also indicative of high quality of deactivation in the used sol–gel GC column. This also demonstrates effective focusing of the analytes at the column inlet after their thermal desorption from the microextraction capillary. For the aldehydes, sol–gel CME-GC with the zirconia–PDMDS coated capillary provided excellent repeatability in peak area (R.S.D. < 5.5%) and retention time (R.S.D. < 0.16%).

Fig. 6 shows a gas chromatogram illustrating CME-GC analysis of several ketones extracted from an aqueous sampl...
Appendix B (Continued)


ple. Like aldehydes, there was no need for derivatization of the ketones, either during extraction or GC analysis. Sharp and symmetrical GC peaks, evident from the chromatogram, show the effectiveness of the used CME-GC system, as well as the practical utility of the mini-union metal connector providing leak free connection between the extraction capillary and the GC column. Excellent reproducibility was achieved in CME-GC of ketones using sol–gel zirconia-PDMS coated capillary as shown in Table 2. The peak area RSD% values for ketones were less than 5.6% and their retention time repeatability on used sol–gel PDMS column was characterized by K.S.D. values of less than 0.27%.

Fig. 7 shows a gas chromatogram illustrating CME-GC analysis of an aqueous sample containing different classes of compounds including PAHs, aldehydes and ketones, and shows that the sol–gel zirconia-PDMS extraction capillary can provide simultaneous extraction of polar and non-polar compounds present in the aqueous sample, and demonstrates the advantage over conventional SPME coatings that often do not allow such effective extraction of both polar and non-polar analyte from the same sample.

In capillary microextraction technique, the amount of analyte extracted into the sorbent coating depends not only on the polarity and thickness of the coated phase, but also on the extraction time. Fig. 8 illustrates the kinetic profile for the extraction of fluorone (a non-polar analyte), heptanone and undecyl aldehyde (both are moderately polar analytes) on a sol–gel zirconia-PDMS-coated microextraction capillary. The CME experiments were carried out using aqueous samples of individual test analytes. The extraction equilibrium for fluorone reached in 10 min, which is much shorter than extraction equilibrium time for heptanone and undecyl aldehyde (both approximately 30 min). This is because fluorone exhibits hydrophobic behavior that has higher affinity toward the non-polar PDMS-based sol–gel zirconia coating than toward water. On the other hand, heptanone and undecyl aldehyde, being more polar and hydrophilic than fluorone showed a slower extraction by the coated non-polar sol–gel zirconia-PDMS sorbent.

Sol–gel zirconia-PDMS coating showed high pH stability, and retained excellent performance after washing with 0.1 M NaOH (pH 13) for 24 h. Chromatograms in Fig. 9a and b show CME-GC analysis of four PAHs before (Fig. 9a) and after (Fig. 9b) zirconia-PDMS extraction capillary was rinsed with 0.1 M NaOH solution.

As is evident from Fig. 9, the extraction performance of the sol–gel zirconia-PDMS capillary remained practically unchanged after rinsing with NaOH as it can be seen in Table 4.

![Fig. 9. CME-GC analysis of PAHs using a sol–gel zirconia-PDMS coated microextraction capillary: before (a) and after (b) rinsing the microextraction capillary with 0.1 M NaOH solution for 24 h. Extraction parameters: 10 cm × 0.25 mm i.d. microextraction capillary; coating thickness 0.3 mm, extraction time 30 min (oven held at room temperature). Other conditions: 10 cm × 0.25 mm i.d. Sol–gel GC PDMS column; splitless desorption; injector temperature from 30 to 300 °C; column temperature program from 30 to 280 °C at rate of 20 °C/min; helium carrier gas: FID 350 °C. Peaks: (1) naphthalene, (2) fluorone, (3) phenanthrene, and (4) pyrene.](image-url)
Appendix B (Continued)

Table 4

| Name of the analyte | Peak area repeatability before rinsing with 0.1 M NaOH (arbitrary unit) | Peak area repeatability after rinsing with 0.1 M NaOH (arbitrary unit) | Relative change in peak area ($|d_b - d_s| / d_s \times 100$ (%) |
|--------------------|--------------------------------------------------------------------------------|-----------------------------------------------------------------------|-----------------------------------------------------|
| Acenaphthene       | 20380.91                                                                      | 20432.76                                                             | 3.12                                                |
| Fluorene           | 34525.63                                                                      | 34528.86                                                             | 0.90                                                |
| Phenanthrene       | 47547.13                                                                      | 46525.33                                                             | 2.15                                                |
| Pyrene             | 33864.61                                                                      | 33621.56                                                             | 0.77                                                |

For comparison, the same experiment was conducted using a 10 cm piece of a conventionally coated commercial PDMS-based GC column as the microextraction capillary. The results are shown in Fig. 10. A drastic loss in extraction sensitivity after rinsing the conventionally coated silica-based microextraction capillary with 0.1 M NaOH solution is obvious (Fig. 10).

These data suggest that the created hybrid sol-gel zirconia-based coatings have significant pH stability advantage over conventional silica-based coatings, and that such coatings have the potential to extend the applicability of capillary microextraction and related techniques to highly basic samples, or analytes that require highly basic condition for the extraction and/or analysis.

4. Conclusion

Sol-gel zirconia-based hybrid organic–inorganic sorbent coating was developed for use in microextraction. Principles of sol–gel chemistry was employed to chemically bind a hydroxy-terminated silicone polymer (polydimethylsiloxane) to a sol–gel zirconia network in the course of its evolution from highly reactive alkoxide precursor (zirconium tetrabutoxide) undergoing controlled hydrolytic polycondensation reactions. For the first time, sol–gel zirconia-PDMS coatings was employed in capillary microextraction. The newly developed sol–gel zirconia-PDMS coating demonstrated exceptional pH stability: its extraction characteristic remained practically unchanged after rinsing with a 0.1 M solution of NaOH (pH 13) for 24 h. Solventless extraction of analytes was carried out simply by passing the aqueous sample through the sol–gel extraction capillary for approximately 30 min. The extracted analytes were efficiently transferred to a GC column via thermal desorption, and the desorbed analytes were separated by temperature programmed GC. Efficient CME-GC analyses of diverse range of solutes was achieved using sol–gel zirconia-PDMS capillaries. Parts per trillion (ppt) level detection limits were achieved for polar and non-polar analytes in CME-GC-FID experiments. Sol–gel zirconia-PDMS coated microextraction capillaries showed remarkable run-to-run repeatability (R.S.D. < 0.27%) and produced peak area R.S.D. values in the range of 1.24–7.25%.
Appendix B (Continued)

[80] WHO, Air Quality Guidelines for Europe, WHO European, Series No. 23, Copenhagen, Denmark, 1997.
TITANIA-BASED COATING FOR CAPILLARY MICROEXTRACTION

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ABSTRACT

A method is presented describing in situ preparation of the titania-based sol-gel PDMS coating and its immobilization on the inner surface of a fused silica microextraction capillary. Sol-gel titania-poly(dimethylsiloxanes) (TiO2-PDMS) coating was developed for capillary microextraction (CME) to perform on-line preconcentration and HPLC analysis of trace impurities in aqueous samples. The sol-gel titania-based coatings demonstrated strong pH stability and enhanced extraction capability over other commercially available GC coatings. Extraction characteristics of a sol-gel titania-PDMS capillary remained practically unchanged after continuous rinsing with a 0.1 M NaOH solution (pH=13) for 12 hours.
ABOUT THE AUTHOR

Tae-Young Kim was born in Seoul, South Korea. He received a bachelor of health science degree in Medical Technology from Yonsei University in 1992. He transferred to the University of Minnesota for dual bachelor degrees in chemistry and microbiology. During his senior year, he conducted undergraduate research on zirconia columns for one year with a graduate student of Dr. Peter Carr, which motivated him to start his career in analytical chemistry. He joined SUNY; college at Buffalo for a master’s degree, and did research in organic synthesis under the direction of Dr. Subodh Kumar. However, he decided to move to the Department of Chemistry, University of South Florida, to pursue a doctorate and to continue his career in analytical chemistry. He joined Dr. Abdul Malik’s research group in 2001 and worked on developing sol-gel titania-based organic-inorganic hybrid coatings for on-line CME-HPLC. He has 2 publications in international journals and 1 US patent application.