Hydrolytic and Nonhydrolytic Sol-gel Zirconia-, Titania-, and Niobia-based Capillary Microextraction Coatings for the Preconcentration and HPLC Analysis of Catecholamine Neurotransmitters and Phosphorylated Peptides

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by

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A dissertation submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy
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Keywords: So-gel chemistry, surface-bonded hybrid organic-inorganic coatings, pH stability, positively-charged sorbent

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Dedication

To my wife, Maali, thank you for your unlimited support, this work was possible because of you.
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Abstract

Sample preparation is the most error-prone step in chemical analysis. A great deal of efforts has been made to develop efficient techniques and protocols for sample preparation to accomplish important goals such as miniaturization and implementation of green analytical methodologies. Solid-phase microextraction (SPME) has successfully eliminated the use of hazardous organic solvents in extraction sampling, sample preparation, preconcentration and sample introduction to the analytical instrument in an effective manner. Ensuring thermal- and solvent-instability of traditional SPME extraction phases represented one of their main drawbacks. This was solved by the introduction of sol-gel SPME phases characterized by enhanced thermal-, solvent-, and stability over a wide pH range. Sol-gel SPME phases (sorbents) facilitated excellent preconcentration effects for a wide range of analytes. In this dissertation, hydrolytic and nonhydrolytic sol-gel routes were explored for the creation of zirconia-, titania-, and niobia-based novel hybrid organic-inorganic sorbents using sol-gel active polymeric ligands. These sorbents were prepared in the form of surface coatings for capillary microextraction and preconcentration of biologically important molecules such as catecholamine neurotransmitters and phosphopeptides. In comparison with other sorbents made only of inorganic transition metal oxides, the presented sol-gel sorbents facilitated efficient desorption of the extracted analytes by LC-MS compatible mobile phases. The sol-gel zirconia- and titania-based hybrid sorbents provided pH-stable (pH range: 0 - 14) and derivatization-free extraction media that effectively overcame the major drawbacks of traditional sorbents for the analysis of catecholamines (silica-
based sorbents suffer from narrow operational pH window while polymer-based sorbents require additional sample derivatization steps). The modification of the terminal hydroxy groups in PPO with ZrCl₄ or TiCl₄ provided an enhanced sol-gel reactivity of the polymer modified-terminals. Such a modification procedure allowed for an efficient incorporation of the polymeric ligand into the evolving sol-gel network. The effectiveness of the PPO modification was also evaluated by a systematic thermogravimetric investigation exploring the loading of the ligand in sol-gel hybrid sorbents, which revealed an enhanced ligand-loading achieved via the nonhydrolytic sol-gel route used with modified-PPO. Sol-gel hybrid sorbents prepared by the nonhydrolytic sol-gel (NHSG) pathway provided excellent microextraction performance for catecholamines: low detection limits (5.6 – 9.6 pM), enhanced run-to-run reproducibility (RSD 0.6 – 5.1 %), excellent desorption efficiency (95.0 – 99.5 %) and high enrichment factors (EF) for epinephrine (EF ~ 1480) and for dopamine (EF ~ 2650) extracted from aqueous and synthetic urine samples at pH 10.5. Run-to-run and capillary-to-capillary reproducibility remained below 5 % when the peak area or the sorbent-mass was used as the reproducibility criterion. Niobia-based sol-gel sorbents prepared with and without organic ligand (polyethylenimine) were utilized as microextraction media for the enrichment of phosphorylated and nonphosphorylated tetrapeptide VYKA. Sol-gel niobia-based sorbents with covalently anchored polyethylenimine showed excellent selectivity toward the phosphopeptide compared to analogous titania-based sorbents. Specific extraction (SE) values were higher by 97.0 % when obtained by niobia-based sorbents. Excellent run-to-run peak area reproducibility (RSD < 5.1 %) and high EF of ~ 4000 were achieved. The sol-gel niobia-based coating facilitated excellent desorption efficiency (97.5 %), which suggests that the surface of the niobia sorbent possesses moderate-strength Lewis acid sites that avoided the need for special elution solvents that are conventionally used for the desorption of phosphorylated
molecules from titania-based sorbents. The sol-gel pathway for the creation of microextraction phases is versatile and capable to provide unique control on the characteristics of the sorbents that are critically important for many sample preparation applications.
Chapter One

Sol-gel Sorbents for Sample Preparation via Microextraction Techniques

1.1. Introduction

Real-life samples are normally acquiring analytes preconcentration, enrichment and cleanup steps prior to the introduction of the sample into the analytical method (usually chromatographic techniques). In principle, these steps are achieved by the sample preparation procedure that comes after the sampling step facilitating the processing of the target analyte/s through the analytical workflow. The later consists of several steps starting from sampling, sample preparation, separation, quantitation and data analysis. Among these steps, sample preparation is most likely to cause errors in the analytical procedure, which creates an increasing demand for innovative and efficient means of sample preparation to achieve maximum precision and accuracy at the end of the analysis. One of the most important goals of the sample preparation step is to extract the target analyte from its complicated matrix and delivering the analyte in a form that is compatible with the analytical instrument used in the subsequent analytical workflow.

Solid-phase extraction (SPE) was introduced as a sorbent-based sample pretreatment method providing an alternative tool to the conventional techniques such as; Soxhlet extraction, accelerated solvent extraction and liquid-liquid extraction that consumes large amounts of
organic solvents. In accordance with the global aims set by regulatory agencies such as the U.S. Environmental Protection Agency (U.S. EPA) for the minimization of the use of hazardous organic solvents in industries and laboratories, the introduction of SPE triggered tremendous efforts toward greener sample pretreatment methodologies.

The first appearance of a sorbent-based separation medium for liquid sample was in 1903 when Mikhail Tswett \(^4\) first used calcium carbonate as sorbent for the separation of plant pigment (Figure 1). Although his work is generally known as the beginning of the field of chromatography, it also allowed for the appearance of column liquid chromatography in the early 1930s, which has evolved and became very popular in many fields and research laboratories.

![Figure 1.1. Chromatographic apparatus from the early work of M. Tswett](adopted with permission from ref.5)

Between 1972 and 1974, Calder and coworkers \(^6,7\) used a miniaturized version of the column LC for the extraction of neutral organic compounds from potable water. Their work was the birth of solid phase extraction and since then it has been widely used and investigated for
sample cleanup, extraction and preconcentration in different areas such as environmental, clinical, forensics and many more applications. The development of wide range of SPE sorbents and formats facilitated the extraction and analysis of various target molecules present in different matrices. SPE became a predominant alternative to other solvent-based extraction techniques such as liquid-liquid extraction (LLE). In principal, the workflow of SPE technique starts with conditioning the SPE cartridge with appropriate solution, then loading the sample in the cartridge. After that, unwanted molecules and sample matrix is washed out with solvents that are typically do not interact with the target analyte taking the untargeted molecules out of the cartridge and leaving behind the target analytes adsorbed on the sorbent surface. Finally, appropriate elution solvent is used for the desorption of the target analyte after the preconcentration and cleanup steps.

Although SPE technique has successfully provided an efficient means of sample pretreatment and has drastically reduced the consumption of pure organic solvents compared to LLE technique, the SPE still requires consequent use of different solvents through its multiple steps. Many developments have occurred on the formats and type of sorbents used in SPE to fulfill the demands of the global environmental, health and safety aims toward greener approaches with minimal (preferably eliminated) use of hazardous pure organic solvents. In this context, the introduction of a solvent-free, environmentally-friendly and non-exhaustive method came as a logical development for SPE as seen in the next section.

1.2. Solid-phase Microextraction (SPME)

The first appearance of SPME was in 1989 when Belardi and Pawliszyn used a fused silica fiber mounted on a Hamilton syringe for the extraction of organic compounds from
aqueous matrix. The technique was then further described in another article \(^\text{14}\) that got about 2700 citations since it was published (according to Scifinder.cas.org as in July 2016). On its original format, SPME syringe-like device (Figure 2) provided an efficient means of extraction of trace-level chlorinated organic molecules using a thermally-activated polyimide outer coating of fused silica fiber that was later introduced to a GC.

Figure 1.2. SPME syringe device as described by J. Pawliszyn (permission taken from ref. 14)

1.2.1. Fundamentals of SPME

The introduction of SPME technique can be considered as one of the most significant advancement in the field of sample preparation, preconcentration, and trace level analysis. SPME integrates very important characteristics such as being non-exhaustive technique, environmentally friendly, and providing a green alternative to the traditional extraction techniques such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE) that often consume large volumes of hazardous organic solvents. Also, SPME technique overcome some of the main shortcomings of the traditional sample preparation methods by either structural features or physicochemical properties of extraction media. The elimination of solvent-consumption is very important advancement offered by SPME for the analytical and environmental laboratories that parallel the increasing emphasis of the regulatory agencies on reducing or even eliminating
the use of hazardous solvents and developing methodologies that produces less waste. SPME is based on the sorption/desorption equilibrium of analytes between the sample matrix and the extraction phase. In this context, the maximum adsorption of the analyte by the extraction phase can be reached when the partitioning equilibrium is established, and since the extraction phase in SPME is a micrometer-sized coating or film, only small portion of the analyte is extracted at equilibrium allowing SPME to be a non-exhaustive technique\textsuperscript{15}.

The microextraction process in SPME is typically performed by either direct immersion of the coated fiber in the sample or by fixing the coated fiber in the head-space of the sample especially when volatile molecules are analyzed (Figure 3). The target analytes get adsorbed on the surface of the SPME extraction phase via intermolecular interactions (e.g., van der Waals forces, dipole-dipole interactions, H-bonding, etc.). For operational simplicity and portability, the coated fiber is installed on the syringe-like device (SPME syringe).

![Figure 1.3. Illustration of the mode of extraction by fiber-SPME](image)

The exposure of the extraction sorbent to the sample matrix facilitates a series of sorption/desorption steps for the analyte molecules on the surface of the coating, until equilibrium is reached. The fact that SPME is an equilibrium-based non-exhaustive extraction technique, has gained SPME an excellent reputation in trace-level analysis. As seen in Equation...
1, the original concentration ($C_o$) of the target analyte can be mathematically calculated based on the amount of the extracted analyte at equilibrium ($n$), the distribution constant ($K_{fs}$), the volume of sorbent on the fiber ($V_f$), and sample volume ($V_s$).

$$n = \frac{K_{fs} \cdot V_f \cdot V_s \cdot C_o}{K_{fs} \cdot V_f + V_s}$$

Equ. 1.1

Considering the fact that $V_f$ is very small compared to $V_s$, under typical SPME conditions ($K_{fs} \cdot V_f) \ll V_s$, Eq. 1.1 can be further simplified to Eq. 1.2.

$$n = K_{fs} V_f C_o$$

Equ. 1.2

SPME is commonly coupled to gas chromatography (GC) and high-performance liquid chromatography (HPLC). The extracted target molecules are thermally desorbed from the sorbent in SPME-GC $^{14,16}$ or by mobile phase in SPME-HPLC $^{17}$. SPME provides an effective means of sample introduction to chromatographic systems using a simple device requiring small volumes of the samples that is sufficient to perform the analysis in few minutes. This particular property of SPME is important when many samples are tested (for example, thousands of samples are tested for drinking water monitoring). Also, the use of SPME for living system was demonstrated and facilitated a promising tool for analysis that do not make any significant disturbance or interference to the biological processes $^{18}$.

1.2.2. Developments in Formats and Extraction Sorbents of SPME

To provide important characteristics to the extraction techniques such as high throughput, high loading capacity, automation, miniaturization and hyphenation to analytical instruments, researchers have developed and employed a variety of formats and extraction sorbents in the area
of SPME. In the past ten years, a good number of review papers appeared in the literature covering important aspects related to SPME such as the recent developments in its formats (in-tube \textsuperscript{19} and thin film \textsuperscript{20} SPME), its hyphenation to liquid chromatography \textsuperscript{21}, SPME calibration methods \textsuperscript{22}, \textit{in-vivo} sampling \textsuperscript{23} and SPME applications in toxicology \textsuperscript{24}, bioanalysis \textsuperscript{18,25-27}, food and flavor analysis \textsuperscript{28}, green analytical chemistry \textsuperscript{29} and water analysis \textsuperscript{30}. In this section, the recent developments in both the formats of SPME devices or in the sorption material are presented.

1.2.2.1. SPME Formats

1.2.2.1.1. Fiber-SPME

Solid-phase microextraction is predominantly used in the fiber-based format. In which, the extraction phase is in the form of a surface coating (~ 1 cm long) on a fiber rod (either fused silica or stainless steel) mounted on a syringe-like device (Figure 1.4). The simplicity of the fiber-SPME device facilitated its portability and suitability for on-field sampling and sample preparation. The process of extraction is typically conducted by either direct immersion of the sorbent coating in the sample or by mounting the coated fiber in the headspace of the sample.
The hyphenation of fiber-SPME to GC is a straightforward approach; the SPME device serves as an efficient mean of sampling, sample preparation and sample introduction to the GC. The needle in the SPME device penetrates the rubber septum in the injection port of the GC and by pressing the plunger of the SPME device, the sorbent coating will be exposed to the stream of the gaseous mobile phase. Thermal desorption is then performed by applying elevated temperature in the injection port, allowing the extracted analytes to leave the extraction phase and travel with mobile phase to the GC column. Fiber-SPME-GC provides solvent-free, non-exhaustive, and environmentally-friendly sampling tool that has been applied in pharmaceutical, environmental, and forensic studies. Recently, new formats for fiber-SPME (Figure 1.5: (a) arrow-SPME and (b) plunger-in-needle SPME) that are practically have a similar working principle to fiber-SPME, have been introduced to providing mechanical stability and a firmer point of penetration of the coated fiber to the analytical instrument such as GC.
Figure 1.5. Illustration of (a) arrow SPME and (b) plunger-in-needle SPME (adopted with permission from references 34 and 35, respectively).

The hyphenation of fiber-SPME particularly to liquid chromatography (LC) can be considered of more importance than other analytical instruments due to the wide reputation of LC in the analysis of thermally labile, low-volatility, and large molecular weight molecules such as proteins, peptides, drugs, and toxins. Chen and Pawliszyn have demonstrated the first attempt to couple fiber-SPME to HPLC through a tee-shape desorption chamber with two ports connected to the Rheodyne 6-port valve in the place of the sample loop. The fiber-SPME needle in introduced through the third port of the tee desorption chamber. One of the advantages offered by this arrangement is the complete desorption (no carry-over) of the extracted PAHs unlike SPME-GC analysis for analogous compounds. The complete desorption was attributed mainly to the solvent-desorption of the analyte by the stream of the mobile phase. Nowadays, specially designed valves are commercially available by Supelco for the SPME/HPLC interface.
1.2.2.1.2. Capillary Microextraction (CME)

Capillary microextraction (CME), also known as in-tube SPME, is the capillary format of SPME where the extraction phase is either in the form of a monolithic rod residing inside the capillary or as a sorbent coating on the inner surface of a capillary or tube. In the early report of in-tube SPME, a piece of GC capillary column coated with polydimethylsiloxane (PDMS) was used for the extraction of polycyclic aromatic hydrocarbons (PAHs) from water in hyphenation to HPLC and thus it has extended the application of SPME for the analysis of thermally labile or nonvolatile molecules that can’t be analyzed by GC. CME solved major shortcomings of fiber SPME, such as the susceptibility to mechanical damage of the sorbent-coated segment of the fiber, scraping of the coated sorbent and operational difficulties. Compared to fiber-based SPME (where the sorbent coating resides on ~ 1-cm of the outer surface of the fiber), the sorbent in CME is immobilized on the inner walls of a 40 to 60 cm fused silica capillary, allowing for the immobilization of a higher amount of the sorbent and thus providing higher sample loading that would definitely facilitate higher extraction capability. In some bioanalytical applications, on-line hyphenation with HPLC is crucially important to avoid sample loss or deterioration. Hyphenation of CME to chromatographic instruments allowed for integration and automation of sample preparation leading to higher sensitivity for the analysis of samples at trace-level concentrations.

1.2.2.1.3. Stir-bar Sorptive Extraction (SBSE)

Cramers et al. introduced the use of a coated stir bar (named stir-bar sorptive extraction SBSE) as an innovative extraction method that integrated the extraction and agitation of the sample in one tool. Their early work utilized a polydimethylsiloxane (PDMS)-coated magnetic
stir bar for the extraction of volatile and semi-volatile molecules from aqueous samples. Compared to SPME, SBSE showed higher loading capacity and better recovery because of the higher amount of the sorbent coated on the outer surface of the stir bar (Figure 1.6). Also, as a result of the higher amount of the sorbent, equilibration time in SBSE can take up to 120 minutes, which is considered longer than typical equilibration time in SPME (40 - 60 minutes). The thermal desorption in SBSE poses another difficulty with this extraction format, a specially designed desorption unit is must be used for the desorption of the extracted analytes from the coated magnetic bar, unlike fiber-SPME and CME, SBSE lacks the ability to integrates extraction and sample introduction to the analytical instruments such as GC.

Figure 1.6. Illustration of stir bar sorptive extraction

1.2.2.1.4. Hollow-fiber SPME

The use of hollow fiber (HF) extraction approach was first reported by Bjergaard and coworker 39 for the extraction of methamphetamine from aqueous solution. This study utilized liquid-phase microextraction approach by filling/impregnating the pores of the hollow fiber (typically, polypropylene hollow fiber) with liquid organic phase for the extraction of the analyte from an immiscible sample. Many other studies have been reported using similar approach for
the extraction of important analytes organochlorine pesticides \(^{40}\) and phthalate esters \(^{41}\) from aqueous samples. Due to the global aims toward the minimization of the use of hazardous organic solvents, researchers are increasingly investigating the use of immobilized sorbents (solid or liquid) as an extraction media. In this context, the logical development of HF-SPME is to incorporate sorptive material in the highly porous structure of the fiber. Polyhydroxy polyparaphenylene (PPP) \(^{42}\) was used to serve as a coating layer on the inner and outer surface (Figure 1.7) of the HF (physically deposited), and used for the extraction of organochlorine pesticides from aqueous samples.

![Figure 1.7. Schematic represents HF-SPME (adopted with permission from ref. 42)](image)

1.2.2.1.5. Thin film Microextraction (TFME)

The principle of TFME was introduced \(^{43}\) aiming to increase the extraction efficiency by utilizing the larger ratio of surface area to extraction phase. Compared to thick-coated fiber-SPME (100 \(\mu\)m PDMS fiber-SPME), the loading capacity and extraction efficiency were noticeably enhanced in TFME (1 cm x 2 cm, \(~25 \mu\)m thickness PDMS thin film) without the sacrifice of the analysis time. Using higher surface area of the extraction phase (\(~40\) times
higher surface area of PDMS) without increasing the thickness of the sorbent as in fiber-SPME or SBSE facilitated efficient means of extraction. The desorption of the extracted analytes in TFME still imposes difficulty for the routine analysis approaches using GC. An additional desorption unit must be used to accommodate the bigger size of the TFME device while in fiber-SPME, the introduction of the extracted analytes is much easier and possible for automation in both GC and LC applications.

The hyphenation of TFME with LC applications has shown better applicability and satisfaction of important parameters for sampling and sample preparation methods such as automation and high throughput. Recently, the use of 96-blade SPME \(^{44}\) system demonstrated high throughput and automated microextraction approach for the analysis of benzodiazepines spiked in human plasma and phosphate-buffered saline solution. The coated portion of each blade provided thin film geometry for the extraction phase that facilitated pronounced extraction efficiency, recovery and reproducibility of a biocompatible C\(_{18}\)-polyacrylonitrile coating \(^{44}\).

Similar approach for the enhancement of the extraction capability by increasing the surface area of the extraction phase was utilized for the introduction of planar SPME (PSPME) \(^{45,46}\). A porous glass fiber filter circles were coated with PDMS and methyltrimethoxysilane using sol-gel technology. The PSPME device was used for the extraction of illicit drugs and explosives from air sample. Compared to static PSPME, dynamic PSPME allowed for faster analysis that is especially beneficial for field-analysis using portable extraction devices.

1.2.2.1.6. In-needle and needle-trapped SPME

The operational principles of needle trap device (NTD) or in-needle capillary adsorption trap (INCAT) are similar to fiber-SPME. The main advantage of these devices is the robustness
and mechanical durability of the stainless steel needle that contains the sorbent (either coated on
the inner surface of the needle or trapped inside the needle). For convenient operational
conditions, the provided needle is easier to handle (compared to the expensive fiber-SPME
device) and considered almost unbreakable. Pawliszyn and coworkers \textsuperscript{47} reported the first use of
needle-trapped sorbent (Figure 1.8) for the analysis of airborne molecules such as PAHs
extracted from drug-inhaler air, insect-repellent spray and diesel exhaust in hyphenation to GC-
MS and simultaneously with fiber-SPME. Versatile performance was reported for the NTD
approach that also provided a cost-effective tool that can be loaded with wide range of sorbents
either commercial or in-laboratory synthesized.

Figure 1.8. Schematic illustration of the needle-trapped sorbent (Adopted with permission from
ref. 47).

1.2.2.2. Advances in SPME Sorbents

In the following sections, different microextraction phases will be discussed and
categorized as follows: conventional SPME sorbents that are commercially available and sol-gel
based in-laboratory synthesized sorbents. Many review papers have been published covering the
sol-gel sorbents employed in solid-phase microextraction applications \textsuperscript{48-55}
1.2.2.2.1. Conventional SPME Coatings

The structure of the syringe-like device used primarily in fiber SPME introduced great convenience of use, portability and facilitated on-site analysis\(^\text{16}\). One to two centimeter of fiber (fused silica fiber or metal alloy) is coated with the extraction phase and housed inside a needle that allows the penetration of the device through the rubber septum in the GC injection port or sample vial. To expose the extraction phase, the fiber is connected to a plunger assisted with a metal spring that when pressed, the coated fiber will be exposed to either sample matrix for analyte adsorption, or for the desorption of the extraction analyte into the stream of the chromatographic mobile phase (either GC or HPLC). The first polymeric sorbent used for the fiber SPME was made of polydimethylsiloxane (PDMS)\(^\text{15}\) which as a stable polymer already gained a wide reputation in gas chromatography. Rapidly, several types of polymer- and co-copolymer-based fiber SPME became commercially available by Supelco Analytical (part of Sigma-Aldrich Inc.). Polar, moderately polar, and nonpolar extraction phases allowed for the application of fiber SPME in the analysis of wide range of analytes. Table 1.1 present the commercially available fiber SPME with their coating type, thickness, phase chemistry, and examples of analyzed molecules. Further information regarding commercially available fiber SPME coatings can be found in extensive reviews recently published summarizing their application in bioanalytical and clinical\(^\text{56}\), metabolomics\(^\text{57}\) and environmental\(^\text{32}\) applications.

Although SPME has provided an efficient means of sample preparation tool that integrates sampling, sample preparation, analyte enrichment and introduction to the chromatographic instruments, it still suffers from several drawbacks mainly rinsing from the instability of the polymeric extraction phases. Swelling and stripping of the extraction phase is a problem seen on the long-term use of the conventional fiber SPME\(^\text{106}\) as a result of lacking
chemical bonding between the extraction phase and the fiber creates. Susceptibility to mechanical damage such as fiber bending and scratching is a problem for first time users as well as experts. High cost is another restraining factor that limit the use of this powerful tool.

Since the introduction of SPME, scientists have realized the importance of this technique because of its effectiveness and convenience, being environmentally friendly, and speedy steps. Many studies and report have been published on the development of novel extraction phases for SPME as well as many SPME device formats have also been introduced filling some of the gaps seen in the conventional fiber SPME either to increase he coating stability, selectivity, and sensitivity, or to provide a more suitable device format to enhance the sample preparation procedure. A great number of newly developed materials and polymers that possessed unique characteristics such as chemical-, thermal-, mechanical- and solvent-stabilities, unique intermolecular interactions or their combinations, target analyte size or shape recognition, and high surface area have been utilized in the fabrication of the extraction phase in SPME. In the coming sections of this chapter, notable achievements in this context are covered and categorized based on the material of SPME extraction phase.

1.2.2.2. Sol-gel based SPME Sorbents

The term sol-gel process is given to reactions where inorganic, modified inorganic or organic precursors (e.g., nitrates, halide salt or alkoxy-based) are used to prepare colloidal sol systems that evolve into oligomers, resulting phase transition to form a gel.
Table 1.1. Summary of the commercially available fiber-SPME sorbents with several examples for their applications

<table>
<thead>
<tr>
<th>Phase Material</th>
<th>Phase Type</th>
<th>Target Analyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polydimethylsiloxane (PDMS)</td>
<td>Nonpolar</td>
<td>Aliphatic hydrocarbons, polycyclic aromatic hydrocarbons PAHs (^{58-64}), haloethers (^{65}), polychlorinated biphenyls (^{66}), polybrominated diphenyls and diphenylethers (^{67}), chemical warfare agents (^{68}), antidepressants (^{69})</td>
</tr>
<tr>
<td>Coating Thickness, (d_f)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(d_f): 7, 30, and 100 (\mu)m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carboxen/polydimethylsiloxane (CAR/PDMS)</td>
<td>Nonpolar</td>
<td>Nitrous oxide (^{80}), volatile organic compounds VOCs (^{71}), volatile nitriles (^{72}), volatile biomarkers (^{73}), volatile alkylated selenium and sulfur (^{74})</td>
</tr>
<tr>
<td>Coating Thickness, (d_f)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(d_f): 75 and 85 (\mu)m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polydimethylsiloxane/divinylbenzene (PDMS/DVB)</td>
<td>Moderate</td>
<td>Semi-volatile soil organic compounds (^{75}), terpenes (^{76}), phthalates (^{77}), non-steroid anti-inflammatory drugs (NSAIDs)(^{78}), gunshot residues (^{79}), polycyclic (^{80}), organophosphate esters (^{81}), urinary metabolites (^{82}), megastigmarienones (tobacco flavors) (^{83}), fragrance allergens (^{84}), carbonyl volatile compounds (^{85})</td>
</tr>
<tr>
<td>Coating Thickness, (d_f)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(d_f): 60 and 65 (\mu)m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS)</td>
<td>Moderate</td>
<td>Coffee aroma (^{86}), fire debris (^{87}), volatile products of lipid oxidation (^{88}), tobacco flavor (^{89}), sulfur volatile compounds (^{90}), terpenes and phenylpropanoids (^{91}), carcinogenic acrylamide (^{92})</td>
</tr>
<tr>
<td>Coating Thickness, (d_f)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(d_f): 50/30 (\mu)m</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1.1 (Continued)

<table>
<thead>
<tr>
<th>Phase Material</th>
<th>Phase Type</th>
<th>Target Analyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbowax – polyethylene glycol</td>
<td>Polar</td>
<td>Chloramphenicol (^{93}), pesticides (^{94}), bisphenol A and phenolic compounds (^{31})</td>
</tr>
<tr>
<td>(CW) (d_f: 60 \text{ \textmu m})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyacrylate</td>
<td>Polar</td>
<td>Phenols and nitrophenols (^{95-97}), pesticides (^{98-101}), 2,4,6-trinitrotoluene (TNT) (^{102}), cationic surfactants (^{103}), nicotine and its metabolites (^{104}), diphenylamine (^{105})</td>
</tr>
<tr>
<td>(PA) (d_f: 85 \text{ \textmu m})</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

During this transition, a solid three-dimensional material is formed, possessing controllable characteristics such as rigidity, permeability, porosity and surface area that can play an important role in applications where such characteristics are essential features in the operation of the application such as chromatographic stationary phases and extraction media. The sol-gel routes provide a simple and suitable approach toward tunability and design of different inorganic-inorganic composites or hybrid organic-inorganic gels and solids modified at the nanoscopic molecular level.
This process is also known as hybridization\textsuperscript{108}, the resulting hybrid material shows new properties that can be useful in different applications; thin film optics\textsuperscript{109}, scratch-resistant coatings\textsuperscript{110}, monolithic column\textsuperscript{111}, catalysts\textsuperscript{112} and many more. Metal/metalloid alkoxides are the predominantly used as sol-gel precursors for the fabrication of sol-gel materials, due to their known chemistry and convenience of use. In sol-gel reactions, metal alkoxides undergo hydrolysis and condensation reactions to form a three dimensional network. These sol-gel processes are depicted in Figure 1.9, which shows typical sol-gel reactions of silicon alkoxide precursors:

![Sol-gel Hydrolysis and Condensation of Silicon Alkoxide](image)

Figure 1.9. Sol-gel Hydrolysis and Condensation of Silicon Alkoxide
In the presence of water, alkoxy groups in the sol-gel precursor are hydrolyzed and displaced with hydroxyl groups. Silicon alkoxide precursors can be hydrolyzed under either acidic or basic condition. Acid-catalyzed hydrolysis mechanism is a bimolecular nucleophilic substitution (S_N2), acid protonation makes the alkoxy group positively charged, making silicon atom susceptible to nucleophilic attack by the oxygen atom in water, a penta-coordinate transition state is formed and later an alcohol is produced as a by-product. Under basic conditions, hydroxyl groups attack the silicon atom directly and alcohol is produced as a by-product 107.

Malik and coworkers 113 succeeded in solving the major instability drawbacks of conventional coating used in fiber SPME (Figure 1.10). Sol-gel organic-inorganic hybrid silica-PDMS coating was chemically surface-bonded on fused silica fiber providing enhanced solvent and thermal (> 320 °C) stabilities and was utilized as an extraction phase for fiber-SPME. The enhanced thermal stability of the sol-gel coating allowed for the analysis of PAHs and other compounds that require high thermal desorption temperatures (in SPME-GC analysis). Also, the integrity of the sol-gel sorbent was practically unchanged due to (i) the chemical bonding between the sol-gel sorbent and the surface of the fused silica fiber and (ii) the covalent bonding between the polymer and the silica network.
Sol-gel Capillary Microextraction \cite{114} was also introduced by the same group, that allowed for the immobilization via chemical-bonding of thin film coatings to the inner surface of fused silica capillary via the condensation of the silanol groups located on the fused silica surface with the hydroxy groups of the hybrid organic-inorganic sol-gel cross-linked network. The chemical bonds between the coating and the substrate provided excellent thermal and solvent stabilities as compared to the conventional coating technology were the coating thin film is physically deposited on a substrate. Several sol-gel extraction phases were prepared for CME in hyphenation to GC or HPLC with distinguished characteristics providing excellent extraction performance of nonpolar, semi-polar, and polar analytes \cite{115-118}.

Sol-gel SBSE was introduced by Liu et al. \cite{119} to overcome stability problems associated with conventional sorbents physically deposited on the surface of the stir bars. Sol-gel PDMS coating provided a crack-free durable thick sorbent that achieved low LODs and excellent
extraction capability, loading capacity and short equilibration time compared to polymer-based SBSE.

In the next sub-sections we conduct a comprehensive revision of the major contributions in the area of SPME mediated by sol-gel sorbents. Due to the intrinsic characteristics that can be tailored by the fine-tuning of the sol-gel materials, the sub-sections of this part of the chapter will be categorized by the type of sol-gel extraction phase: polymer-, ligand-, cavitand-, carbon nanostructure-, metallic-, and ionic liquid-based sorbents in addition to the various approaches to enhance the selectivity of the sorbents such as: molecularly imprinted-, restricted access-, and immunoaffinity-materials will be covered with a special focus on the advantages provided and the drawbacks eliminated by these sorbents.

1.2.2.3.1. Polymer-based Sol-gel SPME Sorbents

Polydimethylsiloxane (PDMS) polymer was in the first report of sol-gel sorbents that provided unique characteristics that were very essential for the microextraction performance of the hybrid organic-inorganic sorbent. Excellent thermal stability (> 350 °C) and notable affinity toward nonpolar, moderately polar, and polar analytes gained PDMS wide popularity as a sorbent for microextraction applications. Many studies utilized PDMS as a sol-gel SPME sorbents for the analysis of PAHs, phenols, amines, phosphates, dextrorphan, BTEX, organochlorine pesticides, TNT, illicit drugs, herbicides, phthalate esters, geosmin, antiestrogens and many other analytes. In these studies, sol-gel PDMS sorbents facilitate low ng/L level of limits of detection (LODs) and excellent thermal and solvent stabilities. Segro and Malik investigated the microextraction performance of polydimethyldiphenylsiloxane PDM DPS (another polymer of the siloxanes family) that in
addition to the expected thermal stability, it was employed for the extraction of polyaromatics utilizing the \( \pi-\pi \) interactions provided by the phenyl groups on the polymer. Excellent reproducibility, LODs, and solvent stability was noticed. Remarkable thermal stability was achieved by this sol-gel sorbent, it showed practically no change in the microextraction performance even after it was rinsed with acetonitrile/water (1/1, v/v %) at up to 200 °C thermal conditions.

Although sol-gel PDMS sorbents have shown ability to extract polar analytes, that capability was enhanced by either the use of a modified PDMS or by preparing a mixed-mode sorbent utilizing PDMS and other ligand, functional material, or polymers to enhance the selectivity and sensitivity of the sol-gel sorbent toward the different analyte. Kulkarni 117 utilized sol-gel cyano-PDMS CME-GC for the extraction of wide variety of analytes (PAHs, aldehydes, ketones and free fatty-acids). Cyano-PDMS stationary phase have been well-known in the area of GC stationary phase for its thermal stability and selectivity toward polar molecules. Table 1.2 illustrate the reported combination of PDMS with various components used as sorbents in SPME applications.

Table 1.2. PDMS-based sol-gel sorbents for microextraction applications

<table>
<thead>
<tr>
<th>Functional material added to PDMS</th>
<th>SPME Format</th>
<th>Target analyte</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metal-organic framework</td>
<td>SBSE</td>
<td>Estrogens</td>
<td>133</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAHs</td>
<td>134,135</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Organophosphorus pesticides</td>
<td>136</td>
</tr>
<tr>
<td>Functional material added to PDMS</td>
<td>Format</td>
<td>Target analyte</td>
<td>Ref.</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>--------</td>
<td>------------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Covalent-organic framework</td>
<td>SBSE</td>
<td>Phenols</td>
<td>137</td>
</tr>
<tr>
<td>(triazine)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenyl- and vinyl-ligand</td>
<td>Fiber-SPME</td>
<td>BTEX</td>
<td>138-140</td>
</tr>
<tr>
<td>(Silane-based precursors)</td>
<td></td>
<td>Aromatics</td>
<td>141</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Organophosphorus pesticides</td>
<td>142</td>
</tr>
<tr>
<td>Open crown ether</td>
<td>Fiber-SPME</td>
<td>BTEX</td>
<td>143</td>
</tr>
<tr>
<td>Poly(vinyl alcohol)</td>
<td>Fiber-SPME</td>
<td>Polychlorinated biphenyl pesticides</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Organophosphorus pesticides</td>
<td>145,146</td>
</tr>
<tr>
<td>Divinylbenzene-butyl methacrylate</td>
<td>Fiber-SPME</td>
<td>Alcohols, fatty acids and esters</td>
<td>147</td>
</tr>
<tr>
<td>Propyl methacrylate-ligand</td>
<td>Fiber-SPME</td>
<td></td>
<td>148,149</td>
</tr>
<tr>
<td>(Silane-based precursor)</td>
<td></td>
<td></td>
<td>150</td>
</tr>
<tr>
<td>Polyethylene-polyvinyl alcohol copolymer</td>
<td>SBSE</td>
<td>Organic sulfur compounds</td>
<td>151</td>
</tr>
<tr>
<td>Cyanopropyl-ligand</td>
<td>SBSE</td>
<td>NSAIDs</td>
<td>152</td>
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<tr>
<td>(silane-based precursor)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Polysiloxane-copolymer</td>
<td>SBSE</td>
<td>Organophosphorus pesticides</td>
<td>153</td>
</tr>
<tr>
<td>Poly(divinyl benzene)-copolymer</td>
<td>Hollow fiber-SPME</td>
<td>Herbicides</td>
<td>154</td>
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<tr>
<td>Octadecyl-SBA</td>
<td>Fiber-SPME</td>
<td>PAHs</td>
<td>155</td>
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</tbody>
</table>
Polyethylene glycol (PEG) is a famous organic polymer that has been used in the area of chromatographic applications for a long time. Under the name Carbowax, many manufacturers have produced PEG-based capillary GC column for the analysis of polar molecules. Due to its excellent selectivity and sensitivity toward polar analytes, and in addition of being used in the conventional fiber-SPME sorbent, hydroxy-terminated PEG (4,000,000 g/mol) has been utilized as a sol-gel SPME sorbent for the analysis of phenols, phthalates, and organochlorine pesticides and has shown significantly higher extraction capability, shorter equilibration time and higher thermal stability compared to commercial Carbowax SPME sorbent. This report, the maximum desorption temperature was up to 300 °C, which is higher than the maximum desorption temperature recommended for the commercial Carbowax SPME by 40 °C. Such higher thermally stability can extend the use of the sol-gel sorbent to semi-volatile and non-volatile analytes that logically require higher desorption temperature. Augusto and coworker investigated the use of a shorter hydroxy-terminated PEG (~ 16,000 g/mol) chains for the preparation of polar sol-gel sorbent for fiber-SPME that also facilitate practically no carry-over and complete desorption of the extracted analytes. Chlorophenols were also analyzed by sol-gel PEG (2000 g/mol) SPME sorbent and nanograms per liter LODs were achieved. All these previous studies successfully utilized the hydrophilic character of PEG in the sol-gel sorbent, but all used lacked the use of efficient means of corporation of the organic polymer to the silica network. The difference in the reactivity between the terminals of the polymer and the silanol groups would definitely lead to poor incorporation of the PEG in the sol-gel materials.

Kulkarni and coworkers addressed this problem and have used a silane-based PEG (Figure 1.11) that can undergo the sol-gel reactions in comparable reactivities that allowed for the preparation of a better PEG-based sorbent. Excellent thermal stability that reported for the first
time for a PEG-based sol-gel sorbent (up to 360 °C) with a nanograms per liter LODs achieved for aldehydes, ketones, amines, phenols and carboxylic acids.

The significant improvement in the microextraction performance of the reported PEG-based sorbent was mainly attributed to the covalent bonding between the silane-based PEG and the silica network. Excellent thermal and solvent stabilities were observed with a high sorbent-to-sorbent reproducibility that confirms the superior performance of sol-gel coating method for the creation of excellent SPME sorbents.

Figure 1.11. A schematic representation of the sol-gel PEG sorbent presented by Kulkarni et al. ref. 118 (adopted with permission from ref. 118)
Yun L. utilized an in-laboratory synthesized diallyl-PEG polymer for the preparation of fiber-SPME sorbent that was later polymerized to vinyltriethoxysilane precursor in the sol solution to provide a direct chemical bonding between the PEG and the sol-gel network. Carasek et al. utilized PEG for the extraction of phthalate esters and achieved ng/L LODs with remarkable stability of the sol-gel sorbent even after 300 extractions. In their work, PEG was reacted with MTMS in the sol solution that was allowed to condense with the zirconia-coated NiTi alloy rod. The chemical bonding between the sol-gel sorbent and the zirconia surface allowed for the noticed operational and thermal (320 °C) stabilities.

Figure 1.12. Illustration of the FPSE (adopted from ref. 162 with permission).
PEG still possesses high popularity because of its availability, cost-effectiveness, and well-known characteristics. Recent study \textsuperscript{161} utilized functionalized-PEG with graphene oxide clusters to enhance the surface area of the sol-gel sorbent and provide it with additional intermolecular forces (\(\pi-\pi\) interactions) that made PEG more suitable for the extraction of aromatic amines. PEG was also used in an emerging format of SPME: fabric phase microextraction (FPSE) \textsuperscript{162}, where the sol-gel PEG is coated on a cellulose fabric substrate providing a cost-effective, flexible and easy to handle format of SPME (Figure 1.12) that achieved excellent extraction efficiency for sulfonamides and biologically important molecules (amphenicols \textsuperscript{163}) and triazine herbicides \textsuperscript{164}.

Gharari \textit{et al}. \textsuperscript{165} introduced a dual mode of fiber-SPME where a thin rod mounted inside a hollow rod and both mounted on a syringe. When the plunger of the syringe is pressed, the both ends are exposed simultaneously providing dual substrates that can be coated with any sorbent coatings to provide simultaneous extraction media. Dual PEG and PDMS were employed for head-space SPME of volatile molecules with wide range of polarity. The performance of the dual sorbents was superior compared to the performance of the PEG or PDMS sorbents individually.

Other polymers in the family of glycols such as polypropylene glycol (PPG), polytetrahydrofuran (poly-THF), and copolymers of PEG and PPG were also used for the preparation of sol-gel hybrid CME sorbents. Although these glycols are amphiphilic polymers (has both hydrophilic and hydrophobic nature), the higher the number carbon atoms in the glycol polymer repeating unit, the more the hydrophobicity character will be dominant. Sol-gel poly-THF \textsuperscript{116,166} sorbent for CME-GC experimentation achieved parts per quadrillion (ppq) LODs for analytes such as aliphatic alcohols that requires an extraction phase that possesses an amphiphilic
character. Excellent thermal and solvent stabilities were demonstrated for sol-gel glycol-based sorbent 167,168.

Conductive polymers such as polypyrrole (Ppy) are gaining an increasing reputation as polar sorbents for the analysis of β-blockers 169, aromatic compounds 170, organophosphorus pesticides 171 or biological VOCs 172. Excellent microextraction performance was noticed for this conductive polymer, especially when it was prepared within the sol-gel composite. Although no prove of direct chemical bonding was established between the Ppy/sol-gel composite, the entrapment of the polymer within the sol-gel network facilitated enhanced thermal and mechanical stabilities that lead to operational durability even after 150 extractions, low ng/L LODs, and fast equilibration time. Polyaniline (PANI) was also a good example for the use of conductive polymer entrapped within the sol-gel network as a hybrid CME coating for the analysis of non-steroidal anti-inflammatory drug (naproxen) 173. The high surface area and the π-π interactions jointly offered by the PANI/sol-gel composite allowed for excellent recovery and enrichment of naproxen from complex biological matrices such as urine and plasma. Analogous properties were obtained by using polyvinylimidazole as a polar organic polymer in the sol-gel sorbent for fiber-SPME. Efficient means of preconcentration for halogenated benzenes 174.

1.2.2.3.2. Carbon Nanostructure-based Sol-gel SPME Sorbents

Wu et al. 175 advantageously utilized the unique characteristics offered by hydroxy-functionalized fullerene (OH-C60) to create sol-gel PDMS-C60 that exhibited significantly higher loading capacity compared to sol-gel PDMS-, sol-gel fullerol-, and commercial PDMS-SPME. The direct chemical bonding between fullerene and the sol-gel network provided an efficient means of immobilization of this π-electronic rich macromolecule that facilitated remarkable
microextraction capability toward polychlorinated biphenyls, phthalic diesters from water used to dip PVC toys and cloths or human perspiration stimulate.

The introduction of carbon nanotubes (CNTs) by Iijima in 1991 initiated various investigation efforts toward the use of these graphitic tubes in different analytical applications such as gas sensing, biosensors, voltammetry, extraction and separation. Sol-gel CNTs SPME sorbent was introduced to utilize the hydrophobicity and high surface area of CNTs in the extraction of BTEX that worked jointly with methyl ligands and PDMS polymer in the hybrid sol-gel sorbent. Compared to pure sol-gel PDMS sorbent, noticeable extraction capability, thermal and solvent stabilities, and highly reproducible fiber-to-fiber and intra-fiber experimentations.

A facile approach was introduced for incorporation of single wall SW-CNTs in the sol-gel network. Acid-functionalized SWCNTs were reacted with silanol-terminated PDMS creating a covalent bonding between the sol-gel active polymer and SWCNTs (Figure 1.13).

Figure 1.13. Schematic representation of the chemically bonded CNTs via the sol-gel approach (adopted with permission from ref. 182)

Further incorporation of the SWCNTs-functionalized polymer in the sol-gel network allowed for the creation of a highly stable sol-gel hybrid sorbent that showed superior microextraction performance compared to commercial PDMS- and PDMS/DVB-SPME phases for the extraction of polybrominated diphenyl ethers.
Multi-wall carbon nanotubes (MWCNTs) \(^{183}\) was later used for SPME as a carbonic material with extremely higher surface area. Compared to carbon aerogels, three-time higher surface area was achieved by the sol-gel MWCNTs sorbent, which was used for the extraction of BTEX and phenols. Table 1.3 illustrates the intensive investigations of the use of CNTs and MWCNTs as an important component of the sol-gel SPME sorbent. In most of these studies, the sol-gel network served as an entrapment network for CNTs, very little investigation has been given to illustrate the chemical bonding between the sol-gel material and CNTs. Also, many of these studies used methyltrimethoxysilane (MTMOS) for the synthesis of the sol-gel network. Many of these studies that utilized CNTs in the extraction sorbent ignored the effect of the methyl group (which is well-known for the extraction of non-polar analytes \(^{184}\)) in their studies attributing all the extraction capabilities to CNTs or CNTs and the organic polymer added in the sol solution. Also, in similar manner, many studies ignored the effect of the used deactivating reagent poly-methylhydrosiloxane (PMHS) to block the silanol groups on the surface of the sol-gel sorbent. PMHS (even at low concentration in the sorbent) is expected to have an extraction capability for non-polar as it resemble the structure of PDMS.

Graphene \(^{209}\) is another carbonaceous material that gained a wide reputation in many fields (e.g., sensing and immunosensing \(^{210,211}\) and sample preparation \(^{212}\)) because of its intrinsic characteristics such as the \(\pi\) -electron rich system and the flat-opened surface (can provide specific surface area up to 2630 m\(^2\)/g). Zhang H. \(^{35}\) utilized graphene in combination with PDMS in a sol-gel sorbent for plunger-in-needle SPME for the analysis of polybrominated diphenyl ethers and UV filters \(^{36}\).
<table>
<thead>
<tr>
<th>Sorbent</th>
<th>SPME device</th>
<th>Mode of extraction (sample matrix)</th>
<th>Target analyte</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MWCNTs / PEG</td>
<td>Fiber-SPME</td>
<td>HS (air)</td>
<td>Methyl t-butyl ether, Monocyclic aromatic amine, Mercury</td>
<td>185, 186, 187</td>
</tr>
<tr>
<td>SWCNTs / PEG</td>
<td>Fiber-SPME</td>
<td>DI (aqueous and urine)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fiber-SPME</td>
<td>DI (aqueous)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fiber-SPME</td>
<td>HS (air)</td>
<td>Furan</td>
<td>188</td>
</tr>
<tr>
<td>SWCNTs / SiO$_2$</td>
<td>Needle trap SPME</td>
<td>Dynamic HS (air)</td>
<td>PAHs</td>
<td>189</td>
</tr>
<tr>
<td></td>
<td>Needle trap SPME</td>
<td>Dynamic HS (urine)</td>
<td>Xylenes, Toluene</td>
<td>190, 191</td>
</tr>
<tr>
<td>Amino-MWCNTs</td>
<td>HF-SPME</td>
<td>DI (aqueous)</td>
<td>Pesticides</td>
<td>192</td>
</tr>
<tr>
<td>COOH-MWCNTs</td>
<td>HF-SPME</td>
<td>DI (aqueous)</td>
<td>Phenobarbital, diethylstilbestrol, Naproxen</td>
<td>193, 194, 195</td>
</tr>
<tr>
<td>PEG-grafted MWCNTs</td>
<td>Fiber-SPME</td>
<td>HS (air)</td>
<td>BTEX, NSAIDs, phenols</td>
<td>196, 197, 198</td>
</tr>
<tr>
<td>PEG-grafted MWCNTs / TiO$_2$</td>
<td>HF-SPME</td>
<td>DI (aqueous)</td>
<td>NSAIDs</td>
<td>199</td>
</tr>
<tr>
<td>SWCNTs / TiO$_2$</td>
<td>Fiber-SPME</td>
<td>Direct Immersion</td>
<td>PAHs</td>
<td>200</td>
</tr>
<tr>
<td>MWCNTs / PDMS</td>
<td>Fiber-SPME</td>
<td>DI (aqueous)</td>
<td>PAHs</td>
<td>203</td>
</tr>
<tr>
<td>MWCNTs / PDMS</td>
<td>SBSE</td>
<td>DI (aqueous)</td>
<td>Co and Cd</td>
<td>204</td>
</tr>
<tr>
<td>MWCNTs / TiO$_2$ or ZrO$_2$</td>
<td>HF-SPME</td>
<td>DI (milk)</td>
<td>Metronidazole</td>
<td>205</td>
</tr>
<tr>
<td>SWCNTs / SiO$_2$</td>
<td>Fiber-SPME</td>
<td>Dynamic HS (air)</td>
<td>Halogenated VOCs</td>
<td>206</td>
</tr>
<tr>
<td>MWCNTs / SiO$_2$</td>
<td>Fiber-SPME</td>
<td>HS (human breath)</td>
<td>VOCs</td>
<td>207</td>
</tr>
<tr>
<td>Glycine-MWCNTs</td>
<td>HF-SPME</td>
<td>DI (urine and aqueous)</td>
<td>Venlafaxine</td>
<td>208</td>
</tr>
</tbody>
</table>
The achieved low ng/L LODs and extraction capacity surpassed those of commercial SPME sorbents for similar analytes. Sol-gel graphene sorbent were used for the analysis of organochlorine pesticides, VOCs, phenols, organophosphate ester and nitrobenzene. PEG-grafted graphene sorbent was also used for the analysis of aromatic amines. Zhang and coworkers introduced an efficient route for the direct immobilization of the graphene nanosheets via direct covalent bonding. Carboxylate-graphene nanosheets were bonded via amide linkage to titanium rod functionalized with aminopropyl-triethoxysilane (APTES). Although the presented pathway require sequential coating steps, it provided remarkable sorbent stability even after 200 extraction cycles and enrichment factors to ~ 8500 for phthalates from aqueous samples. Sehati et. al. investigated the role of the distribution of graphene on different substrates. More efficient performance was notice for graphene-based HF-SPME sorbent immobilized by sol-gel chemistry on TiO$_2$ nanowires compared to TiO$_2$ nanoparticles. This enhancement was attributed to the structure of the nanowires that might have reduced the agglomeration and provided a better distribution of graphene in the sorbent.

1.2.2.2.3.3. Ligand-based Sol-gel SPME Sorbents

Many silane-based sol-gel active precursors are commercially available via several vendors and manufacturers (e.g., Gelest Inc. Morrisville PA) at excellent purity and a variety of functional organic ligands and polymers that are covalently bonded to the silicon atom. The covalent bonding of the ligands to the sol-gel active precursors allowed for facile creation of sol-gel organic-inorganic hybrid sorbents for microextraction purposes.

Kabir and coworkers synthesized a highly-branched phenyl-terminated silane-based dendron precursor for the preparation of π-electron rich sorbent for CME-GC. This new sorbent
was the first non-linear organic ligand used in sol-gel sorbents that provided efficient extraction for wide range of chemicals (polar and non-polar), excellent thermal and solvent stabilities, and high fiber-to-fiber and run-to-run reproducibility (< 7 RSD % and < 8 RSD %, respectively). Azenha et al. utilized a mixture of phenyltrimethoxy- and methyltrimethoxy-silane precursors to create a sol-gel hybrid sorbent that showed comparable performance to thick PDMS sorbent for the extraction of benzene-containing molecules taking advantage of the hydrophobic effect if these ligands and the \( \pi - \pi \) interactions provided by the phenyl ligands which also has been used for the enrichment of Bisphenol A (BPA), polybrominated diphenyl ethers (PBDE) and explosives (TNT). A novel sol-gel phenyl-ended hyperbranched carbo-silane sorbent was synthesized and used as an extraction and separation phases for SPME-GC analysis of BTEX. The hybrid material facilitated excellent analytical performance that was attributed to the free phenyl groups at the end of the branched ligands.

Alkyl-ligands such as butyl-, octyl-, and octadecyl-ligands (C4, C8, and C18, respectively) has been used in the reversed-phase packing materials for LC column for a long time (especially C18). Octyl-silica monolithic capillary was utilized as a sol-gel monolithic CME coupled to \( \mu \)HPLC using capillary packed column for the extraction and analysis of PAHs from aqueous samples. The use octyl-triethoxysilane in the sol solution provided a chemically bonded organic ligand to the silica network via Si-O-Si-C linkage. Segro and Malik investigated the extraction capability of methyl group that was chemically bonded to the sol-gel sorbent (initially from MTMOS). The methyl-based silica sorbent exhibited excellent inter- and intra-fiber reproducibility, solvent and thermal (> 350 °C) stabilities in addition to the ability to extract polar, moderately polar, and nonpolar analytes. Their detailed investigation on the role of the length of the alkyl ligand (C1 vs. C8 vs. C18). Although all these sol-gel sorbent coatings
led to ng/L detection limits, this study also clearly revealed the superior extraction capability provided by the longer alkyl chains. Bagheri et al. \(^{229}\) presented propylmethacrylate-based sorbent using novel strategy that combines electrodeposition and sol-gel chemistry for the creation of the sorbent. Propanthiol-trimethoxysilane was electrodeposited by self-assembled monolayer technique on the surface of gold wire. The thickness of this first was controlled by the electric potential and the deposition time. The immobilization of this first layer was achieved by the strong thiol-Au interaction. Using sol-gel reactions, propylmethacrylate-trimethoxysilane was used to modify the sorbent and provided chemically bonded ligands to a durable and unbreakable substrate that can be a good alternative to fused silica fiber (known as fragile) or stainless steel wires (lacks strong adhesion forces to the sorbent). Similar approach was used for the immobilization of C\(_{12}\) ligand on the surface of Ag wires used in SPME-HPLC-UV analysis of benzophenones \(^{230}\). To achieve a similar goal, Chen et al. \(^{231}\) achieved a uniform formation of octadecyl-containing sol-gel sorbent on TiO\(_2\)/Ti wire to serve as strong and unbreakable substrate for fiber-SPME.

Charged ligands attracted attention for the creation of sol-gel sorbents for the retention of analytes with opposite-charge in different analytical approaches \(^{232}\). Amino groups are easily protonated at a pH medium below 9.0 \(^{233}\), N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AAPTS) \(^{234,235}\) was used for the creation of sol-gel microextraction sorbent for the preconcentration of metal ions from water samples via sol-gel CME coupled online with inductively coupled plasma-mass spectrometry (ICP-MS). The same group used mercaptopropyltrimethoxysilane (MPTS) \(^{236}\) to coat the inner surface of fused silica capillary to create a negatively charged sol-gel sorbent for the enrichment of Cu, Hg, and Pb from biological samples (human urine, human plasma, and food). The extraction was carried out in online
hyphenation to ICP-atomic emission spectrometry (AES). In another study, they combined MPTS and AAPTS to create an intensively charged sol-gel sorbent for the enrichment of arsenic and selenium \(^{237}\). For the creation of positively charged PDMS fiber-SPME, aminopropyltrimethoxysilane (APTMS) \(^{238}\) was used in combination with hydroxy-terminated PDMS in the sol solution to create a sorbent suitable to withstand alkaline conditions for efficient extraction of phenols \(^{238}\), organophosphorus pesticides \(^{239}\) and insecticides \(^{240}\). Monolithic sol-gel capillary \(^{241}\) was created using APTMS for the analysis of metal ions in human hair and urine. The monolithic format of the sol-gel CME microextraction tool facilitated higher sample capacity compared to coating format that led to higher sensitivity and excellent microextraction performance. In a detailed study, NH\(_2\), SH\(_-\), OH\(_-\), double bond- and epoxy-containing silane-based ligands (with and without hydroxy-terminated PEG) were thoroughly investigated for the creation of extraction phases for the analysis of several molecules such as estrogens, herbicides, PAHs, and triazines. No significant difference (for analogous conditions) between the studied ligands was observed, this was attributed to the ability of these ligands to initiate similar intermolecular interactions (e.g., dipole-dipole, dipole-induced dipole, van der Waals, and hydrogen bond interactions). Also, no effect was noticed for PEG in the interaction with these molecules, which also led the authors to conclude that PEG in these sorbents was not effective for the extraction process. Although in this study excellent microextraction performance, high relative recovery, and excellent reproducibility were obtained, at different sampling flow rate and extraction time, but an important parameter (the pH of the sample) was unchanged throughout the study. To maximize the extraction capability of ionizable molecules, the pH of the sample must be adjusted to two pH units above the pKa value for basic molecules (ex. amine-containing molecules), and two pH units below the pKa value for acidic molecules \(^{16}\).
Also, hydroxy-terminated PEG has a poor ability to condense with the sol-gel network, in other study \(^{118}\), sol-gel active PEG played an important role for the extraction of polar analytes. Polymers with low sol-gel activity are expected to have low concentration in the sol-gel sorbent that might lead to minimum effect in the microextraction process.

1.2.2.3.4. Metal oxide-based Sol-gel SPME Sorbents

Malik \textit{et al.} initiated an important line of sorbents, pH-stable sorbents, that added an important contribution for microextraction applications \(^{242,243}\). Their first reported sol-gel pH-stable sorbent coating was comprised of titania-based (titanium oxide TiO\(_2\)) PDMS sorbent for CME-HPLC \(^{243}\). Sub-parts per billion detection limits were achieved for PAHs, ketones and alkylbenzenes. The sol-gel hybrid showed intact performance even after treating the sorbent with highly basic solution (pH 13) for 12 hours. The same group introduced zirconia-based \(^{244}\) polydiphenyldimethylsiloxane (ZrO\(_2\)-PDMDPS) for CME-GC-FID analysis of various analytes that led to low part per trillion detection limits. More importantly, the use of zirconia sol-gel network in the formation of the coating attained the extraction phase an excellent stability at extreme pH conditions (pH range: 1~14), mechanical integrity, and various intermolecular interaction sites (Lewis acid, Bronsted acid and base interactions) in addition to the hydrophobic and \(\pi-\pi\) interactions provided by PDMDPS. These pH-stable sorbents were introduced to fulfill the shortcoming in the conventional SPME sorbent especially when the pH of the sample needs to be adjusted beyond the narrow operational window of silica-based sorbent (pH range: 2~8 \(^{245,246}\)). Sol-gel alumina-based PDMS was then introduced by Zeng \textit{et al.} \(^{247,248}\) exploring the advantages that can be obtained by the alumina for fiber-SPME-GC of polar compounds. Robust performance was facilitated by Al\(_2\)O\(_3\)-PDMS sol-gel sorbent even at extreme pH conditions (pH
range: 7-14) with remarkable reproducibility and thermal stability (~ 360 °C). Although transition metal oxides are known for their adsorptive nature, the ability of the sol-gel hybrid sorbent to withstand elevated temperatures for thermal desorption of the extracted analytes, facilitated excellent desorption and almost no carry-over problems.

The introduction of metal oxides in the fabrication of the sol-gel microextraction sorbents achieved very important enhancements for the integrity and stability of the extraction phases as well as it provided various extraction forces, the inertness of the transition metal oxides toward surface modification poses serious difficulties for the aims to tune and design hybrid organic-inorganic sorbents. Malik et al.249 introduced germania-based sorbents for sample preparation (CME-GC) and GC capillary column stationary phases. As an isostructural analogue to SiO₂, germanium oxide (GeO₂) provided many features to the sol-gel sorbents when compared to other metal oxides. Structural similarities to SiO₂ lead to the formation of homogeneous sol-gel germania-PDMS hybrid sorbents with excellent capillary-to-capillary reproducibility (< 6.0 RSD %). The appropriate control of the hydrolysis and condensation rates of tetramethoxygermane (TMOG) using trifluoroacetic acid (TFA) serving as a chelating agent facilitated the formation of (≡Si-O-Ge≡) that was distinguished by a characteristic IR stretch at ~ 960 cm⁻¹. Also, germania-based sorbent provided a pH stable microextraction sorbent that showed practically unchanged performance after it was continuously rinsed with 0.1 M NaOH (pH 13.0) for 24 hours. The same research 250,251 extended the application of germania-based sorbent for CME-HPLC applications. Remarkable solvent-, thermal- and pH-stability were obtained for sol-gel GeO₂-triblock PEO-PPO-PEO hybrid sorbent coatings. Up to 200 °C solvents were passed through the sol-gel GeO₂-based coated capillary that practically made not change in the performance of the microextraction sorbent. It was concluded that such extremely stable sorbent that could be
beneficial for high temperature liquid chromatography (HT-LC) applications. Various analytes were extracted achieving low detection limits (ng/L) within analysis time of less than 1 hour. The sol-gel germania-based sorbents presented in this work survived continuous exposure to extremely harsh pH conditions (pH range: 0 - 14) for 5 days. Various studies that utilized sol-gel metal oxide sorbents for microextraction applications are presented in Table 1.4.

Table 1.4. Metal oxide-based sorbents for microextraction applications

<table>
<thead>
<tr>
<th>Metal oxide</th>
<th>Analytical technique</th>
<th>Target analyte</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZrO$_2$</td>
<td>CME-ICP-MS</td>
<td>Cr, Cu, Cd, Pb</td>
<td>252</td>
</tr>
<tr>
<td>ZrO$_2$</td>
<td>HF SPME LC-MS</td>
<td>Alkyl phosphonic acids</td>
<td>253</td>
</tr>
<tr>
<td>ZrO$_2$-PEG</td>
<td>Fiber SPME GC-FID/ECD</td>
<td>Halophenols and phthalate esters</td>
<td>160</td>
</tr>
<tr>
<td>ZrO$_2$/TiO$_2$</td>
<td>HF SPME-GC-FID</td>
<td>Dimethylacetamide</td>
<td>254</td>
</tr>
<tr>
<td>TiO$_2$</td>
<td>CME-ICP-MS</td>
<td>V, Cr, Cu</td>
<td>255</td>
</tr>
<tr>
<td>TiO$_2$</td>
<td>HF SPME-ICP-MS</td>
<td>Cd, Co, Ni and V</td>
<td>256</td>
</tr>
<tr>
<td>TiO$_2$</td>
<td>Fiber SPME GC-MS</td>
<td>Phthalate esters</td>
<td>257</td>
</tr>
<tr>
<td>TiO$_2$-PDMS</td>
<td>Fiber SPME-GC</td>
<td>PAHs, phenols, amines</td>
<td>258</td>
</tr>
<tr>
<td>TiO$_2$-PMM</td>
<td>HS SPME-GC-FID</td>
<td>Aliphatic alcohols</td>
<td>260</td>
</tr>
<tr>
<td>Al$_2$O$_3$</td>
<td>CME-ICP-MS</td>
<td>As and Cr</td>
<td>261</td>
</tr>
<tr>
<td>Al$_2$O$_3$</td>
<td>HF SPME ICP-OES</td>
<td>Cu, Mn, Ni</td>
<td>262</td>
</tr>
<tr>
<td>TiO$_2$-PDMS</td>
<td>SBSE HPLC-UV</td>
<td>Amphetamines derivatives</td>
<td>259</td>
</tr>
</tbody>
</table>
Table 1.4 (Continued)

<table>
<thead>
<tr>
<th>Metal oxide</th>
<th>Analytical technique</th>
<th>Target analyte</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Al}_2\text{O}_3/\text{TiO}_2$</td>
<td>HS SPME-GC-FID</td>
<td>PAHs</td>
<td>263</td>
</tr>
<tr>
<td>$\text{Al}_2\text{O}_3/\text{TiO}_2$</td>
<td>HS SPME-GC-ECD</td>
<td>Chlorinated organic solvents</td>
<td>264</td>
</tr>
<tr>
<td>Ag-Zn-PEG</td>
<td>Fiber SPME GC-FID</td>
<td>Styrene</td>
<td>265</td>
</tr>
<tr>
<td>Zn/Al-TiO$_2$</td>
<td>Fiber SPME GC-FID</td>
<td>Valproic acid</td>
<td>266</td>
</tr>
<tr>
<td>ZnO-Graphene</td>
<td>Fiber SPME</td>
<td>Sulfur volatiles</td>
<td>267</td>
</tr>
<tr>
<td>Ta$_2$O$_5$</td>
<td>Packed syringe MALDI-MS</td>
<td>Phosphopeptides</td>
<td>268</td>
</tr>
</tbody>
</table>

1.2.2.3.5. Ionic Liquid and Polymeric Ionic Liquid-based Sol-gel SPME Sorbents

Ionic liquids (ILs) are ionic salts containing organic cation (e.g., imidazolium, pyridinium, pyrrolidinium, tetraalkylphosphonium, alkylammonium) and counteranions (e.g., either inorganic: $\text{Cl}^-$, $\text{PF}_6^-$, $\text{BF}_4^-$ or organic: trifluoromethylsulfonate ($\text{CF}_3\text{SO}_3^-$), bis((trifluoromethyl)sulfonyl)imide (N($\text{CF}_3\text{SO}_3$)), trifluoroethanoate ($\text{CF}_3\text{CO}_2^-$) with low melting. Pino V. et al.\textsuperscript{269} investigated the role of three imidazolium-based ILs for the preconcentration of PAHs prior to their extraction by conventional fiber-SPME coated with PDMS. Their results indicated that the ILs in the solution affected the extraction process because of the micellar aggregates that was formed in the solution and possible adsorption of ILs on the surface of PDMS that hindered the extraction of the analytes. Shearrow, A. and coworkers\textsuperscript{168,270} advantageously used this aggregation behavior of ILs to mediate the synthesis of sol-gel sorbents for CME-GC applications. The presence of the ILs in the sol solution facilitated a more porous morphology and also provided a better microextraction performance for the sol-gel sorbents compared to analogous sorbent prepared without the addition of ILs. Liu, M. et.al.\textsuperscript{271} introduced
a chemically bonded sol-gel ILs-based (Figure 1.14) for the first time as an extraction media for fiber-SPME for polar analytes.

Figure 1.14. Schematic representation of the sol-gel ILs sorbent presented by M. Liu et al. (adopted with permission from ref. 271).

Free radical polymerization facilitated the chemical bonding between the allyl-containing ILs that were mixed with vinyl-containing sol-gel precursors in the sol solution resulting a chemical bonding between the ILs and the sol-gel network that was coated on the surface of the SPME fiber. In addition to the thermal stability, mixed hydrophobic-hydrophilic nature and the good solvating ability provided by ILs, the role of the counteranions were investigated 272 and revealed a significant influence on the thermal stability, selectivity, and the extraction capability of three sol-gel SPME ILs sorbents functionalized with three different counteranions (BF\textsubscript{4}-, PF\textsubscript{6}-, and (N(SO\textsubscript{2}CF\textsubscript{3}))\textsubscript{2}). Sol-gel ILs-based sorbents were utilized for the analysis of various analytes such as PAHs 273, pentachlorophenols 274, estrogens and aromatic amines 275 phthalate esters 276, organophosphate esters 277, NSAIDs 278, methyl-\textit{t}-butyl ether 279 and chlorinated organic compounds 280. In all these studies, ILs-based sorbents illustrated excellent sorption
characteristics, stability and high sensitivity. Thiolene-coupling reaction was used to immobilize aromatic polymeric ILs onto the SPME fiber to enhance the affinity of the ILs sorbent toward PAHs as revealed by the octanol/water distribution coefficient measurements. Sol-gel coating technology assisted the formation of mixed mode SPME extraction phases utilizing ILs with PDMS and carbon nanotubes, calixarene cavitand, crown ether, crown ether, crown ether.

1.2.2.3.6. Sol-gel Host-quest Interaction SPME Sorbents

Crown ethers are comprised of a heteroatoms-ring with electron donor (usually oxygen) that reacts effectively with electron acceptors (such as metals) and also can provide hydrogen bond interaction with polar analytes. Zeng, Z. et al. presented a facile approach for the creation of covalently bonded sol-gel hybrid sorbent for fiber-SPME for the extraction of phenols using hydroxy-terminated dibenzo-14-crown-4 and hydroxy-terminated silicone oil. The size of the crown ether ring and the attached functional groups to the crown ether ring play an important role in determining the selectivity and sensitivity of the sol-gel crown ether-based sorbents as revealed in other studies.

Cyclodextrins (CDs) are cyclic oligosaccharide-based macromolecules providing 6-, 7-, or 8-units (α-, β-, γ-cyclodextrins, respectively) cavity-containing rings. CDs were used in separation science for a long type as chiral stationary phase for enantiomers separation. The first report on using CDs in sol-gel microextraction sorbents simple employed the sol-gel network for the immobilization-by-entrapment of CDs in the sol-gel sorbent for the analysis of NSAIDs by sol-gel CME-HPLC. It was noticed that the presented sol-gel sorbent was a suitable choice except it showed long equilibration time (>100 minutes). Li, G. et al. extended the advantages obtained by CDs as a sorbent for SPME by utilizing an effective pathway to chemically bind the
CDs to the sol-gel network. The fast equilibration time (~ 30 minutes) can be indicative to the easy-access of the target molecules to/from the properly oriented cavity of CDs. The chemical bonding of the CDs to the sol-gel network was investigated by IR spectroscopy studies. Sol-gel CDs-based sorbents were used for the analysis of amphetamines, polybrominated diphenyl ethers, VOCs, and polychlorinated biphenyls.

Calixarene and resorcinarene are another famous cavity-shaped cyclic macromolecule that were used in the preparation of highly selective sol-gel sorbents for SPME. The tunability of the sol-gel sorbents and the functional groups on calixarene structure allowed for the creation of innovative sol-gel hybrid sorbents that provided efficient microextraction performance in a similar manner as for cyclodextrins. Propranolol was efficiently extracted from urine sample using sol-gel calixarene-based sorbents providing remarkable selectivity (inducted by calixarene cavity) and excellent solvent and thermal stabilities (induced by the sol-gel network). Extensive work has been done for the investigation of the role of the functional groups attached on the rim of calixarene or the effect of the cavity size on the selectivity and the extraction capability of the sol-gel sorbents.

1.2.2.3.7. Miscellaneous Sol-gel SPME Sorbents

Sol-gel coating pathway provided efficient routes for the preparation of designable and tunable sorptive materials. The combination of sol-gel coating technology and the new materials is leading to an excellent properties for the extraction phases that wouldn’t be achieved by other means of integration. Ultra-large pore mesoporous cellular foams were for sol-gel fiber SPME for the extraction of bisphenol A and related compounds.
Aptamers, a single strand DNA/RNA molecules synthesized from nucleic acid libraries to demonstrate molecular recognition that can provide precise selectivity and high affinity toward biological molecules (e.g., proteins, peptides, nucleic acids, cells etc.). Sol-gel aptamer-based SPME sorbent was presented for selective enrichment of adenosine from complex matrix. The covalent immobilization for the aptamer molecules to the sol-gel sorbent was achieved via the amide linkage between APTES and the carboxyl group on the aptamer as revealed by the comparison of the adsorption capacity of another sorbent prepared with an aptamer without a carboxyl group.

Metal organic frameworks (MOFs), a new class of hybrid material with metal (or metal cluster) centers linked with organic ligands (e.g., dicarboxylic acid) forming a 3-D network. The intrinsic characteristics of MOFs are derived from the unique ability to design the frameworks selectively to reach optimum characteristics that facilitated the use of MOFs in various application areas such as catalysis, enzymology, and gas separation. Cui et al. reported the first appearance of MOF-199 as a microextraction sorbent for head-space fiber-SPME-GC analysis of alkylbenzenes. Although the MOF-sorbent (physically deposited on the fiber) provided as excellent enhancement factors (> 100,000) and nanograms per liter LODs, but the lack of chemical bonding between the MOF clusters and the fiber might lead to stability problems if an immersion SPME is required for nonvolatile analytes. Hu et al. have utilized the advantages obtained by MOFs (such as large surface area > 5000 m²/g) and sol-gel coating technology for the preparation of sol-gel hybrid MOF-PDMS sorbents for SBSE. The sol-gel network facilitated the immobilization of the MOF into the sorbent and provided adequate porous structure that allowed for the passage of the analyte from the sample matrix to the MOF and vice versa. Recently, Wang et al. proposed another sol-gel strategy for an
efficient immobilization of MOF into the extractive material. Carboxyl-functionalized graphene was mixed with the initial components of MOF-5 and then were subjected to solvothermal treatment identical to the conditions used for the creation of MOF-5. XRD patterns were used for the investigation of the covalent bonding between the graphene and the MOF crystals. Later, carboxyl groups on graphene were reacted with amine groups on APTES providing an amide linkage between the functional material and the sol-gel sorbent. Notable microextraction performance was obtained by the sol-gel graphene/MOF sorbents that was favorably compared to commercial fiber-SPME sorbents (PDMS and PDMS/DVB) for the analysis of triazole fungicides.

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Chapter Two

Nonhydrolytic sol-gel approach to facile creation of surface-bonded zirconia organic-inorganic hybrid coatings for sample preparation. I. Capillary microextraction of catecholamine neurotransmitters

Note to Readers

The enclosed data in this chapter have been previously published in Journal of Chromatography A, 2016, 1468: 23-32, and it has been added to this dissertation with permission from Elsevier.

2.1. Introduction

Analytical tools for efficient extraction, preconcentration and detection of catecholamines (dopamine, epinephrine and norepinephrine) in biological matrices (such as urine) are important from a clinical point of view. Catecholamines and their metabolites have been investigated as potential biomarkers for the diagnosis and monitoring of tumors associated with different types of cancers and neural disorders ¹. Excess production of catecholamines by these tumors can cause “hypercatecholaminemia” which may cause health complications such as cerebrovascular accident, heart failure, cardiomyopathy and other potent impacts on the cardiovascular system ². Catecholamines are excreted in urine mainly in the following forms: unchanged, deaminated...
metabolites, and $o$-methylated amines (metanephrines). Analyzing catecholamines in urine, plasma or blood samples require sample preparation, preconcentration and cleanup steps essential for the minimization of any interfering components that might be present in biological matrices. In current practices, catecholamine sample pretreatments are predominantly performed by solid phase extraction (SPE) utilizing two types of sorbents: (a) polymeric reversed-phase resins and (b) phenylboronic acid-functionalized silica particles. Polymeric sorbents are typically made of $N$-vinylpyrrolidone and divinylbenzene monomers $^3$, and they possess excellent pH stability as well as balanced hydrophilic-hydrophobic characteristics, but low specific affinity toward the polar catecholamines which can be improved through chemical modification (derivatization) of catecholamines prior to their extraction. This is typically accomplished via formation of diphenylboronate-catecholamine complex $^4$ to facilitate their analysis by HPLC $^5$ or capillary electrophoresis $^6$. A notable shortcoming is that extraction beds prepared from organic polymers possess slow mass transfer characteristics analogous to the chromatographic stationary phases prepared from polymeric materials $^7$. This may result in delayed or incomplete desorption of the extracted analytes from the sorbent bed causing sample loss and/or carryover problems.

Silica particles with phenylboronic acid (PBA) ligand have also been widely used in solid-phase extraction (SPE) for cleanup and enrichment of catecholamine samples. PBA ligand has high affinity toward cis-diol groups present in the catecholamines $^8$. The activation of the complexation ligand (phenylboronate, pKa $\sim$ 9.5 $^9$) requires conditioning of the SPE cartridge with high-pH buffer (pH 10-12) $^{10}$ giving rise to the main drawback of PBA-SPE cartridges due to inadequate pH stability of silica-based particles which are known to have a narrow operational pH window (pH 2 – 8) $^{11-14}$. Alumina has been used for the extraction of catecholamines
providing a pH-stable sorbent in the form of SPE sorbent\textsuperscript{15-21}. Extreme strong adsorptive characteristics of alumina requires pH manipulation and the use of phosphate buffers for the desorption and elution of the extracted catecholamines.

Using different metal/metalloid alkoxide precursors, Malik and coworkers\textsuperscript{22-25} have developed a number of sol-gel CME extraction phases providing excellent pH stability (0.0 \textendash{} 14.0) in CME-HPLC as well as enhanced thermal stability in CME-GC operations. They included titania-,\textsuperscript{22,26} zirconia-\textsuperscript{23} and germania-based\textsuperscript{14,24,25} hybrid organic-inorganic CME coatings. The sol-gel coating route provides a simple, convenient and effective approach to synthesizing organic-inorganic hybrid materials\textsuperscript{27}. The key to the success of the sol-gel coating (in addition to the unique physical and chemical properties of the created hybrid materials) is the chemical bonding of the sol-gel coating to the substrate (e.g., fused silica fiber or capillary).

Hydrolytic sol-gel (HSG) route\textsuperscript{11} was used to create those microextraction media. Non-hydrolytic (nonaqueous) sol-gel (NHSG) route has been investigated extensively in the field of catalysis for the creation of metal/metalloid oxides\textsuperscript{28,29}. In a nonaqueous environment, transition metal halide (e.g., ZrCl\textsubscript{4}) concurrently undergoes alcoholysis and condensation reactions leading to the formation of transition metal oxides\textsuperscript{30}. NHSG-generated transition metal oxides possess better water-tolerance, enhanced homogeneity, and more Lewis acid sites than Bronsted acid-base sites\textsuperscript{28,29,31,32}. NHSG route can provide uniformly dispersed transition metal oxide particles in organic solvents and allows for facile surface modification with organic moieties\textsuperscript{33-35}. The later property is important for the use of nonaqueous sol-gel route for the creation of hybrid organic-inorganic material with covalently bonded organic ligands. Here we present a systematic investigation on the synthesis and analytical evaluation of a novel zirconia-based sol-gel hybrid organic-inorganic sorbent to provide a biocompatible extraction medium integrating amphiphilic
properties with enhanced thermal-, mechanical- and pH stability characteristics that are important for the analysis of aqueous samples of free catecholamines and molecules structurally related to their deaminated metabolites.

2.2. Experimental section

2.2.1 Materials and instruments

Zirconium (IV) butoxide, zirconium (IV) chloride, ethanol, 1-butanol, toluene, hydroxy-terminated polypropylene oxide ($M_{\text{avg}}$ 3500), glacial acetic acid, catechol, quinol, resorcinol, 4-hydroxybenzoic acid, benzoic acid, vanillin, acetaminophen, dopamine hydrochloride, epinephrine hydrochloride, and serotonin hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). HPLC grade solvents (methanol, dichloromethane, tetrahydrofuran), polypropylene microcentrifuge tubes and micropipette tips were purchased from Fischer Scientific (Waltham, MA). Fused silica capillary (250 µm i.d.) with polyimide external protective coating was purchased from Polymicro Technologies (Phoenix, AZ). The following chromatographic equipment was used in this study: (a) an Agilent 1100 series HPLC system equipped with a Diode Array Detector (Agilent Inc., Santa Clara, CA), (b) a Varian 3800 model gas chromatograph with a flame ionization detector (currently Varian is a part of Agilent), (c) Rhyeodyne 6-ports valve (IDEX Health & Sciences, Oak Harbor, WA), (d) an in-laboratory built purging/filling system.36,37

2.2.2 Hydrothermal pretreatment of fused silica capillary

A one-meter segment of fused silica capillary (250 µm i.d.) was rinsed with 2 mL each of dichloromethane, methanol, and water using a gas pressure-operated purging/filling system36 at
10 psi. Both ends of the capillary were then sealed using an oxy-acetylene torch. The sealed capillary was placed in the GC oven and conditioned by raising the temperature from 40 °C to 350 °C at a rate of 1°C/min, holding the capillary at 350 °C for 200 min. After thermal conditioning, the capillary was cooled to room temperature and cut open on both ends using an alumina wafer. It was then placed in the GC oven with one end connected to the GC injection port allowing nitrogen gas to flow through the capillary, and the other end was left open and secured in the GC oven. Thermal conditioning of the capillary was performed under nitrogen purge (1 mL/min) as follows: (40 °C to 350 °C at rate of 10 °C/min, 120 min hold time at 350 °C). The capillary was then cooled down to room temperature and its inner surface was ready for coating.

2.2.3 Preparation of sol-gel zirconia-PPO coated capillary via non-hydrolytic sol-gel (NHSG) route

2.2.3.1 Solvents drying

In the NHSG process, the solvents must be free from water. For this, the solvents (butanol, toluene) were dried over molecular sieve (type 4A) by placing 15 mL of each solvent in a separate vial. A 10-gram amount of the molecular sieve was added to each solvent and vortexed for 2 minutes and then left airtight in the hood overnight. Two-mL aliquot of each treated solvent was transferred to a microcentrifuge vial and centrifugation was performed (10,000 rpm for 2 min) to eliminate any possible contamination from the molecular sieve particles. To test if the dried solvents still contained water, 0.5 g of anhydrous copper sulfate (white) was mixed with 1 mL of each dried solvent, then the mixture was thoroughly vortexed. The mixture was centrifuged to precipitate the copper sulfate powder, which would turn blue in
the presence of water. The drying procedure was repeated until no color change of CuSO$_4$ was observed.

2.2.3.2 Modification of organic polymer with zirconium tetrachloride

Prior to the preparation of sol-gel sorbents, the terminal hydroxyl groups of polypropylene oxide (PPO) were modified with zirconium tetrachloride. For this, PPO and ZrCl$_4$ were taken in a 25 mL round-bottom flask in molar ratio of 1:2 (PPO: 0.6 mmol, ZrCl$_4$: 1.2 mmol) and dissolved in anhydrous toluene (300 µL). The solution was stirred for 12 hours at 60 °C and then allowed to reach room temperature before using it for the preparation of sol solution.

2.2.3.3 Preparation of sol solution for the NHSG route

The sol solution was prepared as follows: in a polypropylene centrifuge vial, 46 mg of zirconium tetrachloride was dissolved in 74 µL of dried 1-butanol. In a second vial, 80 mg of modified PPO was mixed with 180 µL of dried toluene and vortexed thoroughly for 1 minute and it was left in the hood for 6 hours. Thereafter, polymer solution was vortexed for 1 minute and then it was transferred to the first vial containing butanolic solution of zirconium tetrachloride. The mixture was vortexed thoroughly to ensure homogeneity. The gelation time of this mixture was ~ 2 hours. Based on this gelation time, the coating of the capillary was performed after the solution was left to undergo reactions in the vial for 30 minutes, allowing further reactions to take place inside the capillary.
2.2.3.4 Creation of CME coating via NHSG route for CME-HPLC

Details of sol-gel coating technology can be found elsewhere. Briefly, a 60-cm piece of hydrothermally treated fused silica capillary was filled with the sol solution using a pressure-operated filling/purging system for coating under 15 psi nitrogen pressure. The exit end of the capillary was sealed with a rubber septum after first few drops of the sol solution came out of the capillary. The sol solution was allowed to reside in the capillary for 30 min. At the end of the in-capillary residence period, the liquid content of the capillary was expelled under 15-psi gas pressure, leaving behind a sol-gel coating on the capillary inner surface. Nine capillaries were prepared with different in-capillary residence times (starting from 10 minutes in-capillary residence and increasing the time by increments of 5 minutes) to optimize the best coating conditions. The coated capillary was thermally conditioned in a GC oven while simultaneously being purged with a flow of nitrogen gas. For this, one end of the capillary was connected to the GC injection port, and the other end was secured in the GC oven. The capillary was heated using a temperature program (40 °C - 150 °C @ 1 °C/min, with a hold of 300 minutes at 150 °C). The conditioned capillary was cooled down to room temperature and rinsed with 2 mL each of n-butanol and methanol with the help of the purging/filling system. Finally, the coated capillary was thermally conditioned in a GC oven under nitrogen purge (40 °C - 150 °C @ 5 °C/min, with a hold time of 300 minutes at 150 °C). At this point, the coated capillary was ready for CME-HPLC experiments.
2.2.4 Preparation of sol-gel zirconia-PPO coated capillary via hydrolytic sol-gel (HSG) route

2.2.4.1 Preparation of sol solution for HSG route

The sol solution was prepared as follows: in a polypropylene centrifuge vial, 70 µL of zirconium butoxide was mixed with 17 µL of glacial acetic acid. In a different vial, 80 mg of modified PPO was mixed with 200 µL of 1-butanol and vortexed thoroughly for 1 minute and left in the hood for 6 hours. The polymer solution was then vortexed thoroughly again for 1 minute and then transferred to the first vial containing zirconium butoxide and glacial acetic acid in solution. The mixture was vortexed thoroughly for 2 minutes, and 8 µL of de-ionized water was added to the mixture and followed by thorough vortexing for 2 minutes to ensure homogeneity of the sol-gel solution. The gelation time of this mixture was about 8 hours. Taking this fact in consideration, the sol solution was first allowed to undergo reactions in the vial for 6 hours before using it for coating CME capillary.

2.2.4.2 Creation of CME coating via HSG route for CME-HPLC

The capillary coating and conditioning procedures for the preparation of sol-gel CME via HSG route were analogous to the one described in the previous section for NHSG route.

2.2.5 Capillary microextraction coupled to high performance liquid chromatography (CME-HPLC)

The CME-HPLC experimental setup was described elsewhere. Briefly, a 40-cm piece of the sol-gel coated CME capillary was installed as an external sampling loop on a 6-port HPLC injection valve. Catecholamines and serotonin stock solution were prepared with serial dilutions with 0.1 M HCl to obtain a 1 mg/L concentration level. For synthetic urine stock solution (the
composition of the synthetic urine was adopted from ref. 38), dopamine and epinephrine were spiked at 1 mg/L concentration level. The samples for the CME experiments were prepared in ammonium hydroxide solution (pH 10.5) at 100 µg/L and 200 µg/L concentration levels for the aqueous and the synthetic urine samples, respectively. The spiked urine stock solution was vortexed for 1 minute and ultracentrifuged at 21,000 g for 2 minutes. The stock solutions of the other test probes were prepared using methanol via serial dilutions to 1 mg/L. These solutions were further diluted to 100 µg/L using deionized water. In the “sampling” position of the injection valve, the aqueous sample was allowed to pass through the CME capillary from an in-laboratory designed gravity-fed sample dispenser 37 via the injection valve. As the sample passed through the capillary, the analytes were extracted by the sol-gel coating on the capillary inner wall. After 40 min of extraction, the HPLC analysis was started by switching the valve to “inject” position, thereby desorbing the extracted analytes by the mixture of the HPLC mobile phases (phase A: methanol, phase B: ammonium acetate 20 mM, pH 3.8) flowing through the capillary and transferring them to the HPLC column (Zorbax C18 2.1 x 150 mm, 5 µm). For the analysis of catecholamines, the mobile phase composition (2/98 v/v % phase A: phase B) while for the test probes (30/70 v/v % phase A: phase B). For the analysis of the synthetic urine sample, the column was flushed with a mobile phase (90/10 v/v % phase A: phase B) for 10 minutes in between the CME-HPLC experiments to clean the HPLC column, which was then equilibrated with the mobile phase (2/98 v/v % phase A: phase B) for 10 minutes prior to next CME-HPLC analysis.
2.2.6 Characterization of the synthesized sol-gel materials

2.2.6.1 Characterization

FTIR and thermogrametric analysis were performed on HSG and NHSG ZrO$_2$-PPO sorbents. For this, freshly prepared sol solutions (as described in previous sections) were mixed with hydrothermally pretreated 5 µm diameter silica particles (0.2 g, 5 w/w % of the sol solution) in microcentrifuge vial and vortexed for 2 minutes. The prepared mixtures were used to coat the inner surface of borosilicate tube (3.6 x 6 mm) following a very similar coating/conditioning method as described in previous sections used for the preparation of the sol-gel CME capillaries. After thermal conditioning, the sol-gel materials were scraped off the tube surface with a stainless steel spatula and were used for FTIR and TGA analysis.

2.2.6.2 Coating thickness and volume

For the determination of the sol-gel CME coating volume, 10 cross-sectional SEM images (using Hitachi Scanning Electron Microscope SU-70) were taken from 10 random segments (~ 1-cm) of the prepared sol-gel CME capillaries and the coating average thickness was used to assess the coating volume. The following equation was used for the coating volume:

$$V = \pi \times L \times (R^2 - r^2),$$

where, L – capillary length, R – fused silica capillary radius from the center to the capillary wall, and r – coated capillary radius from the center to the coating surface.

2.2.6.3 Conversion of peak area to amount of extracted analyte

The chromatographic peak area was used as a quantitative measure of the extracted analytes. Calibration plots for all analytes were constructed by obtaining the average peak area for 3 replicate measurements conducted by directly injecting each of the standard solutions
representing a series of concentrations (0.1-, 0.5-, 1.0-, 5.0-, 25.0-, 50.0-, 75.0-, and 100.0 mg/L). The obtained average peak areas were plotted against the corresponding molar concentration of the injected solutions and the best-fit linear equation was used to convert peak area to molar concentration, and then to the amount of analytes extracted by the CME capillary.

2.2.6.4 Desorption efficiency (DE) %

To evaluate the completeness of desorption of the extracted analytes from the sol-gel CME sorbent, each sample was directly injected into the HPLC system using a 40-cm deactivated fused silica capillary as external sampling loop. The obtained peak areas were converted into analyte amounts using the calibration plots as described in the previous section. Each sample (containing 200 ng of analyte) was allowed to pass through the coated capillary for 40 minutes and the liquid exiting from the capillary was collected. The mass of every analyte in the collected liquid was then determined by direct injection into the HPLC system. The difference in the mass of analyte before and after the extraction (evaluated by direct injection) was considered as the extracted amount. After desorption and analysis of the CME-extracted analytes, the obtained amount of analyte was taken as the desorbed amount. DE % was then calculated using the following equation:

$$\text{Desorption Efficiency (DE) \%} = \frac{\text{Desorbed amount}}{\text{Extracted amount}} \times 100$$

2.3. Results and Discussion

The sol-gel reaction route provides a simple, convenient and effective approach to synthesizing organic-inorganic hybrid materials. Surface-bonded coatings were introduced by our group for open tubular columns in gas chromatography (GC), fiber-based solid phase
microextraction (SPME) \(^{40}\), and capillary microextraction (CME) \(^{37}\). The key to the success of the sol-gel coating (in addition to the unique physical and chemical properties of the created hybrid materials) is the chemical bonding of the sol-gel coating to the substrate (e.g., fused silica fiber or capillary).

Metal/metalloid alkoxides are predominantly used as sol-gel precursors for the fabrication of sol-gel materials, due to their high purity, controllable reactivity, and convenience of use. In hydrolytic route of sol-gel reactions, the sol-gel precursors undergo hydrolysis with practically concurrent polycondensation of the hydrolyzed or partially hydrolyzed precursor species among themselves and/or with other sol-gel active species in the solution. The physico-chemical characteristics of the metal/metalloid atom (size, coordination state and partial positive charge \(\delta M\)), alkoxy group size, together with temperature, solvent, catalyst, etc. represent the most important factors that affect the rate of hydrolysis and condensation of the alkoxide precursors. Compared to silicon alkoxides, zirconium alkoxides undergo significantly faster (by many orders of magnitude) hydrolysis and condensation \(^{11,41,42}\). In \(\text{Zr(OEt)}_4\), the partial positive charge on zirconium is +0.65. By comparison, in \(\text{Ti(OEt)}_4\) the partial charge on Ti is +0.63 and in \(\text{Si(OEt)}_4\) the partial positive charge is +0.32 on Si \(^{43}\). Such differences in partial charges and other parameters for zirconium and silicon result in greatly higher rate of hydrolysis \((k_h \sim 10^{-2} \text{ M}^{-1} \text{ s}^{-1})\) \(^{41}\) for \(\text{Zr(OEt)}_4\) precursor compared to hydrolysis rate for \(\text{Si(OEt)}_4\) \((k_h \sim 5 \times 10^{-9} \text{ M}^{-1} \text{ s}^{-1})\) \(^{11,42}\). In addition to the difference in the rate of hydrolysis, the condensation rate of hydrolyzed zirconium alkoxide precursor is significantly higher than analogous rate for silicon precursors \((k_c \sim 30 \text{ M}^{-1} \text{ s}^{-1} \text{ vs. } 10^{-4} \text{ M}^{-1} \text{ s}^{-1})\) \(^{11,41}\). In a sol-gel system that contains precursors with vastly different reactivities, there exists a great probability of preferential reaction taking place with the participation of chemical species characterized by higher reactivities. Thus, preferential
formation of zirconia is likely to occur when silica-based sol-gel active ligands or organic polymers are mixed with zirconium alkoxide. To create hybrid material systems by integrating different sol-gel-active species, it is important that the chemical reactivities of these species are comparable with each other. To that end, different solvents and chelating agents have been used to slow down the hydrolysis and condensation rates of transition metal alkoxides precursors.

2.3.1. Synthesis and characterization of the sol-gel sorbent coatings

In this study, we employed functionalization of hydroxyl-terminated polypropylene oxide with zirconium tetrachloride (Figure 2.1.) mixed with toluene as a non-oxygen containing reaction medium that has good solubility with ZrCl4, to provide an organic polymer having sol-gel-active terminals with chemical reactivities comparable to that for zirconium-based sol-gel precursors. The modification reaction of PPO was conducted in anhydrous toluene at 60 °C with continuous stirring analogous to a recent study dealing with the derivatization of terminal hydroxy group of PEG by reacting with TiCl4 using microwave-heating of the reaction mixture. Two types of structural and physiochemical characteristics encouraged us to use PPO as the organic component for the proposed hybrid sorbents: (a) the ability to provide H-bonding interactions and its amphiphilic character to provide intermolecular interactions conclusive to the extraction of catecholamines and (b) the ability to provide molecular level interactions (e.g., dipole-dipole, electrostatic forces, etc.) that are important for the creation of homogeneous sol solution composed of organic and inorganic components.

Acetic acid was used as a chelating reagent to reduce the fast hydrolysis rate of zirconium alkoxide precursors for the synthesis of the ZrO2-PPO sorbent via HSG route. The
hydrolysis rate zirconium trichloride groups on the terminals of PPO \((k_h \sim 2.1 \times 10^{-2} \ M^{-1} \ s^{-1})\), is comparable to that of zirconium alkoxide precursors \((k_h \sim 10^{-2} \ M^{-1} \ s^{-1})\). Figure 2.2. illustrates the sol-gel reactions via HSG and NHSG routes in solution and within the hydrothermally pretreated fused silica capillaries. For HSG route, hydrolysis (1-A) of zirconium butoxide takes place after chelation with acetic acid providing species that can undergo condensation reactions, either water condensation (1-B) or alcohol condensations (1-B’) between themselves and with the derivatized PPO (1-C) in the sol solution leading to the formation of a three-dimensional hybrid organic-inorganic network.

Figure 2.1. Illustration of the modification of PPO with zirconium tetrachloride

The reactions taking place in the sol solution during the preparation of the NHSG \(\text{ZrO}_2\)-PPO sorbent are depicted in Figure 2.2. These included alcoholysis (2-A), condensation with alkyl halide elimination (2-B) and polycondensation (2-C) occurred in the presence of the derivatized PPO with zirconium trichloride terminals groups producing sol-gel zirconia-PPO hybrid material. The hybrid organic-inorganic zirconia-based sorbent was synthesized \textit{in situ} by conducting the sol-gel reaction within the capillary where it also had the opportunity to undergo condensation reaction with the silanol groups on the inner surface of fused silica capillary as shown in Figure 2.2.
Figure 2.2. Illustration of HSG and NHSG reactions in solution inside the capillary for the preparation of sol-gel ZrO$_2$-PPO sorbents.
Figure 2.3 illustrates the scanning electron microscopic images of the cross-sectional view for both NHSG and HSG coated capillaries. The average coating thickness calculated for 10 segments of coated capillaries was 1.49 µm for NHSG and 1.81 µm and HSG coating. Based on this data, the calculated internal volume of the NHSG CME capillary was ~ 19.17 µL and that for HSG coated CME capillary ~ 19.07 µL. Knowing that the volume of a 40-cm segment of uncoated fused silica capillary is ~ 19.63 µL, the calculated sorbent coating volume is 0.46 µL for NHSG and 0.56 µL for HSG CME sorbent coatings, respectively.

Figure 2.3. Illustration of scanning electron microscopic images of (a) NHSG ZrO$_2$-PPO coated capillary and (b) HSG ZrO$_2$-PPO coated capillary at 10,000 magnification.

The results from FTIR spectroscopy investigation are shown in Figure 2.4. Here, the peaks at 1555 and 1548 cm$^{-1}$ are indicative of the presence of Zr-O-C bond $^{50}$ in the sol-gel material prepared by NHSG (black) and HSG (red) routes, respectively. The obtained sol-gel materials mimic the compositions and coating conditions used for the preparation of the sol-gel...
sorbents in the CME capillary. As evident from the FTIR spectra, the peak at 867 cm\(^{-1}\) can be attributed to the presence of Zr-O-Si bond \(^{51}\) between the sol-gel material and the silica particles. This data also indicates the feasibility of creating such covalent bonding between the sol-gel zirconia-based sorbents and the fused silica surface of the CME capillary. The sol-gel material prepared via hydrolytic route was treated with water to fully hydrolyze the residual zirconium tetrabutoxide precursors that might have undergone only partial hydrolysis or have not undergone hydrolysis at all during the synthesis. The presence of such species could interfere with the FTIR analysis by showing the presence of Zr-O-C bond between Zr and butoxide groups. This data provides evidence for the successful chemical bonding of PPO to the zirconia sol-gel network and the ability of the presented sol-gel coating routes to create covalently bonded sorbents on fused silica surface.

Figure 2.4. FTIR spectra for sol-gel sorbent prepared via NHSG (black) and HSG (red) routes.
2.3.2. Evaluation of the sol-gel coating pH-stability

To examine the chemical and pH stabilities of the prepared sol-gel sorbent of the present study, CME-HPLC experiments were conducted using a CME capillary coated with NHSG ZrO$_2$-PPO. The capillary was continuously rinsed with 1.0 M HCl aqueous solution for a period of 6 hours followed by rinsing with 50-mL of deionized water. The coated capillary was further rinsed with 1.0 M NaOH aqueous solution for a period of 6 hours and then was washed again with 50-mL of deionized water. Figure 2.5 shows the CME-HPLC chromatograms obtained for the comparison of CME performance of the prepared sol-gel zirconia-PPO coated capillary before and after the exposure to harsh pH conditions. It clearly shows the stability of the prepared sol-gel CME coating since its extraction capability remained practically unchanged. A comparison of the peak areas of these two chromatograms revealed a slight peak area increase (0.9 %, 0.18 %, and 0.37 % for nicotinic acid, serotonin and acetaminophen, respectively) obtained by CME-HPLC experiments conducted after rinsing the capillary with extreme-pH solutions. The slight increase in the extraction capability of the sorbent can be attributed to the renewed availability of some of the buried extraction sites on the surface of the sol-gel sorbent due to removal of possible surface contaminants after rinsing with harsh pH solutions.

2.3.3. Evaluation of the microextraction performance

To determine the time-required to establish the extraction equilibrium of the target analytes between the sol-gel sorbents and the sample matrix, extraction profiles were experimentally constructed. Figure 2.6 presents extraction profiles obtained by NHSG coated capillary.
Benzoic acid, catechol, dopamine, and epinephrine were extracted and the time-required to establish analyte equilibrium between the sample matrix and the sol-gel sorbent was estimated as the point on the time axis that corresponded to the start of the plateau on the extraction curve. The HSG coated capillary provided an analogous extraction behavior for the same analytes.
Catecholamine metabolites such as homovanillic acid (HVA), 3-methoxy-4-hydroxyphenylglycol (MHPG) and vanillylmandelic acid (VMA) possess chemical structures (Figure 2.7.) with similar chemical groups such as hydroxyl- and methoxy-groups, cis-diol, carboxylic acid and benzene ring. The following chemicals, catechol, resorcinol, quinol, vanillin, acetaminophen, benzoic acid and 4-hydroxybenzoic acid, were used as test probes containing similar functional groups as on the catecholamine metabolites.
- **Biomarkers (neurotransmitters)**
  - Dopamine
  - Epinephrine
  - Serotonin

- **Catecholamines metabolites**
  - 4-hydroxy-3-methoxyphenyl glycol (HMPG)
  - Homovanillic acid (HMV)
  - Vanillylmandelic acid (VMA)

- **Analytical probes related to biomarkers and their metabolites**
  - Acetaminophen
  - Vanillin
  - Benzoic acid
  - 4-hydroxybenzoic acid
  - Quinol
  - Catechol
  - Resorcinol

Figure 2.7. Illustration of the chemical structure of catecholamines, their metabolites and chemical analogs related to the biomarkers and their metabolites.
Table 2.1 represents results from CME-HPLC-UV experiments using a sol-gel ZrO$_2$-PPO sorbent obtained via NHSG and HSG routes. The obtained results show excellent run-to-run reproducibility (RSD 1.5 - 3.2 %) and picomolar-level limits of detection ranging from 260 to 820 pM obtained by HSG ZrO$_2$-PPO sorbent. Also, HSG sorbent provided higher affinity toward catechol compared to its isomers (resorcinol and quinol) as revealed by the specific extraction (SE) values (about ~ 90 % higher than quinol and 130 % higher than resorcinol). SE values pertains the extracted analyte mass per unit mass of sorbent, which helps to evaluate the selective interactions of the sorbent toward various analytes with different functional groups allowing for effective comparison of the performance of different extraction media with different ligands and formats. The higher affinity toward catechol can be attributed to the interaction between the two neighboring hydroxyl (cis-diol groups) on catechol with zirconium atom (Lewis acid). The extracted mass of 4-hydroxybenzoic (pKa 4.52) was higher than the extracted mass of benzoic acid (pKa 4.2) by 13.5 %. Since zirconia-based sorbents are oxophilic (interact with oxygen-containing molecules) the observed higher extraction and higher SE value of 4-hydroxybenzoic acid compared to benzoic acid can be attributed to possible additional Lewis acid-base interaction between the hydroxyl group on 4-hydroxybenzoic acid and the Lewis acid sites on the sorbent surface.

NHSG sorbent facilitated significantly better extraction performance and enhancement factor compared to HSG sorbent. Although the run-to-run peak area reproducibility was slightly better for the HSG sorbents as shown in Table 1, the achieved LODs by NHSG sorbent were lower by about 1 ~ 2 order of magnitude. Excellent LODs provided by NHSG sorbents for the deaminated probes ranged between 7.9 – 38.1 pmol/L. The lower limits of detection achieved using NHSG sorbent can be explained by the followings: a) nonhydrolytic route of sol-gel
reactions is known to produce more Lewis-acid sites than Bronsted-base sites on the surface of the sol-gel materials \(^{30}\), (b) nonhydrolytic sol-gel route has incorporated higher amount of the organic polymer into the sol-gel network as revealed by TGA data (Figure 2.8). Lewis acid-base intermolecular interactions (150-400 kJ/mol) \(^{53}\) are stronger than the intermolecular forces associated with hydroxyl groups (H-bonding 5-60 kJ/mol or charge assisted H-bonding 60-120 kJ/mole) \(^{54}\). The presented LOD values represent the analyte concentrations in the original sample that after CME enrichment provided a signal-to-noise ratio 3. Here, both detector response and sorbent enrichment capability are taken into consideration. Since NHSG sorbent provides higher enrichment factors than HSG counterparts, the reported LOD values are lower for the NHSG sorbents.

To investigate the difference in the loading of PPO in HSG and NHSG sorbents, thermogravimetric analysis was performed on these two types of sol-gel sorbents that were scraped off the surface of glass tube as well as on a sample of free PPO. As is evident from the TGA data, the NHSG sorbent contains significantly higher percent of PPO than the HSG sorbent. Furthermore, it was noticed that the pyrolysis temperature of PPO somewhat increased in the case of hybrid organic-inorganic sol-gel sorbents compared with free PPO. This can be attributed to the collective effect of covalent bonding and intercalation of PPO to/within the sol-gel network. Figures 2.9 represents chromatograms obtained in CME-HPLC analysis of quinol, resorcinol, catechol, acetaminophen and 4-hydroxybenzoic acid extracted from an aqueous sample. Excellent performance of the sorbent in simultaneous extraction of multiple target analytes is evident from this chromatogram.
Table 2.1. CME-HPLC-UV results of various analytical probes from aqueous sample.

<table>
<thead>
<tr>
<th>Probe</th>
<th>Run-to-run RSD (%)</th>
<th>LOD (pM)</th>
<th>SE (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechol NHSG</td>
<td>5.6</td>
<td>36.3</td>
<td>24.5</td>
</tr>
<tr>
<td>HSG</td>
<td>1.5</td>
<td>32.0</td>
<td>21.2</td>
</tr>
<tr>
<td>Resorcinol NHSG</td>
<td>6.1</td>
<td>36.3</td>
<td>9.7</td>
</tr>
<tr>
<td>HSG</td>
<td>3.2</td>
<td>70.0</td>
<td>9.7</td>
</tr>
<tr>
<td>Quinol NHSG</td>
<td>1.8</td>
<td>38.1</td>
<td>13.5</td>
</tr>
<tr>
<td>HSG</td>
<td>2.1</td>
<td>60.0</td>
<td>11.2</td>
</tr>
<tr>
<td>Acetaminophen NHSG</td>
<td>7.2</td>
<td>19.2</td>
<td>18.1</td>
</tr>
<tr>
<td>HSG</td>
<td>3.1</td>
<td>27.0</td>
<td>14.4</td>
</tr>
<tr>
<td>4-hydroxybenzoic acid NHSG</td>
<td>2.9</td>
<td>7.9</td>
<td>36.9</td>
</tr>
<tr>
<td>HSG</td>
<td>2.7</td>
<td>44.6</td>
<td>20.5</td>
</tr>
<tr>
<td>Benzoic Acid NHSG</td>
<td>3.1</td>
<td>9.1</td>
<td>28.9</td>
</tr>
<tr>
<td>HSG</td>
<td>1.4</td>
<td>82.0</td>
<td>18.1</td>
</tr>
<tr>
<td>Vanillin NHSG</td>
<td>3.1</td>
<td>10.6</td>
<td>25.5</td>
</tr>
<tr>
<td>HSG</td>
<td>2.1</td>
<td>26.0</td>
<td>19.7</td>
</tr>
</tbody>
</table>

Figure 2.8. Illustration of the TGA curves for unreacted-PPO, NHSG ZrO2-PPO and HSG ZrO2-PPO.
CME-HPLC-UV chromatogram of quinol, resorcinol, parahydroxybenzoic acid, acetaminophen (at 100 µg/L concentration level) and catechol (at 50 µg/L concentration level) extracted from aqueous sample (pH 7.0), using NHSG zirconia-PPO sorbent. Mobile phase composition: minute 0, 10/90 (v/v %) methanol: ammonium acetate (20 mM, pH 3.8), minute 20: 30/70 (v/v %) methanol: ammonium acetate (20 mM, pH 3.8). UV wavelength at 225 nm.

2.3.4. CME-HPLC analysis of catecholamines and serotonin samples using sol-gel coatings

Catecholamines, serotonin, and their metabolites are important diagnostic biomarkers for neuroendocrine cancer types and neurodegenerative diseases. A good number of the recently published studies lack LC-MS compatibility. Some other studies are based on performing lengthy chemical derivatization, which may cause loss of sample. Our study utilizes an LC-MS compatible mobile phase (ammonium acetate buffer, 20 mM) and it is derivatization-free which shows the applicability of the sol-gel ZrO$_2$-PPO coatings in capillary microextraction for the clinical investigations of the catecholamines and their metabolites. To maximize the extraction efficiency of the sol-gel sorbents for free catecholamines, the pH of both the aqueous and the synthetic urine samples containing dopamine and epinephrine were adjusted to pH 10.5 using aqueous ammonia solution (~ two pH units higher than the isoelectric points ($pI$) of catecholamines which range between 8.5 to 9.0). Because of the issues with stability, the use of such a high pH is problematic with silica-based sorbents.
Table 2.2. represents CME-HPLC-UV results for dopamine, epinephrine and serotonin using HSG and NHSG zirconia-PPO sorbents. Excellent desorption efficiency (DE ~ 95 – 99.5 %) was obtained for dopamine, epinephrine and serotonin using NHSG and HSG sorbents. The NHSG ZrO\textsubscript{2}-PPO sorbent provided significantly lower LODs when compared to HSG ZrO\textsubscript{2}-PPO sorbents. NHSG ZrO\textsubscript{2}-PPO achieved LODs of 5.6 pM and 9.59 pM for epinephrine and dopamine, respectively; those for HSG ZrO\textsubscript{2}-PPO sorbents were 270 pmol/L and 350 pM for dopamine and epinephrine, respectively. Also, the sensitivity enhancement factors\textsuperscript{64} for NHSG ZrO\textsubscript{2}-PPO sorbent were 6.0, 27.4 and 4.0 folds higher for epinephrine, dopamine and serotonin, respectively than that of the HSG sorbent. The achieved LODs using the NHSG ZrO\textsubscript{2}-PPO sorbents lower than the LODs for dopamine and epinephrine in many reported studies (54 pM – 27 nM)\textsuperscript{8,10,55-63,65-73}, which can be attributed to both the Lewis acid sites on the surface of zirconia sorbents\textsuperscript{48,74,75} and the ability of the non-hydrolytic sol-gel route to incorporate high content of the PPO into the sorbent coatings. Figure 2.10 illustrates chromatograms of synthetic urine samples: (a) unspiked and (b) spiked with epinephrine and dopamine. The chromatograms were obtained by CME-HPLC experiment using NHSG ZrO\textsubscript{2}-PPO coated capillary. Low picomolar LODs (25 and 32 pM for epinephrine and dopamine, respectively) were achieved with high EFs (1480 and 2650 for epinephrine and dopamine, respectively). The enhanced EFs values obtained after analyzing synthetic urine samples (~ 15 %) compared to the values achieved with aqueous samples can be attributed to the salting effect. The obtained results demonstrate the applicability of the presented sorbent for the analysis of dopamine and epinephrine in complex matrices providing excellent sensitivity. This is important in the analysis of catecholamines and their metabolites, since quantifying the ratio of these biomarkers is critical for the estimation of the state of the cancer in the adrenal gland\textsuperscript{76}
2.4. Conclusion

The newly developed sol-gel ZrO₂-PPO sorbents were utilized in CME-HPLC analysis of free catecholamines and molecules related to their metabolites. Nonhydrolytic sol-gel approach was effectively employed for facile incorporation of PPO ligands in an evolving zirconia network providing zirconia-PPO hybrid organic-inorganic sorbents for CME. Compared to the HSG approach, the NHSG approach provides an effective pathway to chemically incorporate significantly higher amounts of the hydroxy-terminated organic ligand (PPO). NHSG process also facilitates a better extraction of the underivatized catecholamines by making more Lewis acid sites available on the surface for interaction with these basic analytes. These sorbents incorporated structural and compositional features important for the extraction of polar analytes that require extreme pH conditions. Sol-gel hybrid ZrO₂-PPO sorbents offer a pH-stable alternative to conventional silica- or organic polymer-based extraction media. The presented sorbents do not need the derivatization of catecholamines often required for their extraction on polymeric sorbents. The extraction of underivatized catecholamines requires a highly alkaline medium (pH > 10) that is detrimental to traditionally used silica-based sorbents. The newly developed sol-gel ZrO₂-PPO sorbents provided high desorption efficiency (> 95 %) just by using MS-compatible HPLC mobile phases, without the need for pH manipulation or LC-MS non-compatible elution steps often required by traditional sorbents like alumina.
Table 2.2. CME-HPLC-UV results for epinephrine, dopamine and serotonin extracted from aqueous sample (pH 10.5)

<table>
<thead>
<tr>
<th></th>
<th>Run-to-run</th>
<th>LOD (pM)</th>
<th>Mass Extracted (ng)</th>
<th>DE %</th>
<th>SE (µg/g)</th>
<th>Sensitivity Enhancement Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RSD %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NHSG</td>
<td>HSG</td>
<td>NHSG</td>
<td>HSG</td>
<td>NHSG</td>
<td>HSG</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>3.6</td>
<td>2.5</td>
<td>5.6</td>
<td>340.0</td>
<td>72.7</td>
<td>6.1</td>
</tr>
<tr>
<td>Dopamine</td>
<td>5.1</td>
<td>0.6</td>
<td>9.6</td>
<td>270.0</td>
<td>93.3</td>
<td>16.0</td>
</tr>
<tr>
<td>Serotonin</td>
<td>4.7</td>
<td>0.8</td>
<td>9.6</td>
<td>290.0</td>
<td>39.0</td>
<td>11.9</td>
</tr>
</tbody>
</table>
Figure 2.10. CME-HPLC-UV chromatograms (a) unspiked and (b) spiked synthetic urine sample with epinephrine and dopamine at 200 µg/L concentration level. The extraction was conducted using NHSG ZrO$_2$-PPO sorbent. Mobile phase composition: 2/98 (v/v %) methanol: ammonium acetate (20 mM, pH 3.8). UV wavelength at 280 nm.
To the best of our knowledge, the achieved low picomolar limits of detection for dopamine and epinephrine provided by the presented NHSG ZrO$_2$-PPO sorbents compare favorably with the LOD values reported in the literature. The demonstrated exceptional pH stability, excellent sensitivity enhancement factor, desorption efficiency, and the capillary-to-capillary and run-to-run reproducibility suggest that the presented sorbents can be advantageously employed in the analysis of catecholamines and their metabolites representing important biomarkers for neuroendocrine tumors.

2.5. References for chapter two


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Chapter Three

Hydrolytic and nonhydrolytic sol-gel routes to covalently attaching a hydroxy-terminated organic ligand/polymer to a sol-gel titania network evolving within the confines of a fused silica microextraction capillary

3.1. Introduction

In the field of bioanalytical sample preparation, there is a growing need for new sorbent materials that can overcome shortcomings of the existing sorbents. Some of the parameters that need to be considered for the development of new sorbents are: convenience of the preparation method, cost-effectiveness of the prepared materials, chemical and thermal stabilities, selectivity enhancement through exploitation of a variety of intermolecular interactions, adequate sorption capacity, and biocompatibility \(^1\)\(^-\)\(^3\). Since the introduction of solid-phase microextraction (SPME) technique by J. Pawliszyn and coworkers \(^4\), significant advancements in the field of sample preparation, preconcentration, and trace level analysis were achieved by advantageously exploiting the ability of SPME to provide solvent-free sample preparation tool using a sorbent coating either on the outer surface of a fiber (fiber SPME) or on the inner walls of a capillary (in-tube SPME), also known as capillary microextraction (CME) \(^5\),\(^6\). A significant shortcoming of the conventional sorbent coatings used in SPME is that they are held on the substrate surface.
(fiber or capillary) merely by physical forces of adhesion. The lack of chemical bonding between the sorbent coating and the substrate results in reduced thermal- and solvent stabilities.

In 1997, Malik and coworkers\textsuperscript{7} introduced sol-gel extraction media for fiber SPME in the form of surface-bonded coatings\textsuperscript{7} as well as chemically anchored stationary phases for open tubular gas chromatography (GC)\textsuperscript{8}. The same research group also introduced surface bonded sol-gel coatings as sorbents for capillary microextraction (CME)\textsuperscript{5}. Sol-gel coating technology provided enhanced chemical-, thermal-, and solvent stabilities for the chromatographic stationary phase and microextraction media. The enhanced stabilities can be attributed to the chemical bonding of the sol-gel coating to the fused silica substrate (fiber or capillary), and the crosslinking of the sol-gel hybrid organic-inorganic network. A variety of sol-gel microextraction phases were subsequently developed allowing facile coupling of microextraction techniques to GC or HPLC. Metal/metalloid alkoxide precursors were used to create silica\textsuperscript{5,9-11}, titania\textsuperscript{12}, zirconia\textsuperscript{13} and germania-based\textsuperscript{14-16} sorbents that provided excellent detection limits, as well as enhanced performance stability in microextraction. Solvent stability of microextraction coatings is of paramount importance for direct hyphenation of microextraction-based sample preparation with liquid-phase separation techniques (e.g., HPLC and CE). Additionally, titania-, zirconia- and germania-based coatings demonstrated exceptional pH stability characteristics (pH range: 0 – 14) important for extraction and separation under extreme pH conditions encountered in a variety of applications\textsuperscript{17}.

The hydrolytic sol-gel (HSG) pathway typically involves two main reactions: hydrolysis and polycondensation\textsuperscript{18}. The divergence in the rates of either the hydrolysis or the condensation reactions can significantly affect the composition and homogeneity of the sol-gel material
especially when carbon-, silane- and metal-based precursors or sol-gel-active organic ligands are mixed together during the preparation of a sol-gel hybrid material. Metal-based precursors are extremely reactive toward hydrolysis and condensation reactions compared to silane-based precursors and organic ligands (e.g., hydroxy-terminated organic components) that are characterized \(^\text{19}\) by reduced rates for these reactions especially without the use of catalysts and elevated temperature to achieve these reactions. Thus, the functionalization of the evolving sol-gel metal oxide precursors with such organic moieties is difficult to accomplish due to the significant differences in the reaction rates between the metal-based precursors and the functional groups on the organic moieties. In such a reaction environment, the highly reactive metal-based sol-gel precursors and their hydrolysis products (that are also highly reactive) will preferentially react among themselves with very little chemical incorporation of the used organic ligand. Surface modification efforts have been put forward for the functionalization of the metal oxide surfaces with phosphonic acid groups (R-PO(OH)\(_2\)) utilizing the strong and specific interaction of the phosphonic acid with the Lewis acid-base sites on the metal oxide surface \(^\text{20}\). The main drawback of this method is the instability of the electrostatic interaction between phosphonic acid and the metal oxide surface at elevated pH conditions or high concentration buffers causing bleeding of the organic ligands from the prepared sorbent and loss of efficiency \(^\text{21,22}\). Another method for the functionalization of the transition metal oxides is performed by reacting organosilane-based precursors to covalently bind different organic ligands or polymers to the metal oxides network. The organosilane precursors are commercially available with a wide range of different organic ligands and polymers that have been utilized to prepare tailor-made sorbents. Two major drawbacks of this approach are: (a) extreme chemical inertness of metal oxide surfaces that are very difficult to derivatize to generate desired level of ligand
concentration needed for extraction purposes, and (b) the poor stability of siloxane (≡Si-O-) bond in pH conditions above 7.5, leading to an overall instability problems of organosilane-based sorbents in these conditions.

Nonhydrolytic sol-gel (NHSG) route is gaining interest in the synthesis of metal/metalloid oxides due to the sorbent characteristics it provides compared to the hydrolytic sol-gel (HSG) route such as: (a) more control over the reactions for NHSG, (b) higher homogeneity and control of composition, (c) creation of metal oxide with more Lewis acid sites than Bronsted acid-base sites (hydroxyl-associated sites). (d) The possibility of surface-modification with organic moieties to produce homogenously dispersive metal oxide particles in organic solvents. The ability to synthesize stable and covalently bonded sol-gel metal oxide-based hybrid organic-inorganic material can be considered as an important advantage provided by the NSH route. In this study, we conducted an investigation exploring both hydrolytic and nonhydrolytic sol-gel pathways for the synthesis of novel hybrid titania-polypropylene oxide (sol-gel TiO$_2$-PPO) sorbents and evaluated their ability for the incorporation of the organic polymer in the extraction phase and how it reflect on their performance in capillary microextraction (CME) of important neuroendocrine tumor biomarkers.

3.2. Experimental section

3.2.1. Materials and chemicals

Dopamine hydrochloride, epinephrine hydrochloride, serotonin hydrochloride, 2-aminobenzoic acid, 4-aminobenzoic acid, acetaminophen, 4-hydroxybenzoic acid, titanium tetrachloride, ethanol, 1-butanol, toluene, 1-nonanol, polypropylene oxide (M$_\text{avg}$ 3500), glacial acetic acid, were purchased from Sigma-Aldrich (St. Louis, MO). Tetraethoxysilane and titanium
tetrabutoxide were purchased from Gelest (Morrisville, PA). Methanol and dichloromethane (HPLC grade solvents), polypropylene microcentrifuge tubes, 200 and 1000 microliter micropipette tips were purchased from Fischer Scientific (Waltham, MA). Fused silica capillary (250µm i.d.) with polyimide external coating was purchased from Polymicro Technologies (Phoenix, AZ).

3.2.2. Equipment

An Agilent 1100 HPLC system equipped with Diode Array Detector and a Rheodyne 6-ports valve (IDEX Health & Sciences, Oak Harbor, WA) were employed for the chromatographic analysis of the extracted analytes. An in-laboratory built purging/filling system was used to coat the CME capillary and an in-house assembled sample dispenser was employed to carry out the extraction. A Varian 3800 model gas chromatograph with a flame ionization detector (currently Varian is a part of Agilent) was used for thermal conditioning of the coated capillary. Sorvall Legend Micro-21 Microcentrifuge (Thermo Fisher Scientific, Waltham, MA) was employed for the precipitation of proteins from synthetic urine samples prior the CME-HPLC analysis. A Q50 TGA (TA Instruments, New Castle, DE) was employed for the thermogravimetric analysis of the prepared sorbents.

3.2.3. Hydrothermal pretreatment of fused silica capillary

The inner surface of a 1-m of fused silica capillary (250 µm i.d.) was rinsed with 2 mL each of dichloromethane, methanol, and deionized water using the nitrogen-pressure operated purging/filling system at 10 psi. Both ends of the capillary were sealed with an oxy-acetylene torch. The sealed capillary was secured in the GC oven and thermally conditioned (40 °C – 350
°C @ 1 °C/min, with 300 min hold time at 350 °C). The capillary was then cooled down to room temperature and cut-opened on both ends and rinsed again with the solvents as mentioned earlier. Thereafter, the capillary was thermally conditioned in the GC oven while simultaneously purged with nitrogen. For this, one end of the capillary was connected to the injection port, and the other end was left open and secured in the GC oven. Thermal conditioning was performed as follows: 40 °C – 350 °C @ 10 °C/min, with 120 min hold time at 350 °C.

3.2.4. Solvent drying procedure

Butanol and toluene were dried over molecular sieve (type: 4A) by taking 15 mL of each solvent in separate clean and dry vials. Ten grams of molecular sieve (previously conditioned in GC oven at 350 °C for 6 hrs and cooled to room temperature) were added to each solvent and vortexed for 2 min then left airtight in the hood for 1 h. 2 mL aliquots of each solvent were transferred to a microcentrifuge vial and vortexed thoroughly with 0.5 g of anhydrous copper sulfate powder (white). Centrifugation was performed (10,000 rpm for 2 min) to precipitate CuSO$_4$ and any possible contamination from the molecular sieve particles that were subjected to visual inspection for any color change to blue that would indicate the presence of water. The drying process was repeated (if necessary) until no color change (white to blue) for CuSO$_4$ was observed.

3.2.5. Sol-gel reactivity enhancement via the derivatization of PPO terminals

Prior to the use of hydroxy-terminated polypropylene oxide (PPO) in the preparation of the TiO$_2$-PPO sol-gel sorbents, the PPO terminals were modified with titanium tetrachloride. The reaction of PPO and TiCl$_4$ was conducted using a method analogous to a recently reported
procedure utilized polyethylene oxide (PEG) with TiCl₄ but with appropriate modification. A 1.6 g amount of PPO was dissolved with 250 µL of dried toluene and thoroughly vortexed for 2 min. The polymer solution was left in the hood for 24 hrs to facilitate the formation of solution with homogenously distributed polymer chains. Thereafter, the polymer solution was vortexed for 1 min and the vial was placed inside the filling/purging device with a deactivated fused silica capillary immersed in the solution. At 10 psi of nitrogen, the polymer solution was drop-wise added to sealed 25 mL round-bottom flask containing a TiCl₄-toluene solution (100 µL of TiCl₄ and 150 µL toluene). The content of the flask was continuously stirred providing a 1:2 molar ratio for PPO: TiCl₄. The reaction mixture was stirred for 12 h at 60 °C then it was allowed to reach room temperature before it was used in the preparation of the sol solution.

3.2.6. Preparation of TiO₂-PPO coated capillary via nonhydrolytic sol-gel (NHSG) route

3.2.6.1. Preparation of sol solution for NHSG route

Two sol solutions were prepared for the creation of NHSG CME sorbents one with modified PPO (NSH-MOD) and the other with unmodified PPO (NSH-UNM). Each sol solution was prepared separately using 2-mL microcentrifuge vials as follows: 40 µL of TiCl₄ was mixed with 150 µL of dried toluene in a microcentrifuge vial and vortexed for 1 min. In a different vial, 80 mg of modified PPO was mixed with 125 µL of 1-butanol and vortexed thoroughly (2 min). The polymer-containing solution was transferred to the vial containing TiCl₄-toluene solution and vortexed thoroughly for 2 min. The gelation time of this sol solution was ~ 2 h.
3.2.6.2. Preparation of NSH TiO$_2$-PPO coated capillaries

A freshly prepared sol solution (as described in section 2.5.1) was used to fill a 1 meter long piece of hydrothermally pretreated fused silica capillary using the filling/purging system. The sol solution was allowed to reside in the capillary for 45 min after the exit-end of the capillary was sealed with a rubber septum. The unbounded portion of the sol solution was then expelled from the capillary by applying 15-psi of nitrogen pressure, leaving behind a surface-bonded layer of sol-gel sorbent. The coated capillary was thermally conditioned in the GC oven under nitrogen purge. For this, one end of the capillary was connected to the injection port and the other end was left secured in the GC oven. The GC oven temperature was programmed as follows: 40 °C – 150 °C @ 1 °C/min, 300 min hold time at 150 °C. In an analogous way, a second capillary was coated with a fresh sol solution prepared with unmodified PPO.

3.2.7. Preparation of TiO$_2$-PPO coated capillary via hydrolytic sol-gel (HSG) route
3.2.7.1. Preparation of sol solution for HSG route

The process of sol-gel coating utilizing hydrolytic sol-gel pathway was used for the preparation of two CME-capillaries, one with modified PPO (HSG-MOD) and the other with unmodified PPO (HSG-UNM). The molar ratios of titanium tetrabutoxide, water, glacial acetic acid and 1-butanol in the sol solution were: 1: 1.6: 3.8: 10.5, respectively. In a 2-mL polypropylene microcentrifuge vial, 50 µL of titanium tetrabutoxide was vortexed with 14 µL of glacial acetic acid for 1 min. In a different vial, 80 mg of the modified PPO was mixed with 140 µL of 1-butanol and vortexed thoroughly for 1 min. The polymer solution was transferred to the other vial containing titanium precursor-acetic acid solution. After thorough vortexing (for 2 min), 8 µL of deionized water was added to the mixture followed by thorough vortexing.
3.2.7.2. Preparation of HSG TiO$_2$-PPO coated capillaries

A freshly prepared HSG sol solution as described in section (2.6.1.) was allowed to reside inside a hydrothermally pretreated fused silica capillary for 30 min. The sol-gel coated capillary was purged with nitrogen and thermally conditioned in GC oven as described in section (2.5.2). A second HSG TiO$_2$-PPO coated capillary was prepared using a sol solution containing unmodified PPO.

3.2.8. Preparation of sol-gel silica coated capillary

For comparison with titania-based sorbents, sol-gel silica coated capillary was prepared as follows: in a microcentrifuge vial, 140 µL of 1-butanol and 50 µL of nonanol/toluene mixture (1:1 v/v) were mixed with 65 µL volume of TEOS and the mixture was vortexed thoroughly for 1 min. Thereafter, 30 µL of trifluoroacetic acid (5 % water) was added and the mixture was vortexed thoroughly for 2 min. The gelation time for this sol solution was ~ 3 h. A freshly prepared SiO$_2$ sol solution was used to coat a 1 meter long piece of hydrothermally pretreated capillary as described in section (2.5.2.) with in-capillary residence time of sol solution for 60 min.

3.2.9. Preparation of samples and synthetic urine

Stock solutions of analytes of interest were prepared in methanol (except for catecholamines and serotonin that were prepared in 0.1 M HCl) at 10 mg/mL concentration. Aliquots of 10 µL of the stock solution were diluted with deionized water to 1mL total volume (100 mg/L concentration). To prepare 100 µg/L aqueous samples, aliquots of 100 µL of the diluted sample were further diluted in a volumetric flask to 100 mL with deionized water (18
mΩ). For the preparation of the catecholamines or serotonin samples, a similar procedure was followed with the exception that in this case 0.1 M HCl was used as a solvent for the dilution of the stock solution, deionized water was used for further dilution in preparing the final solution. Synthetic urine was prepared in the lab mimicking the major components in normal human urine. In a volumetric flask, the following chemical ingredients were dissolved in 1 liter of deionized water (18 mΩ): albumin 65 mg, myoglobin 1 mg, chymotrypsin 10 mg, tyrosine 100 mg, ascorbic acid 10 mg, creatinine 210 mg, DL-methionine 100 mg, cysteine 130 mg, benzylimidazole 50 mg, urea 2500 mg, uric acid 60 mg, sodium bicarbonate 100 mg, sodium phosphate 100 mg, sodium citrate 130 mg, potassium bicarbonate 100 mg, magnesium carbonate 20 mg, ammonium sulfate 130 mg, potassium chloride 500 mg, sodium chloride 2500 mg. Prior to the microextraction experiment, dopamine, epinephrine and serotonin were spiked at 200 μg/L level in the synthetic urine samples which were vortexed for 1 min and the spiked urine crude samples were ultracentrifuged at 21,000 g for 2 min. The urine sample was then stored at -18 °C.

3.2.10. Coupling capillary microextraction to high performance liquid chromatography (CME-HPLC)

The CME-HPLC experimental setup was described elsewhere. Briefly, a 40-cm piece of the sol-gel coated capillary was installed as an external sampling loop on a Rheodyne 6-port injection valve of the HPLC system. The aqueous sample was allowed to pass through the capillary under gravity from an in-laboratory constructed sample dispenser. The extraction was continued until the system reached extraction equilibrium (for most analytes it took under 1 h). Thereafter, the HPLC mobile phase (phase A: methanol, phase B: ammonium acetate 20 mM, pH 3.8) was directed to pass through the coated capillary by switching the injection valve to
“inject” position. The organo-aqueous mobile phase (the mobile phase composition of phase A: phase B for the analysis of catecholamines: 2/98 v/v %, and for other test probes: 15/85 v/v %) facilitated the desorption of the extracted analytes from the sol-gel coating and their introduction into the HPLC column (Zorbax C18 2.1 x 150 mm, 5 µm) for chromatographic analysis. In between the CME-HPLC analysis of the synthetic urine samples, the HPLC column was cleaned with a mobile phase (90/10 v/v % phase A: phase B) for 10 min and then equilibrated with the mobile phase (2/98 v/v % phase A: phase B) for 10 min prior to next CME-HPLC analysis.

3.2.11. Characterization of the sol-gel sorbents
3.2.11.1. Evaluation of coating thickness and volume

The thickness and volume of the coated sorbent on the inner surface of the fused silica capillaries were estimated by Scanning Electron Microscopy (SEM) Imaging. For this, 10 random segments (~ 1-cm) of the coated capillaries were cut using alumina wafer. Cross-sectional images were taken for the coated capillary segments and the average coating thickness was used for calculating the coating volume on a 40-cm long coated capillary. The following equation was used for the calculation of coating volume: \( V = \pi L \times (R^2 - r^2) \), where \( L \) – length of the CME capillary, \( R \) – inner radius of the uncoated capillary and \( r \) – inner radius of the coated capillary.

3.2.11.2. FTIR and TGA characterization of the prepared sol-gel sorbents

For characterization purposes, sol-gel TiO2-PPO materials prepared via HSG and NHSG routes were coated on the inner surface of a glass tube (36 x 6 mm i.d.) with some adjustment in the procedure for the preparation of the coating solution: in the vial containing PPO solution, 0.2
g of hydrothermally pretreated silica particles (5 μm diameter) were added and the mixture was vortexed thoroughly for 2 min. After coating, the sol-gel sorbent-like materials were scraped off the coated glass tubes using stainless steel spatula then taken for FTIR and TGA analysis.

3.2.11.3. Conversion of peak area to extracted amounts

Calibration plots were constructed to convert chromatographic peak areas to mass of analyte extracted by CME. For this, samples of each analyte were prepared at a series of concentrations (0.1, 0.5, 1.0, 5.0, 25.0, 50.0, 75.0, 100.0 mg/L; for each analyte, the concentration was converted to molar concentration for constructing the calibration plot). Three replicate measurements were made for peak area of each analyte using 5 μL external sampling loop at each molar concentration level. The obtained average peak areas were plotted against the corresponding molar concentration of the injected sample and the best-fit linear plot was constructed.

3.2.11.4. Evaluation of the desorption efficiency (DE %)

An experimental parameter (DE %) was introduced to evaluate the desorption efficiency of the prepared sol-gel sorbents. The original concentration (prior extraction) of aqueous samples was validated by direct injection into the HPLC system using a 40-cm deactivated fused silica capillary as external sampling loop and the constructed calibration plots. Then, the sample (100 mL) was allowed to pass completely through the CME capillary and the liquid exiting from the CME capillary was collected in an ice-cooled and parafilm-sealed flask. The mass of every analyte in the collected liquid was then evaluated by direct injection into the HPLC system. The extracted amount is then calculated by subtracting the mass of analyte before
and after the extraction. After performing the CME-HPLC experiment, the desorbed amount of the CME-extracted analytes was obtained. DE % was then calculated using the following equation:

\[
\text{Desorption Efficiency (DE) \%} = \frac{\text{Desorbed amount}}{\text{Extracted amount}} \times 100 \quad (\text{Eqn. 3.1})
\]

3.3. Results and discussion

Metal oxides-based organic-inorganic hybrid sorbents must have an adequate surface concentration of the organic ligands. Preferably, these ligands should be chemically bonded to the surface of the metal oxide. Serious drawbacks and instability problems (pH or solvent instability) exist in the reported means of derivatizing the metal oxide surface with either silane- or phosphate-based organic ligands\textsuperscript{21}. Siloxane bond (=Si-O-) suffers from instability in pH level above 7.5\textsuperscript{17,18,35}, which translates into instability of the organosilane ligands at such conditions. The interaction between Lewis acid sites on the metal oxide surface and phosphate groups can be reversed at elevated pH conditions or with strong buffers\textsuperscript{36} and thus organophosphate ligands would also suffer from instability problems.

3.3.1. The synthesis of surface-bonded sol-gel hybrid sorbents

It is well-known that hydroxy-terminated organic ligands/polymers suffer from low sol-gel reaction rates compared to metal/metalloid-based precursors (the reaction rates for metal oxide-based precursors are higher than hydroxy-terminated ligands by at least 5 orders of magnitude\textsuperscript{37,38}). A proper means of sol-gel-activation for the hydroxy-terminated ligands is required to overcome this incompatibility problem. For this, here we describe the synthesis of covalently bonded sol-gel titania-PPO hybrid sorbents. The hydroxy-terminated PPO was treated
with TiCl₄ (as shown in Figure 3.1) to produce a modified-PPO with terminals that can undergo sol-gel reaction with either TiCl₄ or Ti(OBu)₄ via hydrolytic or nonhydrolytic routes, respectively. The modification of the terminals of PPO facilitated an effective means of incorporation of PPO in the sol-gel network. This modification process avoided the use of silane- or phosphate-based organic ligands, and has generated titanium trichloride on the terminals of PPO that can undergo hydrolysis and condensation at reaction rates (kₜ ~ 5.1 x 10⁻² M⁻¹ s⁻¹ and kₙ ~ 30 M⁻¹ s⁻¹)¹⁸,³⁸,³⁹, which are comparable with the reaction rates of the titanium alkoxide precursors°³⁷,³⁸.

![Figure 3.1. Illustration of the modification of the PPO hydroxyl terminals with titanium tetrachloride.](image)

In hydrolytic sol-gel (HSG) approach, the rate of hydrolysis of the metal alkoxide precursor (titanium tetrabutoxide) was reduced by a chelating agent (acetic acid) to prevent immediate precipitation of metal oxide particles and for a better control of the overall process. A schematic representation of the hydrolysis reaction with Ti(OBu)₄ precursor is shown in Figure 3.2-a. Concurrently, the hydrolyzed or partially-hydrolyzed precursor species underwent different types of condensation reactions in the sol solution: (a) water condensation (where hydroxyl groups of the hydrolyzed precursor molecules or modified PPO reacted together
producing water), (b) alcohol condensation (where the alkoxy group of the nonhydrolyzed or partially hydrolyzed precursor reacted with hydroxyl groups of fully or partially hydrolyzed precursors) \(^{18}\). These hydrolysis and condensation reactions (as depicted in Figure 3.2) took place in the sol solution within the hydrothermally pretreated fused silica capillary, allowing the sol-gel material to undergo condensation reaction with the silanol groups on the inner surface of fused silica capillary.

Figure 3.2. Illustration of the hydrolytic sol-gel reactions inside the capillary: (a) hydrolysis of titanium tetrabutoxide in the presence of acetic acid as a chelating reagent, (b) condensation of hydrolyzed precursors, (c) polycondensation of hydrolyzed and partially hydrolyzed precursors with modified PPO and (d) condensation of the sol-gel sorbent with the surface silanol groups.
In a water-free environment, nonhydrolytic sol-gel (NHSG) route can be used for the synthesis of metal oxides using metal halide precursors (e.g., TiCl₄)\(^{26}\). In this work, NHSG reaction of TiCl₄ with 1-butanol (as a solvent and source of oxygen) in the sol solution within the hydrothermally pretreated fused silica capillary underwent several steps as depicted in Figure 3.3: (a) alcoholsysis of titanium tetrachloride (HCl elimination, forming Ti-O-Bu species)\(^{40}\), (b) polycondensation of the alcoholsyzed species either the titanium-based precursor molecules or (c) with the modified terminals of PPO (forming Ti–O–Ti bridges)\(^{26,33}\). Finally, (d) condensation of the evolving sol-gel material with the silanol groups on the inner surface of fused silica capillary. As a result of this process, the hybrid organic-inorganic sol-gel coatings gets chemically bonded to the inner surface of the fused silica capillary as illustrated in Figure 3.3.

Figure 3.3. Illustration of the nonhydrolytic sol-gel pathway for the formation of titania-PPO hybrid coating inside the capillary: (a) alcoholsysis of titanium tetrachloride, (b) condensation of alcoholsyzed with metal halide precursors, (c) polycondensation of the evolving sol-gel network and the modified PPO, and (d) condensation of the sol-gel sorbent with silanol groups on the inner surface of the capillary.
3.3.2. Characterization of the sol-gel sorbents

As a proof of formation of such hybrid and chemically bonded material, a freshly prepared HSG and NHSG sol solutions (mixed with hydrothermally pretreated silica particles, 5 μm diameter) were coated on the inner surface of glass tube and conditioned similarly to the coated capillary. The produced sol-gel materials were then characterized by FTIR. The black trace (trace # 1) in Figure 3.4 illustrates the FTIR spectra for NHSG TiO$_2$-PPO sorbent. The signal at 1010 cm$^{-1}$ can be attributed to the presence of the Ti–O–C linkage which indicates that the analyzed material is a hybrid organic-inorganic sol-gel sorbent with a covalently bonded PPO to sol-gel titania network. The FTIR analysis was also conducted for the HSG TiO$_2$-PPO sorbent (trace #2 in Fig. 3.4) after thorough treatment with deionized water to achieve complete hydrolysis of any possible butoxytitanium moieties that might be still present in the sol-gel TiO$_2$-PPO material. The presence of such species in the unhydrolyzed form can affect the FTIR interpretation, by showing the presence of additional Ti–O–C bonds in the created sorbent. Both findings indicate the usefulness of the presented sol-gel pathways for the synthesis of covalently bonded hybrid organic-inorganic sorbents. The peak at 928 cm$^{-1}$ can be attributed to Si–O–Ti bonds in the sol-gel TiO$_2$-PPO material condensed with the hydrothermally pretreated silica particles that were dispersed in the sol solution during the synthesis. This data also indicates the ability of the presented sol-gel routes to create a sol-gel TiO$_2$-PPO CME coating chemically bonded to the inner surface of fused silica capillary.
Figure 3.4. FTIR spectra for hybrid TiO$_2$-PPO sorbents prepared via NSH (black trace, #1) and HSG (red line, #2) pathways.

Figure 3.5 illustrates cross-sectional images obtained for NSH TiO$_2$-PPO coated capillary showing the thickness of the sol-gel sorbent coating on the inner surface of a fused silica capillary. Figure 3.6 illustrates a cross-sectional image of NSH TiO$_2$-PPO at 300x magnification and the corner frame illustrates the surface morphology of the coating at 10,000x magnification. The internal volumes of the coated capillaries were $\sim 19.26 \, \mu$L and $\sim 19.44 \, \mu$L for NSH and HSG coated capillaries, respectively.
Figure 3.5. Illustration of cross-sectional scanning electron microscopic image of NSH TiO$_2$-PPO coated capillary at 10,000x magnification.

Figure 3.6. Illustration of scanning electron microscopic image of NSH TiO$_2$-PPO coated capillary at 300x magnification and 10,000x magnification (left frame) showing the morphology of the sorbent surface.
3.3.3. The evaluation of capillary microextraction performance

The time required to achieve the maximum extraction was evaluated by performing a series of extraction experiments performed in triplicates over 10-, 20-, 30-, 40-, 50-, and 60 min periods generating extraction profiles (Figure 3.7) for five analytes (2-aminobenzoic acid, 4-hydroxybenzoic acid, catechol, dopamine and epinephrine). The microextraction process reached the equilibrium for catechol, both 4-hydroxybenzoic acid and 2-aminobenzoic acid, and the catecholamines (dopamine and epinephrine) within 20-, 30-, and 40 min, respectively. The slightly longer equilibrium time for catecholamines can be attributed to the stronger intermolecular interactions between the polar functional groups of catecholamines and the Lewis-acid sites on the surface of the titania-based sorbents.

![Graphical representation of the extraction profile constructed by plotting the SE values of the extracted analytes vs. the time of the extraction period.](image)

Figure 3.7. Graphical representation of the extraction profile constructed by plotting the SE values of the extracted analytes vs. the time of the extraction period.
Table 3.1 shows the reproducibility of the sorbent mass formed on the inner surface of five HSG sol-gel coated CME capillaries as well as five NHSG coated capillaries. The relative standard deviation (RSD %) of the sorbent masses was used as a measure for the reproducibility of the creation of surface-coated sorbent. Excellent reproducibility was noticed (RSD < 4.0 %) which reveals the usefulness of the sol-gel coating technology for reproducing sorbents. The reproducibility of the microextraction performance of these coated capillaries was also investigated. CME-HPLC experiments were performed on catechol-containing aqueous sample as a test probe. Excellent capillary-to-capillary reproducibility (RSD < 4.5 %) in terms of HPLC peak area was obtained (shown in Table 3.1). Knowing that the extraction process is affected by various factors such as the intermolecular interactions between the analyte and the extraction sites on the sorbent surface, the kinetics of mass transfer, porosity and surface area and other factors, the obtained reproducibility results illustrate the excellent capability of sol-gel coating method for creating reproducible microextraction sorbents with analogous characteristics and extraction capability.

A newly developed parameter, specific extraction (SE)\textsuperscript{43}, that can serve as an objective measure of the microextraction performance was used in this work. SE provides the ratio of the CME extracted mass of the analyte to the unit mass of sorbent used in the extraction process independently to the used analytical instrument. Such an objective parameter can be used to evaluate the extraction capability of sorbents prepared by similar method. SE is defined as follows (Equ. 3.2):

\[
SE = \frac{\text{mass of extracted analyte}}{\text{mass of sorbent}} \quad \text{Equ. (3.2)}
\]
Table 3.1. Evaluation of the sol-gel creation method by capillary-to-capillary reproducibility (n = 5) evaluated by measuring the mass of TiO$_2$-PPO hybrid sorbents prepared via HSG and NHSG pathways.

<table>
<thead>
<tr>
<th>Cap. #</th>
<th>NHSG</th>
<th>HSG</th>
<th>NHSG</th>
<th>HSG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>86.0</td>
<td>66.7</td>
<td>1.69</td>
<td>1.83</td>
</tr>
<tr>
<td>2</td>
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<td>1.67</td>
</tr>
<tr>
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<td>1.74</td>
</tr>
<tr>
<td>Avg.</td>
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<td>67.44</td>
<td>1.64</td>
<td>1.73</td>
</tr>
<tr>
<td>STD</td>
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<tr>
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<td>4.32</td>
<td>4.35</td>
<td>3.66</td>
<td>3.68</td>
</tr>
</tbody>
</table>

Figure 3.8 illustrates a comparison of SE values obtained by four sol-gel TiO$_2$-PPO coated CME capillaries. Both HSG and NHSG routes were used to prepare two CME capillaries. For each route, two capillaries were prepared: one capillary was coated using a sol solution containing modified PPO (MOD) while the other using a sol solution containing unmodified PPO (UNM). For simplicity, the four capillaries were denoted as NHSG-MOD, NHSG-UNM, HSG-MOD and HSG-UNM. The purpose of this comparison is to evaluate the usefulness of the modification of the hydroxyl-terminals of PPO to provide a sol-gel-active PPO whose terminals provided sol-gel reactivity comparable with the reactivity of titanium-based precursors. NHSG-MOD capillary showed notably superior extraction performance among the four tested capillaries. The SE values achieved by NHSG-MOD were ~23 – 27 % higher than NHSG-UNM, which can be attributed to the use of modified-PPO that translates into higher loading of
PPO in the sorbent as evident by TGA data (Figure 3.9). NHSG-MOD provided ~ 60% and 300% higher SE values compared to HSG-MOD and HSG-UNM, respectively. In addition to the higher PPO loading, the superior extraction performance of NSH-MOD can be attributed to the notion that NHSG route produces more Lewis-acid sites than Bronsted acid-base sites compared to analogous sol-gel materials prepared via HSG pathway. These interaction sites are responsible for stronger intermolecular forces (Lewis acid-base interactions are ~15 times stronger than hydroxyl-associated forces). The performance of the HSG-UNM was the lowest among all the tested sol-gel sorbents. This lower extraction capability can be attributed to the vast reactivity gap between the terminal hydroxyl groups of unmodified PPO and titanol groups, which translates into ineffective chemical incorporation of PPO in the resulting sol-gel sorbent.

![Diagram](image)

Figure 3.8. Comparison of the specific extraction (SE) values achieved by CME-HPLC experiments using sol-gel TiO2-PPO prepared via HSG and NHSG using modified and unmodified PPO.
Figure 3.9. Illustration of the TGA data obtained for four sol-gel TiO$_2$-PPO sorbents prepared using NSH route with modified-PPO (NSH-MOD), modified PPO (NHSG-UNM), and via HSG route with modified-PPO (HSG-MOD), or modified PPO (HSG-UNM).
3.3.4. Online CME-HPLC for the neuroendocrine tumor biomarkers

Catecholamines and their deaminated metabolites (such as homovanillic acid, 3-methoxy-4-hydroxyphenylglycol and vanillylmandelic acid) represent important diagnostic biomarkers for tumors associated with different types of cancers and neural diseases. A capillary coated with NSH-MOD sorbent was used for the preconcentration of epinephrine, dopamine, serotonin, and molecules structurally-related to their metabolites (catechol, nicotinic acid, 2-aminobenzoic acid, 4-aminobenzoic acid, acetaminophen and 4-hydroxybenzoic).

To evaluate the role of the organic and inorganic components in hybrid sol-gel sorbent, three chromatograms (Figure 3.10) were obtained by CME-HPLC-DAD experiments performed on aqueous sample containing a mixture of catechol, 4-aminobenzoic acid, acetaminophen, and 4-hydrobenzoic acid using three sol-gel CME capillaries coated with (a) sol-gel SiO$_2$, (b) sol-gel TiO$_2$, and (c) NSH-MOD, respectively. As shown in Table 2, the SE values for these probes achieved by sol-gel TiO$_2$ are higher by ~ 160 – 200% compared to the sol-gel SiO$_2$ sorbent. The higher affinity of the titania-based sorbent towards the probes can be attributed to the chelating effect of the carboxylate groups or the cis-diol groups on these probes with the titania-based sorbent. These structural properties allowed these probes to interact with the Lewis-acid sites on the surface of the sol-gel TiO$_2$ sorbent more efficiently compared to sol-gel silica sorbent. Furthermore, the chelation effect was stronger for carboxylic acids compared to cis-diol groups, resulting ~ 30 – 60% higher SE values for carboxylic acids than catechol (shown in Table 2).

The presented results indicate the usefulness of the titania-based sorbent for the extraction and preconcentration of molecules with carboxylic acid, hydroxyl or amino groups as in catecholamines and their metabolites. Also, it was noticed that the extraction capability of the titania-based sorbent was significantly enhanced by the effective incorporation of PPO. The
microextraction performance obtained by NSH-MOD sorbent, as measured by SE values, was 4-7 folds higher compared to NSH titania-based sorbent (PPO-free). The effective incorporation of PPO facilitated a profound sample capability and variety of microextraction forces (e.g., hydrophilic interactions, H-bonding, van der Waals forces, and hydrophobic interactions with the aromatic ring) in addition to the interactions provided by the titania the resulting sol-gel hybrid organic-inorganic sorbent. The completeness of the desorption of the analytes was evaluated by measuring the desorption efficiency (DE %) of the prepared sorbents. Metal oxides are known for the strong adsorption on their surface mostly via the interaction with oxygen or nitrogen atoms. It was also noticed that DE of the target analytes from the surface of the hybrid organic-inorganic NSH-MOD sorbent was 10 % higher compared to sol-gel TiO\textsubscript{2} sorbent using analogous desorption conditions.

Because of the excellent chemical stability of titania-based sorbents at extreme pH conditions, NSH-MOD was used for the extraction of dopamine, epinephrine and serotonin from aqueous sample modified to pH 10.5 with ammonia solution. The extraction of these underivatized polar organic molecules can be achieved at maximum efficiency when the analytes are close to their neutral state. For this, the extraction of these neuroendocrine tumor biomarkers (catecholamines have an average pKa ~ 8.5 – 8.9) was performed at sample pH 10.5, which would be problematic for conventional silica-based extraction media due to the instability of silica-based sorbents at pH conditions above 7.5.
Table 3.2. Experimental data collected by the CME-HPLC-UV experiments using sol-gel SiO$_2$, sol-gel TiO$_2$, and NHSG-MOD coated capillaries.

<table>
<thead>
<tr>
<th></th>
<th>Mass Extracted (ng)</th>
<th>Run-to-run RSD % n=5</th>
<th>LOD (pM) S/N = 3</th>
<th>SE (ng/mg)</th>
<th>DE %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SiO$_2$ TiO$_2$ NSH-MOD</td>
<td>SiO$_2$ TiO$_2$ NSH-MOD</td>
<td>SiO$_2$ TiO$_2$ NSH-MOD</td>
<td>SiO$_2$ TiO$_2$ NSH-MOD</td>
<td>SiO$_2$ TiO$_2$ NSH-MOD</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>0.5 1.3 4.9</td>
<td>4.1 2.5 2.9</td>
<td>797 319 58</td>
<td>0.5 1.3 5.02</td>
<td>98 91 99</td>
</tr>
<tr>
<td>Catechol</td>
<td>1.4 3.4 17</td>
<td>5.2 3.8 3.5</td>
<td>279 107.5 27.5</td>
<td>1.3 3.4 17.4</td>
<td>99 90.5 98</td>
</tr>
<tr>
<td>4-aminobenzoic acid</td>
<td>1.5 3.8 19</td>
<td>4.0 5.8 4.6</td>
<td>248 101 19.7</td>
<td>1.4 3.9 19.5</td>
<td>99 90 96.5</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>1.1 2.8 20</td>
<td>3.7 3.8 3.8</td>
<td>445 166 24.4</td>
<td>1.2 3.6 20.5</td>
<td>99.5 89 97</td>
</tr>
<tr>
<td>2-aminobenzoic acid</td>
<td>2.2 5.6 28</td>
<td>2.7 5.7 4.9</td>
<td>171 68 22.6</td>
<td>2.0 5.7 28.7</td>
<td>98.5 88 95.5</td>
</tr>
<tr>
<td>4-hydroxybenzoic acid</td>
<td>1.8 4.6 31</td>
<td>3.6 4.3 2.9</td>
<td>418 146.5 25.7</td>
<td>1.6 4.6 31.7</td>
<td>98 91.5 94.8</td>
</tr>
</tbody>
</table>
Figure 3.10. CME-HPLC-UV chromatograms for catechol, 4-aminobenzoic acid, acetaminophen, and 4-hydroxybenzoic acid at a concentration level of 100 μg/L using sol-gel SiO$_2$- (a), sol-gel TiO$_2$- (b), and NSH-MOD- (c) coated capillaries. HPLC conditions: mobile phase 15/85 v/v % (methanol: ammonium acetate, 20 mM, pH 3.8). UV detector: 280 nm.

The reported LODs in this study for the preconcentration of these biomarkers are comparable to recently published studies on the analysis of catecholamines (summarized in Table 3.3). Table 3.4 represents the LOD, sensitivity enhancement factors (EFs), DE % and SE measurements for dopamine, epinephrine and serotonin. Excellent DE % were achieved for the NSH-MOD hybrid sorbent in the range of 95 – 98 % indicating that the presented sorbent practically does not suffer from any significant carry-offer or irreversible adsorption problem. Low picomolar LODs obtained for these important neuroendocrine biomarkers that are comparable the recently published studies (Table 3.3). Also, the presented study have provided a convenient analysis approach that avoided chemical modification or derivatization that are
usually performed on the catecholamines to increase their affinity toward conventional polymeric or octadecyl-based sorbents.

Table 3.3. Summary of selected published studies for the analysis of catecholamines E: epinephrine, DA: dopamine, CX-SPE: cation exchange-solid phase extraction, EC: electrochemical, RAM-SPE: restricted access material-solid phase extraction, IP: ion pairing, PBA: phenylboronic acid. *(for convenience of comparison, units presented in original publication have been converted to molarity)

<table>
<thead>
<tr>
<th>Catecholamines</th>
<th>L.O.D.</th>
<th>Sample Preparation</th>
<th>Separation Technique</th>
<th>Derivatization</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>/ MS compatibility</td>
<td></td>
<td></td>
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<tr>
<td>DA</td>
<td>0.5-1  nM*</td>
<td>C18 trap column</td>
<td>LC-UV</td>
<td>N/a</td>
<td>49</td>
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<td></td>
<td></td>
<td></td>
<td>/ Not compatible</td>
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<tr>
<td>DA</td>
<td>1.7</td>
<td>N/a</td>
<td>Micro-CE-ED</td>
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<tr>
<td>E</td>
<td>µM</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>0.45</td>
<td>µM</td>
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<td>DA</td>
<td>10.0   nM</td>
<td>Fe3O4 nanoparticles</td>
<td>CE-UV</td>
<td>Diphenyl boronic complex</td>
<td>51</td>
</tr>
<tr>
<td>E</td>
<td>9.0    nM  /PBA</td>
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<td></td>
<td>/ Not compatible acid complex</td>
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</tr>
<tr>
<td>DA</td>
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<td>PBA-SPE</td>
<td>IP-LC-EC</td>
<td>Diphenyl boronic complex</td>
<td>52</td>
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<tr>
<td>E</td>
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<td>DA</td>
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<td>µM*</td>
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<td></td>
<td>1.42</td>
<td>nM*</td>
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<td>E</td>
<td>0.054</td>
<td>CX-SPE</td>
<td>LC-MS-MS</td>
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<td>54</td>
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<td></td>
<td>nM*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>0.546</td>
<td>Alumina-SPE</td>
<td>LC-MS-MS</td>
<td>N/a</td>
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<tr>
<td></td>
<td>nM*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA</td>
<td>0.033  nM</td>
<td>Microdialysis</td>
<td>UPLC-MS-MS</td>
<td>Diethyl labeling</td>
<td>56</td>
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<td>Catecholamines</td>
<td>L.O.D.</td>
<td>Sample Preparation</td>
<td>Separation Technique</td>
<td>Derivatization</td>
<td>Ref.</td>
</tr>
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<td>-----</td>
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<tr>
<td>DA</td>
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<tr>
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<tr>
<td>DA</td>
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<td>MIP-SPME</td>
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<td>DA</td>
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<td>Alumina-96 wells</td>
<td>LC-MS-MS</td>
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<tr>
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<td>0.03 nM</td>
<td>plate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA</td>
<td>1.3 nM*</td>
<td>Packed-tip SPE</td>
<td>HPLC-ED</td>
<td>Diphenyl boronic acid-complex</td>
<td>60</td>
</tr>
<tr>
<td>E</td>
<td>1.3 nM*</td>
<td>/ Not compatible</td>
<td></td>
<td></td>
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<tr>
<td>DA</td>
<td>130 nM*</td>
<td>N/a</td>
<td>HPLC-ED</td>
<td>N/a</td>
<td>61</td>
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<tr>
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<td>nM*</td>
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<td></td>
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<tr>
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<td>130 nM*</td>
<td></td>
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<tr>
<td>E</td>
<td>53 nM*</td>
<td>MEPS</td>
<td>HPLC-ED</td>
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Table 3.4. CME-HPLC-UV results for neuroendocrine biomarkers extracted from aqueous ammonia solution at pH 10.5 using NSH TiO₂-PPO coated capillary.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Mass Extracted (ng)</th>
<th>RSD %</th>
<th>LOD (pM)</th>
<th>DE %</th>
<th>SE (ng/mg)</th>
<th>EFs</th>
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</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>18.85</td>
<td>4.01</td>
<td>50.6</td>
<td>97.0</td>
<td>20.31</td>
<td>409</td>
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<tr>
<td>Dopamine</td>
<td>20.10</td>
<td>2.41</td>
<td>62.7</td>
<td>94.8</td>
<td>21.65</td>
<td>403</td>
</tr>
<tr>
<td>Serotonin</td>
<td>19.07</td>
<td>1.8</td>
<td>53.9</td>
<td>98.0</td>
<td>20.55</td>
<td>389</td>
</tr>
</tbody>
</table>

3.3.5. Enrichment of neuroendocrine tumor biomarkers from synthetic urine samples

Synthetic urine samples were used to mimic complex biological sample matrix for extraction of dopamine, epinephrine and serotonin using the capillary with NSH-MOD sorbent. Figure 3.11 illustrate the CME-HPLC-UV chromatograms of spiked (biomarkers-added: dopamine, epinephrine and serotonin) synthetic urine (Fig 3.11 – a) and unspiked synthetic urine (Fig 3.11 – b). The prepared sorbent provided excellent sensitivity enhancement factor for these biomarkers. Thus, 435-, 460- and 400-folds enrichment were achieved for epinephrine, dopamine and serotonin from synthetic urine (modified to pH 10.5), respectively. Significant selectivity of the NSH-MOD sorbent toward the catecholamines was observed compared to serotonin. This can be attributed to the affinity of the TiO₂ surface toward cis-diol groups on catecholamines.
The obtained sensitivity enhancement factors from synthetic urine by the NSH TiO$_2$-PPO sorbent favorably compare with the state-of-the-art reported literature data obtained by aminophenylboronic acid functionalized magnetic nanoparticles $^{63}$ (13~17-fold for DA: dopamine and E: epinephrine), electrophoretic stacking $^{64}$ (130-fold for DA), MIP-SPME $^{58}$ (100-fold for DA and E), dynamic pH junction in CE $^{65}$ (~100-fold for DA and E), and boronate affinity micro-SPE $^{66}$ (~220-fold for DA and E). The obtained results are indicative of the applicability of the presented sorbent to preclinical applications related to catecholamines and serotonin.

Figure 3.11. CME-HPLC-UV chromatograms for spiked synthetic urine (a) and unspiked synthetic urine (b) at pH 10.5 and at 200 μg/L concentration level for dopamine, epinephrine and serotonin using NSH-MOD coated capillary. HPLC conditions: mobile phase 2/98 v/v % (methanol: ammonium acetate, 20mM, pH 3.8). UV detector: 280 nm.
3.4. Conclusion

Sol-gel titania-polypropylene oxide hybrid organic-inorganic sorbents were prepared for the first time via hydrolytic and nonhydrolytic sol-gel pathways providing covalent bonding between the organic polymer and the inorganic network. Chemical modification of the hydroxyl groups of the PPO was performed to sol-gel-activate the terminals of the organic polymer with titanium tetrachloride species to overcome the vast gap in reactivity between the sol-gel components. The presented sol-gel sorbents were used as microextraction phases for the preconcentration of an important neuroendocrine biomarkers for adrenal gland tumors (catecholamines) providing a favorably sample preparation tool compared to the state-of-art techniques. Compared to other sorbents that are prepared from metal oxides such as alumina, the presented sorbents provided adequate adsorption forces that allowed for easy desorption of the extracted analytes with LC-MS compatible mobile phase, avoiding the conventional elution methods such as pH manipulation and the use of phosphate buffer. The prepared NSH-MOD sorbent facilitated excellent extraction performance that was achieved through derivatization-free sample preparation and at elevated pH conditions that would be problematic for conventional silica-based sorbents.

3.5. References for chapter three


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613, 108.

(65) Tang, W.; Ge, S.; Gao, F.; Wang, G.; Wang, Q.; He, P.; Fang, Y. Electrophoresis
2013, 34, 2041.

Enrichment of Phosphopeptides using a Positively Charged Sol-gel Niobia-polyethylenimine Surface-bonded Coatings for Capillary Microextraction Coupled to High Performance Liquid Chromatography

4.1. Introduction

Phosphorylation is an extremely important biochemical process that has gained wide attention due to the intrinsic control it provides to the cell regulations and signaling [1, 2]. The phosphorylation process occurs at a very low concentration level in the cell, which necessitate the use of a selective and efficient method for the preconcentration and enrichment of the phosphorylated proteins or peptides from the biological samples [3]. Sorbents such as metal oxides are widely used for the enrichment of phosphorylated molecules, replacing the conventional methods such as immunoaffinity-based techniques that suffer from complication problems, use of expensive antibodies, and the possibility for sample and sorbent degradation. In contrast, metal oxides provide low cost, chemical stability, tunability, and diversity of sorbents either singular- or multi-component materials via different formats such as nano-particles [4, 5], nanotubes [6], core-shell microspheres [7], coated open tubular [8] and monolithic [9] capillaries, crystal clusters [10], that provide excellent efficiency and selectivity toward phosphorylated proteins or peptides.
Titanium oxide (TiO₂) is widely used as a preferred sorbent for the enrichment of phosphorylated peptide/proteins. TiO₂, like other metal oxide, possesses Lewis acid sites providing Lewis acid-base interactions with phosphate groups in the biosamples. This interaction can be established at a low pH medium (pH ~ 2.5 – 3.0) and then can be reversed at high pH elution conditions (pH 10) [11]. TiO₂ has been heavily investigated for its surface chemistry and intermolecular interactions, and has been found to have a robust performance for the enrichment of phosphorylated samples [12]. In analytical separations, TiO₂ has been used as chromatographic support either in the form of particles or monolithic beds [13-16]. For separation applications, the functionalization of TiO₂ surface with octadecyl ligands was achieved via the interaction between TiO₂ and organophosphate ligands [17]. TiO₂-phosphate interactions were found to be strong and not completely reversible even at pH 10 [17]. In this context and for sample preparation purposes, such strong interaction can cause sample loss, analytical errors, and sample carryover problems.

Niobium oxide (Nb₂O₅) has posed great potential in many research areas such as catalysis [18, 19] and solar cells [20, 21] because of the favorable properties and characteristics of the nanomaterials synthesized from Nb₂O₅. Compared to TiO₂, Nb₂O₅ has a better chemical stability [22-24] (the dissociation energy of Nb – O bond is higher (D₀ 7.93 eV) than Ti-O bond (D₀ 6.87 eV) [25]. Furthermore, as shown by a detailed IR spectroscopic study [26], niobia surface has a lower abundance of hydroxyl groups compared to titania. This is an important feature of niobia-based sorbents that allowed for the creation of more Lewis-acid sites rather than Bronsted-base sites on niobia surface. Also, it provides Nb₂O₅ with a better water-tolerance [18] due to the lower abundance of hydroxyl groups on the surface, which translates into lower chances of nonspecific interaction with the non-targeted molecules. Such information can serve as a key
factor for the use of metal oxides in the enrichment of phosphorylated samples from protic and polar matrices. Ficarro et al. [27] explored the use of niobium oxide (a commercial powder) for the extraction of phosphopeptides and reported a significant divergence in the selectivity of the Nb_2O_5 particles compared to TiO_2 counterparts. The Nb_2O_5 powder used that was used as purchased, which was thermally treated at 400 °C resulting nanocrystalline patches in the amorphous structure [28]. Treating a metal oxide at temperature above 200 °C can cause an alteration of its amorphous structure toward crystalline structure [29-31], which lowers the surface area of the metal oxide and potentially decreases its extraction capability.

The use of polyethylenimine (PEI) investigated [32] as a positively charged moiety that was coated on the outer surface of magnetic Fe_3O_4 nanoparticles to facilitate the enrichment of phosphopeptides via the electrostatic interaction between the positively charged PEI and the negatively charged phosphate groups. Although the sorbent used in the previous study showed good sensitivity toward phosphopeptides, it lacked any covalent bonding between the Fe_3O_4 nanoparticles and PEI. Furthermore, in order to immobilize PEI to the nanoparticles, the outer surface of Fe_3O_4 nanoparticles was coated with a layer of silica using tetraethoxysilane (TEOS) to provide a negatively charged surface that can create an electrostatic interaction with PEI. This PEI immobilization method brings two major disadvantages for this enrichment method: (a) the negative charge on the nanoparticle surface may apply repulsive forces to the phosphate groups and (b) these surface negative charges are also simultaneously consuming the positive charge on PEI to establish its immobilization on the surface of the particles.

The physicochemical characteristics of Nb_2O_5 encouraged us to investigate the use of sol-gel Nb_2O_5 and sol-gel Nb_2O_5-PEI sorbents as surface coatings for the enrichment of phosphorylated peptides via capillary microextraction. In this study, we examine the usefulness
of sol-gel niobia-based sorbents prepared under mild conditions with and without the addition of PEI in the extraction phase for the enrichment of phosphorylated molecules. To the best of our knowledge, this is the first study that utilizes sol-gel niobia and sol-gel-active PEI for the creation of a positively charged surface and covalently-bonded hybrid sorbent coatings for the enrichment of phosphopeptides.

4.2. Experimental Section

4.2.1. Equipment

An Agilent 1100 HPLC (Agilent Inc., Santa Clara, CA) system equipped with diode array detector, and a Rheodyne 6-ports injection valve (IDEX Health & Sciences, Oak Harbor, WA) were used for the CME-HPLC analysis. A Varian 3800 gas chromatograph (currently Varian is a part of Agilent Inc.) was used for the hydrothermal pretreatment and conditioning of the sol-gel coated capillaries. An in-laboratory built filling/purging device [33] and sample dispenser were used for coating the CME capillaries and for delivering the aqueous samples to the CME capillary, respectively.

4.2.2. Materials and chemicals

Fused silica capillary (250 µm i.d.) with polyimide external protective coating was purchased from Polymicro Technologies (Phoenix, AZ). Titanium (IV) butoxide, 1-butanol, toluene, 1-nonanol, glacial acetic acid, and hydrochloric acid were purchased from Sigma-Aldrich (St. Louis, MO). Niobium (V) ethoxide and trimethoxysilylpropyl-modified polyethylenimine (modified-PEI) were purchased from Gelest Inc. (Morrisville, PA). HPLC grade methanol, polypropylene microcentrifuge vials (2 mL) and pipette tips (200 and 1000 µL)
were purchased from Fischer Scientific (Waltham, MA).

4.2.3. Hydrothermal pretreatment of fused silica capillary

A 2-meter long fused silica capillary (250 µm i.d.) was rinsed with 2 mL each of dichloromethane, methanol, and water using an in-laboratory made nitrogen pressure-operated purging/filling system [33] at (10 psi). An oxy-acetylene torch was used to seal both ends of the fused silica capillary. The sealed capillary was secured in a GC oven and thermally conditioned by programming the oven temperature as follows: 40 °C – 350 °C @ 1 °C/min, with 200 min hold-up time at the final temperature 350 °C. The capillary was then cut-opened on both ends and rinsed again with 2 mL each water and methanol. Finally, the capillary was placed in the GC oven with one end connected to the injection port, and the other secured in the GC oven. Thermal conditioning was then performed with the flow of nitrogen through the capillary and simultaneous temperature programming as follows: 40 °C – 300 °C @ 10 °C/min, with 100 min hold-up time at 300 °C.

4.2.4. Preparation of sol-gel coated capillaries

4.2.4.1. Preparation of the sol solution for sol-gel niobia-based Sorbents.

Five niobium (V) ethoxide-based sol solutions were prepared by mixing the following molar ratios: Nb(OEt)₅, Acetic acid, H₂O, EtOH were as 1: 1.05: 0.03: 9.2, respectively. Trimethoxysilyl-modified polyethylenimine (modified-PEI) was added to four of the five sol solutions leaving one of the sol solutions free of modified-PEI (sol solution-i). The molar ratios of modified-PEI to Nb(OEt)₅ in the four sol solutions were as follows: 0.05, 0.10, 0.25 and 0.50, which will be referred as sol solution-ii, -iii, -iv, and -v, respectively. For sol solution-i, 330
µL of ethanol (containing 1% water) was mixed with 50 µL of toluene/1-nonanol (v/v 1:1) and vortexted vigorously in a 2-mL polypropylene microcentrifuge vial (after the addition of each component of the sol solution, the mixture was vortexed for 1 min). In another microcentrifuge vial, 33 µL of glacial acetic acid was mixed with 50 µL of Nb(OEt)₅ and the mixture was vortexed thoroughly. The content of the first vial was transferred to the niobium ethoxide/acetic acid-containing vial and the mixture was vortexed thoroughly. The gelation time for sol solution-i was ~ 80 min.

Table 4.1 shows the amounts of the sol solution components used for the preparation of solutions-ii, -iii, -iv and -v using a procedure analogous to the one described for the preparation of sol solution-i but with the addition of modified-PEI (Figure 4.1) to the first microcentrifuge vial as the first component.

4.2.4.2. Preparation of the sol solution for sol-gel titania-based sorbents

Titanium butoxide-based sol solutions were prepared following a procedure very similar to the one described in the previous section, but with minor changes. The molar ratios of the components of five sol solutions were: Ti(OBu)₄, acetic acid, H₂O, BuOH as 1: 1.07: 0.03: 9.37. Sol solution-i was left free of modified-PEI while the other four sol solutions were prepared with the following molar ratios of modified-PEI to Ti(OBu)₄: 0.05, 0.1, 0.25 and 0.50 in sol solution-ii, -iii, -iv, and -v, respectively. For the preparation of titanium butoxide-based sol solution-i, 220 µL of 1-butanol (containing 1.5% of water) was transferred to a microcentrifuge vial and mixed with 50 µL of toluene/1-nonanol (v/v 1:1) and vortexed thoroughly. In another microcentrifuge vial, aliquot of 22 µL of glacial acetic acid was mixed with 50 µL of titanium tetrabutoxide and the mixture was vortexed thoroughly.
Table 4.1. Summarization of the amounts of reagents used for the preparation of the sol solution (in µL) and their gelation time.

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<tr>
<th></th>
<th>Nb(OEt)$_3$ (µL)</th>
<th>Glacial Acetic Acid (µL)</th>
<th>(Nonanol/toluene v/v 1:1) (µL)</th>
<th>Ethanol (µL)</th>
<th>PEI/molar ratio (µL)</th>
<th>Gelation time (min)</th>
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<tr>
<td>Sol solution-i</td>
<td>50</td>
<td>37</td>
<td>50</td>
<td>330</td>
<td>0 /0.00</td>
<td>80</td>
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<td>Sol solution-ii</td>
<td>50</td>
<td>37</td>
<td>50</td>
<td>300</td>
<td>30 /0.05</td>
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<td>Sol solution-iii</td>
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<td>37</td>
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<td>270</td>
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<td>Sol solution-iv</td>
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<td>37</td>
<td>50</td>
<td>179</td>
<td>151 /0.25</td>
<td>130</td>
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<tr>
<td>Sol solution-v</td>
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<td>37</td>
<td>50</td>
<td>28</td>
<td>302 /0.50</td>
<td>~ 230</td>
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<table>
<thead>
<tr>
<th></th>
<th>Ti(OBu)$_4$ (µL)</th>
<th>Glacial Acetic Acid (µL)</th>
<th>(Nonanol/toluene v/v 1:1 %) (µL)</th>
<th>Butanol (µL)</th>
<th>PEI/molar ratio (µL)</th>
<th>Gelation time (min)</th>
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<tbody>
<tr>
<td>Sol solution-i</td>
<td>50</td>
<td>25</td>
<td>50</td>
<td>220</td>
<td>0 /0.00</td>
<td>65</td>
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<tr>
<td>Sol solution-ii</td>
<td>50</td>
<td>25</td>
<td>50</td>
<td>197</td>
<td>23 /0.05</td>
<td>75</td>
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<tr>
<td>Sol solution-iii</td>
<td>50</td>
<td>25</td>
<td>50</td>
<td>175</td>
<td>45 /0.10</td>
<td>100</td>
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<tr>
<td>Sol solution-iv</td>
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<td>25</td>
<td>50</td>
<td>109</td>
<td>111 /0.25</td>
<td>150</td>
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<tr>
<td>Sol solution-v</td>
<td>50</td>
<td>25</td>
<td>47</td>
<td>0</td>
<td>223 /0.50</td>
<td>~ 210</td>
</tr>
</tbody>
</table>
Figure 4.1. Illustration of the chemical structure of trimethoxysilylpropyl-modified polyethylenimine (PEI)

The content of the butanolic solution was transferred to the titanium butoxide/acetic acid-containing vial and the mixture was vortexed thoroughly. For the preparation of titania-PEI sol solutions, an analogous procedure to the one described for sol solution-i was used but in this case, modified-PEI was added as the first component in the first microcentrifuge vial. The gelation times for all five titanium butoxide-based sol solutions are shown in Table 4.1.

4.2.4.3. Creating sol-gel coatings on the inner surface of fused silica capillaries

Ten sol-gel coated capillaries were prepared using the 10 sol solutions (5 of each niobia- and titania-based sorbents) that were freshly prepared as described in the previous section and table 1. A 1-meter piece of hydrothermally treated fused silica capillary (thoroughly dried and accurately preweighed) was installed on a filling/purging device [33]. This device was used to fill each of the ten capillaries with a sol solution by applying 15-psi nitrogen pressure. As the sol solution started to drip out of the exit end of the capillary, the capillary was sealed with a rubber septum allowing the sol solution to reside inside the capillary (for all the sol-gel coated capillaries, the sol solution in-capillary residence time was kept at 30 min). Following this, the rubber septum was removed and the liquid content of the sol solution was expelled from the capillary using nitrogen pressure leaving behind a surface-bonded sol-gel coating on the capillary
inner wall. The coated capillary was then thermally conditioned in a GC oven carried out with simultaneous purging of the capillary with nitrogen flow. The temperature program of the GC oven was as follows: 40 °C – 150 °C @ 1 °C/min, 300 min hold-up time at 150 °C). After thermal conditioning, the coated capillary was rinsed with 2 mL of methanol using the purging/filling system. Finally, the coated capillary was thermally conditioned with nitrogen flow using the same temperature programming a second time. At this point, the coated capillary was ready for capillary microextraction experiments in hyphenation with HPLC.

4.2.5. Online sample extraction and analysis by CME-HPLC

A 40-cm piece of sol-gel coated capillary was installed on the 6-port HPLC injection valve as an external sampling loop for online-hyphenation of CME to the HPLC system [34]. To perform the CME-HPLC experiment, the injection port was kept at “Load” position allowing the aqueous sample to pass through the sol-gel coated capillary by gravity from an in-laboratory designed sample dispenser. After 40 min of extraction, which was enough time for the system to reach extraction equilibrium, the valve was switched to “Inject” position to facilitate the transfer of the extracted analytes to the HPLC column (HPLC C18 Luna column 250 x 2.1 mm) by desorbing them with the organo-aqueous HPLC mobile phase (water (0.1 % TFA): methanol (0.1 % TFA) 90/10 % v/v). A UV (at wavelength: 265 nm) detector was used to analyze the target analytes after their separation in HPLC column.

4.2.6. FTIR and TGA characterization of the sol-gel materials

Freshly prepared sol solutions were used for the creation of surface coatings on the inner surface of hydrothermally pretreated glass (borosilicate) tubes (36 x 6 mm). The
coating/conditioning method used for the coated glass tubes here was analogous to the methods used to prepare the sol-gel coated CME capillaries. The sol-gel materials were scraped off the glass tube inner surface with a stainless spatula then were taken for FT-IR and TGA analysis.

4.2.7. Gravimetric evaluation of the coating mass and volume

The weight of a thoroughly dried sol-gel coating created on the inner surface of fused silica capillary was determined. For this, the weight of a dried capillary (2 meters) before creating the sol-gel coating was subtracted from the weight of the same piece of capillary after coating its inner surface and performing thermal conditioning as described in section 4.2.4. The resulting value of the weight difference is the weight of the sol-gel coating on the 2-meter coated capillary (referred as \( W_{2m} \)), thus, the weight of the coating on a 40-cm CME capillary is calculated by dividing the \( W_{2m} \) by five.

The volume of the sol-gel coating was determined gravimetrically. The filling/purging device was placed in an in-laboratory designed glove box made of a Styrofoam cooler box. A 2-meter uncoated capillary (sealed on both ends with Restek capillary column glass caps) was weighed at room temperature before and after it was filled with methylene chloride (DCM) using the filling/purging device placed inside the glove box. Before filling the capillary with methylene chloride, the temperature of the glove box was cooled to 17 \(^\circ\)C using cold airflow passing through rubber tubing that were connected to an airtight icepacks-container that is placed inside the glove box allowing cold air to blow inside the glove box. This arrangement of cooling the glove box allowed for preventing evaporation of methylene chloride especially at the ends of the capillary during the process of filling the capillary with DCM. The 2-meter capillary was then coated with sol-gel sorbent as described earlier. Then the sol-gel coated capillary was filled with
DCM as described at 17 °C and carefully sealed with glass caps. The weight of the DCM-filled coated capillary was taken at room temperature. The volumes of DCM in the uncoated capillary and the sol-gel coated capillaries were calculated using the specific gravity of DCM at room temperature (S.G. at 25 °C is 1.379 g/mL). The difference in the volumes of DCM obtained from these measurements is equivalent to the volume sol-gel coating created on 2-meter capillary and referred as $V_{2m}$. The volume of the sol-gel coating on 40-cm capillary used for CME-HPLC experiments can then be calculated by dividing $V_{2m}$ by five.

4.2.8. Conversion of peak area to mass of extracted peptide

The peak areas obtained by CME-HPLC experiments were used as a quantitative measure of the extracted analytes. Calibration plots for both phosphorylated and non-phosphorylated VYKA peptides were constructed by obtaining the average peak area of triplicates injections of standard solutions of series of concentrations (5.0, 7.5, 10.0, 12.5 and 25.0 mg/L) using a 5-µL external sample loop. The obtained average peak areas were plotted against the corresponding molar concentration and the best-fit linear equation was constructed. For each of the peptides, the average peak area of the extracted sample was substituted in the corresponding equation to convert the peak area to molar concentration, then converted to mass of extracted analyte.

4.3. Result and discussion

Sol-gel coating technology gained excellent reputation for its convenience, high reproducibility and ability of create a wide range of sol-gel sorbents utilized in sample preparation [35-37] or analytical separation [33, 38-41]. In the field of analytical microextraction of trace analytes, Malik et al. has introduced a variety of sol-gel sorbents that demonstrated
excellent chemical and thermal stabilities while being utilized as surface coatings for fiber-based solid-phase microextraction [42] and capillary microextraction [43-45].

4.3.1. Synthesis of the sol-gel sorbents

Hydrolytic sol-gel route [46] for the preparation of metal oxides from metal alkoxides precursors is the predominantly used sol-gel route because of the convenience of the reaction conditions and the availability of very pure commercial metal alkoxide precursors. In the presence of water, the transition metal precursors undergo hydrolysis and condensation reactions as depicted in Figure 4.2 (general example for a tetravalent metal alkoxide precursor). The transition metal-based precursors tend to have an extremely high reactivity with water leading to immediate precipitation of metal oxide particles. The reactivity of transition metal precursors toward water can be decreased using a chelating agent such as carboxylic acids or diketones (in this study, acetic acid was used). For instance, acetic acids can achieve a dual role of decreasing the reactivity of the precursors toward hydrolysis and condensation reactions by the chelating effect and reducing the pH environment of the reaction. Low pH conditions can hinder the condensation reaction as described by Livage et al. [47] by forming more aquo-ligands (-OH₂) than hydroxo-ligands (-OH) around the metal atom. The tendency of aquo-ligands to be poor nucleophiles translates into a decreased possibility of nucleophilic addition reactions to proceed. As a result, the condensation reactions are hindered. In this study, the chelation ratio \( x \) (chelation ratio is the molar ratio of acetic acid to Nb(OEt)₅ or Ti(OBu)₄ precursors) was kept ~ 1, it was found that the ratio \( 1 \leq x < 2 \) gives a moderate slow-down of the metal alkoxide reactivity, while a ratio of \( x > 2 \) led to excessive chelation effect that permanently hindered the condensation reaction for niobia- and titania-based sol-gel precursors. These observations are consistent with
earlier sol-gel studies using transition metal precursors [47].

Figure 4.2. Illustration of the sol-gel reactions via hydrolytic route: (a) hydrolysis and (c) condensation for a tetravalent metal alkoxide

As described in the experimental section, the sol solution volume and the molar concentration of the sol-gel precursors were kept similar (0.427 M and 0.423 M for Nb(OEt)₅ and Ti(OBu)₄, respectively) to provide analogous conditions for the preparation of these two types of the sol solutions (one is niobium pentoxide-based and the other is titanium butoxide-based) that were used in this study. Figure 4.3 depicts the surface bonded sol-gel coated sorbents on the inner surface of fused silica capillaries in the form of niobia, niobia-PEI, titania and titania-PEI coatings. As shown in Figure 4.3, modified-PEI was allowed to condense with the evolving sol-gel network to facilitate covalent bonding to the sol-gel niobia or titania networks, such bonding provided chemical stability to the sorbent compared to PEI immobilization via electrostatic interactions [32]. The sol-gel coated capillaries were conditioned in the GC oven for a speedy completion of the sol-gel reactions that might be still occurring in the sol-gel coating created on the inner surface of the fused silica capillary. Also, heating the coated capillary up to
only 150 °C simultaneously with continuous flow of nitrogen facilitated the drying process of the sol-gel coatings from solvents and porogenic mixtures. Such a low drying temperature was chosen to prevent stress on the sol-gel porous structure during the evaporation of solvents and by-products to produce an amorphous structure with minimal crystalline regions in the sol-gel network [31, 48].

4.3.2. Characterization of the sol-gel sorbent

Figure 4.4 illustrates the FTIR data obtained for the sol-gel sorbents created on the inner surface of a glass tube and conditioned using analogous procedures to the one used for the sol-gel coated capillaries. The FTIR peak at 950 cm\(^{-1}\) (for sol-gel Nb\(_2\)O\(_5\)-PEI, Figure 4.4-b) can be attributed to the presence of the chemical linkage (Nb – O – Si) [49] between niobia-based sol-gel network and the silicon atom on the sol-gel-active PEI providing an evidence for the presence of the covalent bonding between PEI and the sol-gel niobia network. This data also indicates that covalent bonding between niobia-based sorbents and the silanol groups on the inner surface of the fused silica capillary is also achievable. Figure 4.4-c illustrate the FTIR data for sol-gel TiO\(_2\)-PEI. The peak at 921 cm\(^{-1}\) is very close to the peak assigned (925 cm\(^{-1}\)) to the Ti – O – Si linkage [49]. This data proves the establishment of the chemical bonding between the sol-gel titania network and PEI.
Figure 4.3. Illustration of the sol-gel surface coatings prepared by sol-gel niobia (a), niobia-PEI (b), titania (c), and titania-PEI (d) sorbents chemically bonded to the fused silica capillary inner surface.
The gravimetric analysis (described in section 4.2.7) of the sol-gel coated capillaries (40-cm) revealed that the coatings volumes were ~ 0.13 µL for both Nb₂O₅- and TiO₂-based sorbents while the volumes of Nb₂O₅- and TiO₂-PEI sorbents were 0.5 and 0.53 µL, respectively. For the proof of concept, after the formation of the sol-gel sorbents on the inner surface of fused silica capillaries, three cross-sectional scanning electron microscopic images were taken for short segments of sol-gel Nb₂O₅ and Nb₂O₅-PEI coated capillaries (Figure 4.5 and Figure 4.6, respectively). The coating volume was calculated using the average coating thickness and the length of capillary. Paired t-Tests (at 95 % confidence level) were conducted and revealed that the results obtained by these two methods (gravimetric and SEM imaging) for the determination of the sol-gel coating volumes do not differ significantly.

4.3.3. The extraction of phosphopeptide VYKA using niobia- and titania-based sol-gel sorbents

For the minimization of the non-specific interaction between non-targeted molecules with the sorbent, the pH of the sample was adjusted to pH 2 by the addition of trifluoroacetic acid. The adjustment to a pH level lower than the isoelectric point (IEP) of both niobia and titania (IEP 4.1 and 6.1 for sol-gel Nb₂O₅ and sol-gel TiO₂, respectively [50, 51]) facilitated the protonation of the hydroxyl groups on the surface of the sol-gel sorbents providing a positively charged metal oxides in addition to the surface Lewis-acid sites.
Figure 4.4. Illustration of the FTIR data obtained for unreacted PEI (a), sol-gel niobia-PEI (b) and sol-gel titania-PEI (c) sorbents.
Figure 4.5. Illustration of cross-sectional scanning electron microscopic image of a sol-gel niobia coated capillary

Figure 4.6. Illustration of cross-sectional scanning electron microscopic image of a sol-gel niobia-PEI coated capillary
Also, PEI is expected to be positively charged at such a low pH level. Here, the sol-gel sorbents were designed to be positively charged coatings that are suitable for the microextraction of phosphopeptide via the interaction with the negatively charged phosphate group in addition to the specific interaction between phosphate groups and the Lewis acid sites. Figure 4.7 illustrates CME-HPLC chromatograms obtained by sol-gel niobia coated capillary (a) and sol-gel titania coated (b) capillary. The sol-gel sorbents used here are free of PEI. To provide a quantitative comparison, a newly introduced parameter (specific extraction, SE [52]) that pertains the mass of extracted analyte to the unit mass of sorbent. Niobia-based sorbents achieved higher SE values of phosphorylated peptide VYKA by 96.7 % compared to titania-based sorbent. The higher SE value obtained by the sol-gel niobia-based sorbent can be attributed to the surface nature of niobia-based oxide that facilitated the creation of more Lewis acid sites than Bronsted acid sites (Lewis acid-base interactions are stronger by ~ 15 times than Bronsted acid sites [53]) on niobia surface as revealed by a detailed IR spectroscopic studies elsewhere [26]. These additional interaction sites provided more Lewis acid-base interactions between sol-gel niobia-based sorbent and the phosphate group on the peptide that translated into higher extraction capability.

4.3.4. Polymer-loading role in the extraction capability of the sol-gel sorbents

The extraction capability of the presented sol-gel sorbents for phosphorylated and nonphosphorylated peptide VYKA was significantly enhanced after incorporating the positively charged PEI in the sol-gel sorbent (Figure 4.8). The positively charged sites (hydroxyl-associated sites and quaternary amine-containing polymer) and the Lewis acid sites on the hybrid sol-gel sorbents provided dual intermolecular interactions (electrostatic and Lewis acid-base interactions) that facilitated higher extraction performance of the sol-gel or hybrid sorbents.
compared to the sol-gel inorganic sorbents. It was noticed that the gradual increase of the molar ratio of PEI (as shown in the Figure 4.8) in the sol-gel sorbents resulted a significant drop in the SE difference between Nb$_2$O$_5$-PEI and TiO$_2$-PEI sorbents. The percentage of the difference in SE values dropped from 96.7 % to 48.0 %, 22.9 %, 13.3 % then 11.9 % as the molar ratio of PEI was increased from 0.0 to 0.05, 0.1, 0.25 and 0.5, respectively. Possible blockage could have occurred to a good number of the Lewis acid sites on the metal oxide surface by the horizontally-oriented PEI after increasing PEI ratio in the sorbent that led to the declining difference between Nb$_2$O$_5$-PEI and TiO$_2$-PEI sorbents.

Figure 4.7. CME-HPLC Chromatogram that illustrates the microextraction of VYKA phosphopeptide using sol-gel niobia-based coating (blue) vs. sol-gel titania-based (red) coating from aqueous sample at 100 µg/L concentration level (pH 2, adjusted with TFA). HPLC conditions; mobile phase composition: 90:10%, solvent A: water (0.1% TFA), solvent B: methanol (0.1% TFA), at room temperature. Detector conditions: UV wavelength at 265 nm. HPLC C$_{18}$ Luna column (250 x 2.1 mm).
Figure 4.8. Comparison of SE values for sol-gel niobia-based (a) CME sorbents vs. sol-gel titania-based (b) sorbents with different molar ratios of the added PEI. The right frame illustrate the percentage of the difference in SE values vs. the molar ratio of the added PEI.

4.3.5. CME-HPLC experiments via sol-gel sorbents

Figure 4.9 illustrates the extraction profile for VYKA phosphopeptide as an investigation of equilibration time for the sorption-desorption process. The adsorption equilibrium of VYKA phosphopeptide between the aqueous sample and the sol-gel titania coating was reached within 15 min, while it took about 30 min to reach equilibrium using the niobia-based coating. Figure 4.10 illustrates CME-HPLC chromatogram obtained for VYKA phosphopeptide using the sol-gel Nb_2O_5-PEI and TiO_2-PEI coated capillaries. These two sorbents) were prepared with equivalent molar ratio of modified-PEI and molar concentration of the sol-gel precursors resulting an equivalent loading of PEI as evident by the TGA data (Figure 4.11) obtained for sol-gel materials used to coat these two CME capillaries (sol solution-iii). From the TGA data, the higher affinity toward VYKA phosphopeptide achieved by niobia-PEI sorbent can be attributed to the inorganic component (niobia) in the sol-gel niobia-PEI. The same sol-gel CME capillaries were used for
the extraction nonphosphorylated VYKA peptide, comparable performance of both sorbents was revealed by the obtained CME-HPLC experiment (Figure 4.12).

Table 2 shows the CME-HPLC results obtained by the four sol-gel sorbents for the analysis of phosphorylated and nonphosphorylated VYKA peptides. Excellent run-to-run peak area reproducibility was obtained (RSD ≤ 5.1 %) that was in consistence with previously published studies that showed similar remarkable reproducibility of the sol-gel sorbents [54-57]. The higher affinity of the sol-gel niobia-based sorbents toward the phosphorylated peptide allowed for the achievement of ~ 60 % higher sensitivity enhancement factor [57] compared to sol-gel titania-based sorbents.

![Extraction profile](image)

Figure 4.9. Extraction profile of VYKA phosphorylated peptide using sol-gel niobia and sol-gel titania coatings with chromatographic conditions similar as in Figure 4.7.
Figure 4.10. CME-HPLC chromatogram that illustrates the microextraction of VYKA phosphopeptide using sol-gel niobia-PEI coating (blue) vs. sol-gel titania-PEI coating (red) from aqueous sample at 100 µg/L concentration level (pH 2, adjusted with TFA). Mobile phase composition: 95/5 v/v % solvent A: water (0.1 % TFA), solvent B: methanol (0.1% TFA). UV detector: 265 nm. HPLC C18 Luna column (250 x 2.1 mm)
Figure 4.11. Illustration of TGA curves obtained for sol-gel niobia-PEI (red line) and sol-gel titania-PEI (black line) sorbents
Figure 4.12. CME-HPLC chromatogram that illustrates the microextraction of nonphosphorylated VYKA peptide using sol-gel niobia-PEI coating (blue) vs. sol-gel titania-PEI coating (red) from aqueous sample at 100 µg/L concentration level (pH 2, adjusted with TFA).

A better desorption efficiency (DE %) [58] for niobia-based sorbent (97.5 %) compared to titania-based sorbent (90.4 %) was observed, this can be attributed to several factors: (a) “softer” nature of the niobia-Lewis acid sites as a result of the larger atomic radius of niobium.
atom (198 pm for Nb, while 174 pm for Ti atom) [59]. (b) Strong TiO₂-phosphate interaction, which led to a lower DE % of the titania sorbent. These strong interactions could have caused an irreversible adsorption on the surface of TiO₂-based sorbent (usually reversed by high pH elution solvent, 10 % aqueous ammonia [10, 49]).

4.4. Conclusion

Surface coatings of sol-gel niobia and titania were prepared to be chemically bonded on the inner surface of fused silica capillary for capillary microextraction coupled to high performance liquid chromatography for the enrichment of phosphorylated VYKA peptide. Both coatings were prepared with a covalently bonded polyethylenimine polymer as a positively charged ligand to provide enhanced intermolecular interactions with the negatively charged phosphate groups. Sol-gel niobia coating showed a higher affinity towards to the target phosphopeptide compared to titania counterpart. This study showed the applicability of sol-gel coating method for fine tuning the sorbents by utilizing charged ligands and soft Lewis acid metal oxides for selective and easily desorbed interactions with phosphorylated molecules. Such efficient microextraction medium can serve as a potential extraction phase for sample preparation purposes of important biomolecules.
Table 4.2. CME-HPLC results for phosphorylated and nonphosphorylated VYKA peptides obtained by niobia-, niobia-PEI-, titania- and titania-PEI-based sol-gel sorbents.

<table>
<thead>
<tr>
<th></th>
<th>VYKA Phosphorylated</th>
<th>VYKA Nonphosphorylated</th>
<th>VYKA Phosphorylated</th>
<th>VYKA Nonphosphorylated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nb₂O₅</td>
<td>Nb₂O₅-PEI</td>
<td>TiO₂</td>
<td>TiO₂-PEI</td>
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<tr>
<td>Average peak area (n=3)</td>
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<td>148</td>
<td>12.5</td>
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<td></td>
<td>1.8</td>
<td>2.5</td>
<td>5.1</td>
<td>2.8</td>
</tr>
<tr>
<td>Run-to-run RSD %</td>
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<td>395.3</td>
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<td>246</td>
<td>367</td>
<td>2466</td>
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</table>

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4.5. References for chapter four


Appendices

Appendix A:

Nonhydrolytic Sol-gel Approach to Facile Creation of Surface-bonded Zirconia Organic-inorganic Hybrid Coatings for Sample Preparation. I. Capillary Microextraction of Catecholamine Neurotransmitters
enrichment of catecholamine samples. PBA ligand has high affinity toward cis-diol groups present in the catecholamines [8]. The activation of the chelating ligand (phenylboronic acid, PBA) requires conditioning of the SPE cartridge with high-pH buffer (pH 10–12) [10] giving rise to the main drawback of PBA-SPE cartridges due to inadequate pH stability of silica-based particles which are known to have a narrow operational pH window (pH 2–8) [11–14]. Alumina has been used for the extraction of catecholamines providing a pH-stable sorbent in the form of SPE sorbent [15–21]. Extensive strong adsorbent characteristics of alumina require pH manipulation and the use of phosphate buffers for the desorption and elution of the extracted catecholamines.

Using different metal/metalloid alkoxide precursors, Malik and coworkers [22–25] have developed a number of sol-gel CME extraction phases providing excellent pH stability (5.0–14.0) in CME-HPLC as well as enhanced thermal stability in CME-GC operations. They included titania- [22,26], zirconia- [23] and germanium-based [14,24,25] hybrid inorganic-organic CME coatings. The sol-gel coating route provides a simple, convenient and effective approach to synthesizing organic-inorganic hybrid materials [27]. The key to the success of the sol-gel coating (in addition to the unique physical and chemical properties of the cresteched hybrid materials) is the chemical bonding of the sol-gel coating to the substrate (e.g., fused silica fiber or capillary).

Hydrosilylated sol-gel (HSG) route [11] was used to create those microextraction media. Non-hydrolytic (nonaqueous) sol-gel (NIHS) route has been investigated extensively in the field of catalysis for the creation of metal/metalloid oxides [28,29]. In a nonaqueous environment, transition metal halide (e.g., ZrCl₄) concurrently undergoes alkoxyl and condensation reactions leading to the formation of transition metal oxides [30]. NIHS-generated transition metal oxides possess better water-tolerance, enhanced homogeneity, and more Lewis acid sites than Bronsted acid base sites [28,29,31,32]. NIHS route can provide uniformly dispersed transition metal oxide particles in organic solvents and allows for facile surface modification with organic moieties [33–35]. The later property is important for the use of nonaqueous sol-gel route for the creation of hybrid organic-inorganic material with covalently bonded organic ligands. Here we present a systematic investigation on the synthesis and analytical evaluation of a novel zirconia-based sol-gel hybrid organic-inorganic sorbent to provide a biocompatible extraction medium integrating amphiphilic properties with enhanced thermal-, mechanical- and pH stability characteristics that are important for the analysis of aqueous samples of free catecholamines and molecules structurally related to their deaminated metabolites.

2. Experimental section

2.1. Materials and instruments

Zirconium (IV) butoxide, zirconium (IV) chloride, ethanol, 1-butanol, toluene, hydroxyl-terminated polypropylene oxide (M₉₅000), glacial acetic acid, cretechol, quinol, resorcinol, 4-hydroxynaphthalic acid, boric acid, vanillin, acetaminophen, deumine hydrochloride, epinephrine hydrochloride, and serotonin hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). HPLC grade sorbents (methanol, dichloromethane, tetrahydrofuran), polypropylene microcentrifuge tubes and micropipette tips were purchased from Fisher Scientific (Waltham, MA). Fused silica capillary (250 μm i.d.) with polyethylene external protective coating was purchased from Polymicro Technologies (Phoenix, AZ). The following chromatographic equipment was used in this study: (a) an Agilent 1100 series HPLC system equipped with a Diode Array Detector (Agilent Inc., Santa Clara, CA); (b) a Varian 3800 model gas chromatograph with a flame ionization detector (currently Varian is a part of Agilent). (c) Rheodyne 6-port valve (IDEX Health & Sciences, Oak Harbor, WA) an in-house built purging/filling system [36,37].

2.2. Hydrothermal pre-treatment of fused silica capillary

A one-meter segment of fused silica capillary (250 μm i.d.) was rinsed with 2ml each of dichloromethane, methanol, and water using a gas pressure-operated purging/filling system [36] at 16psi. Both ends of the capillary were then sealed using epoxy-acrylate trench. The sealed capillary was placed in the GC oven and conditioned by raising the temperature from 40°C to 350°C at a rate of 1°C/min, holding the capillary at 350°C for 20min. After thermal conditioning, the capillary was cooled to room temperature and cut open on both ends using an alumina wafer. It was then placed in the GC oven with one end connected to the GC injection port allowing nitrogen gas to flow through the capillary, and the other end was left open and secured in the GC oven. Thermal conditioning of the capillary was performed under nitrogen purge (1 ml/min) as follows: 40°C–350°C at rate of 10°C/min. 120 min hold time at 350°C. The capillary was then cooled down to room temperature and its inner surface was ready for coating.

2.3. Preparation of sol-gel zirconia–PPO coated capillary via non-hydrolytic sol–gel (NIHS) route

2.3.1. Solvents drying

In the NIHS process, the solvents must be free from water. For this, the solvents (butanol, toluene) were dried over molecular sieve (type 4A) by placing 15 ml of each solvent in a separate vial. A 10-g amount of the molecular sieve was added to each solvent and vortexcited for 2 min and then left airight in the hood overnight. 25 ml aliquot of each treated solvent was transferred to a microcentrifuge vial and centrifugation was performed (10000 rpm for 2 min) to eliminate any possible contamination from the molecular sieve particles. To test if the dried solvents still contained water, 0.15g of anhydrous copper sulfate (white) was mixed with 1ml of each dried solvent, then the mixture was thoroughly vortexed. The mixture was centrifuged to precipitate the copper sulfate powder, which would turn blue in the presence of water. The drying procedure was repeated until no color change of CuSO₄ was observed.

2.3.2. Modification of organic polymer with zirconium tetrachloride

Prior to the preparation of sol-gel sorbents, the terminal hydroxyl groups of polypropylene oxide (PPO) were modified with zirconium tetrachloride. For this, PPO and ZrCl₄ were taken in a 25 ml round-bottom flask in molar ratio of 1:2 (PPO: 0.5 mmol, ZrCl₄: 1.2 mmol) and dissolved in anhydrous toluene (300 μl). The solution was stirred for 12 h at 60°C then allowed to reach room temperature before using it for the preparation of sol solution.

2.3.3. Preparation of sol solution for the NIHS route

The sol solution was prepared as follows: in a polypropylene centrifuge vial, 46 mg of zirconium tetrachloride was dissolved in 74 μl of dried 1-butanol. In a second vial, 380 mg of modified PPO was mixed with 180 μl of dried toluene and vortexed thoroughly for 1 min and it was left in the hood for 6h. Thereafter, polymer solution was vortexed for 1 min and then it was transferred to the first vial containing butanonic solution of zirconium tetrachloride. The mixture was vortexed thoroughly to ensure homogeneity. The gelation time of this mixture was ~2 h. Based on this gelation time, the coating of the capillary was performed after the solution was
left to undergo reactions in the vial for 30 min, allowing further reactions to take place inside the capillary.

2.3.4. Creation of CME coating via HSG route for CME-HPLC

Details of the sol-gel coating technology can be found elsewhere. [27] Briefly, a 60-cm piece of hydrothermally treated fused silica capillary was filled with the sol solution using a pressure-operated filling/purging system for coating under 15 psi nitrogen pressure. After the end of the capillary was sealed with a rubber septum after first few drops of the sol solution came out of the capillary, the sol solution was allowed to reside in the capillary for 30 min. At the end of the in-capillary residence period, the liquid content of the capillary was expelled under 15 psi gas pressure, leaving behind a sol-gel coating on the capillary inner surface. Nine capillaries were prepared with different in-capillary residence times (starting from 10 min in-capillary residence and increasing the time by increments of 5 min) to optimize the best coating conditions. The coated capillary was thermally conditioned in a GC oven while simultaneously being purged with a flow of nitrogen gas. For this, one end of the capillary was connected to the GC injection port, and the other end was secured in the GC oven. The capillary was heated using a temperature program (40°C–150°C @ 1°C/min, with a hold of 300 min at 150°C). The conditioned capillary was cool down to room temperature and rinsed with 2 mL each of n-butanol and methanol with the help of the purging/filling system. Finally, the coated capillary was thermally conditioned in a GC oven under nitrogen purging (40°C–150°C @ 5°C/min, with a hold time of 300 min at 150°C). At this point, the coated capillary was ready for CME-HPLC experiments.

2.4. Preparation of sol-gel zirconium-PFO coated capillary via hydrolytic sol-gel (HSG) route

2.4.1. Preparation of sol solution for HSG route

The sol solution was prepared as follows: in a polypropylene centrifuge vial, 20 μL of zirconium butoxide was mixed with 17 μL of glacial acetic acid. In a different vial, 80 μg of modified PFO was mixed with 200 μL of n-butanol and vortexed thoroughly for 1 min and left in the hood for 5 h. The polymer solution was then vortexed thoroughly again for 1 min and then transferred to the first vial containing zirconium butoxide and glacial acetic acid in solution. The mixture was vortexed thoroughly for 2 min, and 6 μL of de-ionized water was added to the mixture and followed by thorough vortexing for 2 min to ensure homogeneity of the sol-gel solution. The gelation time of this mixture was about 8 h. Taking this fact in consideration, the sol solution was first allowed to undergo reactions in the vial for 6 h before using it for creating CME capillary.

2.4.2. Creation of CME coating via HSG route for CME-HPLC

The capillary coating and conditioning procedures for the preparation of sol-gel CME via HSG route were analogous to the one described in the previous section for HSG route.

2.5. Capillary microextraction coupled to high performance liquid chromatography (CME-HPLC)

The CME-HPLC experimental setup was described elsewhere. [22] Briefly, a 40-cm piece of the sol-gel coated CME capillary was installed as an external sampling loop on a 6-port HPLC injection valve. Catecholamines and serotonin stock solution were prepared with serial dilutions with 0.1 M HCl to obtain a 1 mg/L concentration level. For synthetic urine stock solution (the composition of the synthetic urine was adopted from Ref. [38]), dopamine and epinephrine were spiked at 50 μL/L concentration level. The samples for the CME experiments were prepared in ammonium hydroxide solution (pH 10.5) at 100 μg/L and 200 μg/L concentration levels for the aqueous and the synthetic urine samples, respectively. The spiked urine stock solution was vortexed for 1 min and ultracentrifuged at 21,000 g for 2 min. The stock solutions of the other test probes were prepared using methanol via serial dilutions to 1 mg/L. These solutions were further diluted to 100 μg/L using deionized water. In the “sampling” position of the injection valve, the aqueous sample was allowed to pass through the CME capillary from an in-laboratory designed gravity-fed sample dispenser [37] via the injection valve. As the sample passed through the capillary, the analytes were extracted by the sol-gel coating on the capillary inner wall. After 40 min of extraction, the HPLC analysis was started by switching the valve to “inject” position, thereby desorbing the extracted analytes by the mixture of the HPLC mobile phases (phase A: methanol, phase B: ammonium acetate 20 mM, pH 3.6) flowing through the capillary and transferring them to the HPLC column (Zorbax C18 2.1 × 150 mm, 5 μm). For the analysis of catecholamines, the mobile phase composition (298/70/20 phase A: phase B) while for the test probes (207/20/70 phase A: phase B). For the analysis of the synthetic urine sample, the column was flushed with a mobile phase (90/10/0 phase A: phase B) for 10 min. In both the between the CME-HPLC experiments to clean the HPLC column, which was then equilibrated with the mobile phase (298/20/70 phase A: phase B) for 10 min prior to next CME-HPLC analysis.

2.6. Characterization of the synthesized sol-gel materials

2.6.1. Characterization

FTIR and thermogravimetric analysis were performed on HSG and NHSG ZrO2-PFO sorbents. For this, freshly prepared sol solutions (as described in previous sections) were mixed with hydrothermally pretreated 5 μm diameter silica particles (0.2 g, 3% w/s of the sol solution) in microcentrifuge vial and vortexed for 2 min. The prepared mixtures were used to coat the inner surface of borosilicate tube (1.6 × 6 mm) following a very similar coating/conditioning method as described in previous sections used for the preparation of the sol-gel CME capillaries. After thermal conditioning, the sol-gel materials were scraped off the tube surface with a stainless-steel spatula and were used for FTIR and TGA analysis.

2.6.2. Coating thickness and volume

For the determination of the sol-gel CME coating volume, 10 cross-sectional SEM images (using Hitachi Scanning Electron Microscope SU 70) were taken from 10 random segments (~1 cm) of the prepared sol-gel CME capillaries and the coating average thickness was used to assess the coating volume. The following equation was used for the coating volume: \( V = \pi \times l \times (R^2 - r^2) \), where \( l \) = capillary length, \( R \) = fused silica capillary radius from the center to the capillary wall, and \( r \) = coated capillary radius from the center to the coating surface.

2.6.3. Conversion of peak area to amount of extracted analyte

The chromatographic peak area was used as a quantitative measure of the extracted analytes. Calibration plots for all analytes were constructed by obtaining the average peak area for 3 replicate measurements conducted by directly injecting each of the standard solutions representing a series of concentrations (0.1, 0.5, 1.0, 5.0, 25.0, 50.0, 75.0, and 100.0 μg/L). The obtained average peak areas were plotted against the corresponding molar concentration of the injected solutions and the best-fit linear equation was used to convert peak area to molar concentration, and then to the amount of analytes extracted by the CME capillary.

2.6.4. Desorption efficiency (DE) %

To evaluate the completeness of desorption of the extracted analytes from the sol-gel CME sorbent, each sample was directly
injected into the HPLC system using a 40-cm deactivated fused silica capillary as external sampling loop. The obtained peak areas were converted into analyte amounts using the calibration plot as described in the previous section. Each sample (containing 200 ng of analyte) was allowed to pass through the coated capillary for 40 min and the liquid exiting from the capillary was collected. The mass of every analyte in the collected liquid was then determined by direct injection into the HPLC system. The difference in the mass of analyte before and after the extraction (evaluated by direct injection) was considered as the extracted amount. After desorption and analysis of the CME-extracted analytes, the obtained amount of analyte was taken as the sorbed amount. DES wasthen calculated using the following equation:

\[
\text{Desorption Efficiency (DE)} \% = \frac{\text{Desorbed amount}}{\text{Extracted amount}} \times 100
\]

3. Results and discussion

The sol-gel reaction route provides a simple, convenient, and effective approach to synthesizing organic-inorganic hybrid materials [27]. Surface-bonded coatings were introduced by our group for open tubular columns in gas chromatography (GC) [39], fiber-based solid phase microextraction (SPME) [40], and capillary microextraction (CME) [37]. The key to the success of the sol-gel coating (in addition to the unique physical and chemical properties of the created hybrid materials) is the chemical bonding of the sol-gel coating to the substrate (e.g., fused silica fiber or capillary).

Metal/metalloid alkoxide precursors are predominantly used as sol-gel precursors for the fabrication of sol-gel materials, due to their high purity, controllable reactivity, and convenience of use. In hydrolytic route of sol-gel reactions, the sol-gel precursors undergo hydrolysis with practically concurrent polycondensation of the hydrolyzed or partially hydrolyzed precursor species among themselves and/or with other sol-gel active species in the solution. The physico-chemical characteristics of the metal/metalloid alkoxides (size, coordination state, and solid phase charge 8M), alkoxide group size, together with temperature, solvent, catalyst, etc. represent the most important factors that affect the rate of hydrolysis and condensation of the alkoxide precursors. Compared to silicic acid, all zirconium alkoxides undergo hydrolysis and condensation more rapidly [11,41,42]. In Zr0.63, the partial charge on zirconium is +0.65. However, in Ti0.63, the partial charge on Ti is +0.63 and in Si0.63, the partial charge is −0.32 on Si [43]. Such differences in partial charges and other parameters for zirconium and silicon result in greater rates of hydrolysis (kH ≈ 10−2 M−1 s−1) [41] for Zr0.63 precursor compared to hydrolysis rate for Si0.63 (kH ≈ 10−4 M−1 s−1) [11,42]. In addition to the difference in the rate of hydrolysis, the condensation rate of hydroxylated zirconium alkoxide precursors is significantly higher than analogous rates for silicon precursors (kH ≈ 10−7 M−1 s−1 vs. 10−9 M−1 s−1) [11,41]. In a sol-gel system that contains precursors with vastly different reactivities, there exists a great probability of preferential reaction taking place with the participation of chemical species characterized by higher reactivities. Thus, preferential formation of zirconium is likely to occur when silica-based sol-gel active ligands or organic polymers are mixed with zirconium alkoxide.

To create hybrid material systems by integrating different sol-gel-active species, it is important that the chemical reactivities of these species are comparable with each other. To that end, different solvents and chelating agents have been used to slow down the hydrolysis and condensation rates of transition metal alkoxides precursors [23,44−46].

3.1 Synthesis and characterization of the sol-gel sorbent coatings

In this study, we employed functionalization of hydroxyl-terminated polypropylene oxide with zirconium tetrachloride (Fig. 1) mixed with toluene as a non-oxygen containing reaction medium that has good solubility with ZrCl4 [47], to provide an organic polymer having sol-gel-active terminals with chemical reactivities comparable to that for zirconium-based sol-gel precursors. The modification reaction of PPO was conducted in anhydrous tolune at 60 °C with continuous stirring analogous to a recent study [35] dealing with the derivatization of terminal hydroxyl group of PEG reacting with TC4 using microwave-heating of the reaction mixture. Two types of structural and physicochemical characteristics encouraged us to use PPO as the organic component for the proposed hybrid sorbents: (a) the ability to provide H-bonding interactions and its amphiphilic character to provide intermolecular interactions conducive to the extraction of catecholamines and (b) the ability to provide molecular level interactions (e.g., dipole-dipole, electrostatic forces, etc.) that are important for the creation of homogeneous sol solution composed of organic and inorganic components.

Acetic acid was used as a chelating reagent to reduce the fast hydrolysis rate of zirconium alkoxide precursors for the synthesis of the ZrO2−PO sorbent via HSG route [23,48]. The hydrolytic rate zirconium trichloride groups on the terminals of PPO (kH ≈ 1 × 10−2 M−1 s−1) [48] is comparable to that of zirconium alkoxide precursors (kH ≈ 10−2 M−1 s−1) [41]. Fig. 2 illustrates the sol-gel reactions via HSG and NISG routes in solution and within the hydrothermally pretreated fused silica capillaries. For HSG route, hydrolysis (1-A) of zirconium butoxide takes place after chelation with acetic acid providing species that can undergo condensation reactions, either water condensation (1-B) or alcohol condensations (1-C) between themselves and with the derivatized PPO (1-C) in the sol solution leading to the formation of a three-dimensional hybrid organic-inorganic network.

The reactions taking place in the sol solution during the preparation of the NISG ZrO2−PO sorbent are depicted in Fig. 2. These included alcoholysis (2-A), condensation with alkyl halide elimination (2-B) and polycondensation (2-C) occurred in the presence of the derivatized PPO with zirconium trichloride terminals groups producing sol-gel zirconia−PPO hybrid materials. The hybrid organic-inorganic zirconia-based sorbent was synthesized in situ by conducting the sol-gel reaction within the capillary where it also had the opportunity to undergo condensation reaction with the silanol groups on the inner surface of fused silica capillary as shown in Fig. 2.

Fig. 3 illustrates the scanning electron microscopic images of the cross-sectional view for both NISG and HSG coated capillaries. The average coating thickness calculated for 10 segments of coated capillaries was 1.49 μm for NISG and 1.81 μm for HSG coating. Based on this data, the calculated internal volume of the NISG CME capillary was ~19.17 μL and for HSG coated CME capillary ~19.07 μL. Knowing that the volume of a 40-cm segment of uncoated fused silica capillary is ~10.63 μL, the calculated sorbent coating volume is 0.46 μL for NISG and 0.56 μL for HSG CME sorbent coatings, respectively.

The results from FTIR spectroscopy investigations are shown in Fig. 4. Here, the peaks at 1555 and 1548 cm−1 are indicative of the presence of Zr−O−C bond [50], in the sol-gel material prepared by NISG (black) and HSG (red) routes, respectively. The obtained sol-gel materials mimic the compositions and coating conditions used for the preparation of the sol-gel sorbents in the CME capillary. As evident from the FTIR spectra, the peak at 867 cm−1 can be attributed to the presence of Zr−O−Si bond [51] between the sol-gel material and the silica particles. This data also indicates the feasibility of creating such covalent bonding between the
Fig. 1. Illustration of the modification of PPO with zirconium tetrachloride.

1- Hydrolytic sol-gel Route

2- Nonhydrolytic sol-gel Route

Fig. 2. Illustration of ISGC and NIGC reactions in solution inside the capillary for the preparation of sol-gel ZrO₂-PPO sorbents.

Fig. 3. Illustration of scanning electron microscopic images of (a) NHSC ZrO₂-PPO coated capillary and (b) ISG ZrO₂-PPO coated capillary at 10,000 magnification.

sol-gel zirconia-based sorbents and the fused silica surface of the CME capillary. The sol-gel material prepared via hydrolytic route was treated with water to fully hydrolyze the residual zirconium tetraoxide precursors that might have undergone only partial hydrolysis or have not undergone hydrolysis at all during the synthesis. The presence of such species could interfere with the FTIR analysis by showing the presence of Zr—O—C bond between Zr and butoxide groups. This data provides evidence for the successful
chemical bonding of PPO to the zirconia sol-gel network and the ability of the presented sol-gel coating routes to create covalently bonded sorbents on fused silica surface.

3.2. Evaluation of the sol-gel coating pH-stability

To examine the chemical and pH stabilities of the prepared sol-gel sorbent of the present study, CME-HPLC experiments were conducted using a CME capillary coated with NHSG ZrO$_2$-PPO. The capillary was continuously rinsed with 1.0 M HCl aqueous solution for a period of 6 h followed by rinsing with 50-ml of deionized water. The coated capillary was further rinsed with 1.0 M NaOH aqueous solution for a period of 6 h and then was washed again with 50-ml of deionized water. Fig. 5 shows the CME-HPLC chromatograms obtained for the comparison of CME performance of the prepared sol-gel-zirconia-PPO coated capillary before and after the exposure to harsh pH conditions. It clearly shows the stability of the prepared sol-gel CME coating since its extraction capability remained practically unchanged. A comparison of the peak areas of these two chromatograms revealed a slight peak area increase (0.9%, 0.18%, and 0.37% for nicotinic acid, serotonin and acetaminophen, respectively) obtained by CME-HPLC experiments conducted after rinsing the capillary with extreme-pH solutions. The slight increase in the extraction capability of the sorbent can be attributed to the renewed availability of some of the buried extraction sites on the surface of the sol-gel sorbent due to removal of possible surface contaminants after rinsing with harsh pH solutions.

3.3. Evaluation of the microextraction performance

To determine the time required to establish the extraction equilibrium of the target analytes between the sol-gel sorbents and the sample matrix, extraction profiles were experimentally constructed. Fig. 6 presents extraction profiles obtained by NHSG coated capillary. Benzoic acid, catechol, dopamine, and epinephrine were extracted and the time required to establish analyte equilibrium between the sample matrix and the sol-gel sorbent was estimated as the point on the time axis that corresponded to the start of the plateau on the extraction curve. The HSG coated capillary provided an analogous extraction behavior for the same analytes.
To investigate the difference in the loading of PPO in HSG and NHSG sorbents, thermogravimetric analysis was performed on these two types of sol-gel sorbents that were scraped off the surface of glass tube as well as on a sample of free PPO. As is evident from the TGA data, the NHSG sorbent contains significantly higher percent of PPO than the HSG sorbent. Furthermore, it was noticed that the pyrolysis temperature of PPO somewhat increased in the case of hybrid organic-inorganic sol-gel sorbents compared with free PPO. This can be attributed to the collective effect of covalent bonding and intercalation of PPO to within the sol-gel network. Fig. 8 represents chromatograms obtained in CME-HPLC analysis of quinol, resorcinol, catechol, acetaminophen and 4-hydroxybenzoic acid extracted from an aqueous sample. Excellent performance of the sorbent in simultaneous extraction of multiple target analytes is evident from this chromatogram.

3.4. CME-HPLC analysis of catecholamines and serotonin samples using sol-gel coatings

Catecholamines, serotonin, and their metabolites are important diagnostic biomarkers for neuroendocrine cancer types and neurodegenerative diseases. A good number of the recently published studies [8, 15, 24-23] lack LC-MS compatibility. Some other studies are based on performing lengthy chemical derivatization, which may cause loss of sample. Our study utilizes an LC-MS compatible mobile phase (ammonium acetate buffer, 20 mM) and it is derivatization-free which shows the applicability of the sol-gel ZrO2-PPO coatings in capillary microextraction for the clinical investigations of the catecholamines and their metabolites. To maximize the extraction efficiency of the sol-gel sorbents for free catecholamines, the pH of both the aqueous and the synthetic urine samples containing dopamine and epinephrine were adjusted to pH 10.5 using aqueous ammonia solution (~two pH units higher than the isoelectric points (pl) of catecholamines which range between 8.5 and 9.0) [15]. Because of the issues with stability, the use of such a high pH is problematic with silica-based sorbents.

Table 2 represents CME-HPLC-UV results for dopamine, epinephrine and serotonin using HSG and NHSG ZrO2-PPO sorbents. Excellent desorption efficiency (DE ~95-99.5%) was obtained for dopamine, epinephrine and serotonin using NHSG and HSG sorbents. The NHSG ZrO2-PPO sorbent provided significantly lower LODs when compared to HSG ZrO2-PPO sorbents. NHSG ZrO2-PPO achieved LODs of 5.5 pM and 9.59 pM for epinephrine and dopamine, respectively, those for HSG ZrO2-PPO sorbents were 270 pmol/l and 350 pM for dopamine and epinephrine, respectively. Also, the sensitivity enhancement factors [64] for NHSG ZrO2-PPO sorbent were 60. 27.4 and 40 folds higher for epinephrine, dopamine and serotonin, respectively than that of the HSG sorbent. The achieved LODs using the NHSG ZrO2-PPO sorbents lower than the LODs for dopamine and epinephrine in many reported studies [54 pM -27 nM] [8, 16, 23, 63, 65-73] which can be attributed to both the Lewis acid sites on the surface of zirconia sorbents [48, 74, 75] and the ability of the non-hydrolytic sol-gel route to incorporate high content of the PPO into the sorbent coatings. Fig. 9 illustrates chromatograms of synthetic urine samples: (a) unsnipped and (b) spiked with epinephrine and dopamine. The chromatograms were obtained by CME-HPLC experiment using NHSG ZrO2-PPO coated capillary. Low picomolar LODs (25 and 32 pM for epinephrine and dopamine, respectively) were achieved with high EFs (1480 and 2650 for epinephrine and dopamine, respectively). The enhanced EFs values obtained after analyzing synthetic urine samples (~15%) compared to the values achieved with aqueous samples can be attributed to the salting effect. The obtained results demonstrate the applicability of the presented sorbents for the analysis of dopamine and epinephrine in complex matrices providing excellent sensitivity. This is important in the analysis of catecholamines and their metabolites, since quantifying the ratio of these biomarkers is critical for the estimation of the state of the cancer in the adrenal gland [76].

4. Conclusion

The newly developed sol-gel ZrO2-PPO sorbents were utilized in CME-HPLC analysis of free catecholamines and molecules related to their metabolites. Nonhydrolytic sol-gel approach was effectively employed for facile incorporation of PPO ligands in an evolving zirconia network providing zirconia-PPO hybrid organic-inorganic sorbents for CME. Compared to the HSG approach, the NHSG approach provides an effective pathway to chemically incorporate significantly higher amounts of the hydroxy-terminated organic ligand (PPO). NHSG process also facilitates a better extraction of the derivatized catecholamines by making more Lewis acid sites [30].
Table 2
CME-HPLC-UV results for epinephrine, dopamine and serotonin extracted from aqueous sample (pH 13.5).

| Substance | NH3 | HSO | NH3 | HSO | NH3 | HSO | NH3 | HSO | NH3 | HSO | NH3 | HSO | NH3 | HSO |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Epinephrine | 3.6 | 2.5 | 6.1 | 2.5 | 340.6 | 72.7 | 6.1 | 340.6 | 72.7 | 6.1 | 340.6 | 72.7 | 6.1 | 340.6 | 72.7 |
| Dopamine | 5.1 | 2.5 | 9.3 | 2.5 | 270.6 | 35.0 | 11.0 | 270.6 | 35.0 | 11.0 | 270.6 | 35.0 | 11.0 | 270.6 | 35.0 |
| Serotonin | 4.7 | 6.0 | 11.0 | 6.0 | 200.6 | 200.6 | 200.6 | 200.6 | 200.6 | 200.6 | 200.6 | 200.6 | 200.6 | 200.6 | 200.6 |
| Mass Extracted (µg) | 124.4 | 19.0 | 2312.0 | 85.0 | 218.0 | 39.0 | 2312.0 | 85.0 | 218.0 | 39.0 |

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2016.09.086.

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