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Sol-gel Niobia-based Sorbents for the Enrichment of Organophosphorus Compounds by Capillary Microextraction Online Coupled to High Performance Liquid Chromatography

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Sol-gel Niobia-based Sorbents for the Enrichment of Organophosphorus Compounds
by Capillary Microextraction Online Coupled to High Performance Liquid Chromatography

by

Sheshanka Kesani

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy
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Key words: Capillary Microextraction, Organophosphorus Compounds, Niobia sorbents, Sol-gel.

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Abbreviations

1. SPME  Solid phase microextraction
2. CME   Capillary microextraction
3. HPLC  High performance Liquid chromatography
4. GC    Gas Chromatography
5. FTIR  Fourier transform infrared spectroscopy
6. SEM   Scanning electron microscope
7. TGA   Thermogravimetric Analysis
8. SE    Specific extraction
9. DE    Desorption efficiency
10. polyTHF Polytetrahydrofuran
11. C_{18}(+ve) N-Octadecyldimethyl[3-(trimethoxy silyly) propyl]ammonium Chloride
12. C_{18} Octadecyltrimethoxy silane
13. SPE   Solid phase extraction
14. DSPE  Dispersive solid phase extraction
15. LLE   Liquid-Liquid extraction
16. SBSE  Stir bar sorptive extraction
17. MEPS  Microextraction by packed sorbent
Abstract

Sample preparation is a key step in chemical analysis, and includes isolation of target analytes, removal of interferences, preconcentration, and/or modification of target analytes (if needed). Sample preparation is also the most time-consuming and error-prone step in the whole analytical process. Traditional sample preparation techniques involve hazardous solvents. Considering the environmental and health safety, it is desirable to reduce or eliminate organic solvents in sample preparation. Solid phase microextraction (SPME) was introduced as a solvent free sample preparation technique. Capillary microextraction (CME) is one of the formats of SPME that can be easily coupled to high performance liquid chromatography (HPLC). In SPME and CME a solvent free sample preparation is accomplished by using a sorbent coating instead of hazardous organic solvents commonly used in conventional extraction techniques. This research is focused on the development and systematic examination of novel niobia-, titania- and silica-based organic-inorganic hybrid sol-gel sorbents for CME. Conventionally silica and titania based precursors were used in organi-inorganic hybrid sol-gel sorbents for CME, here novel niobia based precursor was used in creating organic-inorganic hybrid sol-gel sorbents. Poly tetrahydrofuran (polyTHF) as well as electrically neutral and charged organic ligands were used to prepare the sorbents for CME coupled to HPLC. Characterization of created sol-gel sorbents, evaluation of extraction performance, and enrichment of environmentally and biomedically important analytes including organophosphorus compounds were performed. CME performances of the created sorbents were characterized by specific extraction (SE) (a measure of extraction efficiency) and desorption efficiency (DE) (a measure of completeness desorption of extracted analytes). Scientific findings
of this research has shown that sol-gel niobia-polyTHF sorbent provides 60 to 70 % higher SE values for different environmentally important analytes compared to analogously prepared silica-polyTHF sorbent. This superior extraction performance can be attributed to the presence of surface Lewis acid sites undergoing Lewis acid-base interactions with analytes representing Lewis bases. The prepared sorbents also have the ability to undergo van der Waals interactions due to the presence of polyTHF. Absence of Lewis acid sites on silica surface resulted in inferior extraction efficiency compared to niobia-polyTHF sorbents. Extraction efficiency of the created sol-gel based niobia-polyTHF was also explored in the enrichment of organophosphorus pesticides and compared with that of the state-of-the-art titania-based sorbent. Sol-gel niobia-polyTHF sorbent has provided 40 to 50 % higher SE values in the enrichment of organophosphorus pesticides compared to sol-gel titania-polyTHF sorbent which can be attributed to the presence of bronsted acid sites on niobia surface (but lacking on titania) along with Lewis acid sites. To explore relative contributions of electrostatic, Lewis acid-base and van der Waals interactions between sol-gel sorbents and analytes, two sol-gel sorbents, one containing a positively charged octadecyl ligand and the other a neutral octadecyl ligand were created. Positive charge was imparted by using N-octadecyldimethyl [3-(trimethoxysilyl) propyl] ammonium chloride (C_{18}(+ve)) as ligand bearing co-precursor. Similarly N-octadecyl trimethoxysilane was used to impart a neutral C_{18} ligand in sol-gel coating. Experimental results have shown that sol-gel Nb_{2}O_{5}-C_{18}(+ve) sorbent has superior extraction efficiency compared to sol-gel based Nb_{2}O_{5}-C_{18} and purely inorganic Nb_{2}O_{5} sorbents in enrichment of organophosphorus compounds (nucleotides and organophosphorus pesticides). Electrostatic interactions between the positive charge of organic ligand (C_{18}(+ve)) and negative charge of phosphate group has contributed to the higher extraction performance of sol-gel based Nb_{2}O_{5}-C_{18}(+ve) sorbent. TiO_{2}-C_{18}(+ve) sorbent was also created to compare with the novel sol-
gel niobia based sorbents, since titania-based sorbents are considered as the state-of-the-art extraction material in the enrichment of organophosphorus compounds. Established research results has shown that sol-gel based Nb₂O₅-C₁₈ (+ve) sorbent has provided 40 to 50 % higher specific extraction values for organophosphorus compounds compared to sol-gel based TiO₂-C₁₈ (+ve) sorbent. Desorption efficiency of sol-gel Nb₂O₅-C₁₈ (+ve) and TiO₂-C₁₈ (+ve) sorbents were 96% vs 90%. This superior DE of sol-gel Nb₂O₅-C₁₈ (+ve) sorbent can be attributed the higher Lewis acid strength of titania than nioiba. The developed sol-gel niobia based sorbents have also shown high pH stability compared to traditional sol-gel silica based sorbents. The created sol-gel sorbents were characterized by less than 5% run to run RSD values and also less than 5% capillary to capillary RSD values which indicated the high reproducibility of developed method. The developed sol-gel niobia sorbents are applicable to sample preparation in different fields including biomedical, environmental, forensic, defense etc.
1. CHAPTER ONE

1.1 Sample preparation

In general an analytical process includes sampling, sample preparation, separation, quantification, statistical evaluation, and finally decision making. Errors in any one of the above steps leads to the inaccuracy in whole analytical process. As all steps involved in analytical process are dependent on each other, by reducing the time for most time consuming step ultimately fastens the whole analytical process. So all steps needed to be considered for high throughput of the analytical process. Here in this research one of the early step of analytical process, sample preparation is explored. Evolution of sample preparation can be traced back to beginning of analytical chemistry when complex samples like samples from natural sources, living body were needed to be analyzed. Sample preparation being one of the early steps in analytical process even a small error in this step leads to inaccurate performance of whole process. In general a cleanup process of samples leads to more efficient detection and separation of analytes. At the same time a poorly cleaned sample can invalidate the whole process. The cost and time of assay also can be reduced by proper sample clean up.

The main purpose of sample preparation includes removing the potential interfering components from the sample matrix, concentration of analyte, if needed modification of the analyte for proper detection and separation, finally production of a reproducible method for analysis of analytes. Excellent description was provided in the form of books, reviews, regarding the importance of sample preparation [1-6]. Extraction of analytes can be considered as primary
step involved in the sample preparation. The basic concept involved in extraction is preconcentration of the analyte in one phase. An analyte is distributed between two phases depending on temperature, distribution constant and relative volumes of the phases. Extraction rate of analyte depends on temperatures and diffusion rates in between two phases. In all extraction methods a balance must be obtained between extraction of selected analyte and complete extraction of all organic components. In general principles involved in extraction procedure can be either exhaustive process or equilibrium process. In exhaustive process majority of the target analytes are expected to be extracted by extraction phase so calibration is not needed in this process. Where as in equilibrium process concentration of the target analyte reaches equilibrium between extraction phase and sample matrix, here calibration is needed.

Two classic sample extraction techniques are Liquid liquid extraction (LLE) and Solid phase extraction (SPE). These are the most popular techniques used for decades (LLE since in 1870 [2, 7] in sample preparation. The urge to analyze large scale samples led to develop microscale extraction techniques involving lower consumption of organic solvents, higher selectivity, faster speed, and higher efficiency. Some of the microextraction techniques based on solvent and sorbent are solid phase microextraction (SPME) [8] single-drop microextraction, [9-11] dispersive solid phase extraction (DSPE) [12] stir-bar sorptive extraction (SBSE) [13], Microextraction by packed sorbent (MEPS) [14]. The driving force for the development of above mentioned microextraction techniques can be attributed to environmental applications, increased demand for analysis of food, natural products and biological samples. Other motivations for advancement of these extraction techniques can be attributed to the miniaturization, integration and hyphenation. From above mentioned microextraction techniques, SPME is one of the most widely explored technique in past two decades.
Here in this research one of the formats of SPME (that is CME) was used for enrichment of organophosphorus pesticides and nucleotides. First a brief description about SPME, principle involved in SPME, its calibration methods, then coupling of SPME to different analysis techniques, finally application of SPME in different fields is provided. Then capillary microextraction (one of the format of SPME) which was used in this research, its parameters are described. Details about Sol-gel technology and its applications in CME are discussed later in this chapter.

1.2 Solid phase microextraction

Solid phase microextraction was developed to facilitate rapid sample preparation. So evolution of solid phase microextraction occurred in Janusz Pawliszyn lab [8] in a situation to retain time efficiency advantages of high speed separation instruments by reducing the time for sample preparation. Evolution of solid phase microextraction technique was well described in a review by Pawliszyn et. al [15]. SPME is a solid phase extraction in which a fiber coated with extracting phase that is either a sorbent or polymer is used to extract different kinds of analytes from different media (liquid or gas). In early stages of invention of SPME, fused silica optical fibers were coated with liquid and solid polymeric phases and used for extraction [8]. Later coated fibers were incorporated into a micro syringe which allowed the exposure of coated fiber during extraction and desorption and protecting of it during storage by help of plunger [16]. Figure 1.1 represents the schematic representation of SPME fiber
Figure 1.1: Schematic representation of Solid phase microextraction fiber.

SPME can be applied in different ways for analysis of target analytes. Figure 1.2 represents the different versions of SPME.
Figure 1.2: Graphical representation of different modification of solid phase microextraction (adapted figure: Reprinted with permission from (Current developments and future trends in solid-phase microextraction techniques for pharmaceutical and biomedical analyses). Copyright (2017) Japan society for Analytical Chemistry”.

1.2.1 Principles involved in SPME

The main principle involved in solid phase microextraction technique is the concentration equilibrium that is established between sample matrix and extraction phase.
Primary difference between classical technique like solid phase extraction and solid phase microextraction is the objective of SPE, which involves exhaustive extraction. In more detailed way the extraction process in SPME can be explained as follows: (a) extraction of analytes starts in SPME once the coated fiber is kept in sample (b) extraction is completed once an equilibrium is reached in between sample matrix and extraction phase (c) finally the extracted analytes can be injected to chromatographic techniques like gas chromatography (GC) or high performance liquid chromatography (HPLC) for separation and analysis. Analytes reaching equilibrium between sample matrix and extraction phase can be explained as extracted amount is constant within limits of experimental error and no further increase in extraction time is necessary. The equilibrium conditions can be explained as below [17]

\[
n = \frac{K_{fs} V_f V_s C_o}{K_{fs} V_f + V_s}
\]

**Equation:1**

Where \( n \) = no of moles extracted by the coating

- \( K_{fs} \) = fiber coating/sample matrix distribution constant
- \( V_f \) = fiber coating volume
- \( V_s \) = sample volume
- \( C_o \) = initial concentration of a given analyte in the sample

Equation 1 indicates that once the equilibrium is reached there is a direct proportional relationship between analyte concentration and amount of analyte extracted, which is the basis for analyte quantification.
Equation 1 considers sample matrix as single homogenous phase, but the equation can be modified with existence of other components by considering the volumes of individual phases and appropriate distribution constants [15].

For large volumes of samples the above equation was modified as

\[ n = K_f s V_f C_0 \]

**Equation:2**

Equation 2 indicates that the concentration of analyte extracted is independent of the sample volume. This confirms that the sampling step can be integrated with sample preparation leading to acceleration of the whole analytical process. Also the errors associated with sampling like adsorption, decomposition during sampling process can be avoided. One another advantage of SPME technique is, it integrates sampling, sample preparation, preconcentration and sample introduction into one single step before instrumental analysis. Therefore SPME technique can be considered as the most convenient and user friendly technique. To understand any extraction process including SPME technique, just perceiving the principle involved in SPME will not be enough, the type of interactions between sample matrix and extraction phase is also important. SPME efficiency is explained by different intermolecular interactions like, van der Waals, dipole-dipole, acid-base interaction, electrostatic interactions between analytes in sample matrix and extraction phase.

**1.2.2 Calibration methods for SPME**

The three traditional calibration methods for SPME can be described as [18]

(i) External standard (calibration curve)

(ii) Standard addition

(iii) Internal standard
The above mentioned methods include both advantages and disadvantages for SPME calibration but they can play major role in laboratory purposes. In literature we can find some reports where these calibration methods were used for on-site applications. By applying the above mentioned quantification methods of SPME two approaches equilibrium and pre-equilibrium can be performed. In one approach partitioning equilibrium is attained between sample matrix and extraction phase where convection conditions doesn’t affect the amount of analyte extracted. In the second case convection/agitation is constant and the amount extracted depends on time. Pre-equilibrium approach will be preferred rather than equilibrium approach when equilibration time is too long for extraction. In external standard calibration, standard solutions of sample matrix are prepared to develop a relationship between peak response and target standard concentrations. Subsequently samples are extracted under same conditions and concentrations of target analytes of samples can be calculated from the equation of calibration curve. This method is most widely used method in environmental [19] [20] biological [21] [22] and food samples [23] [24] with SPME. Calibration curve method will not require extensive sample preparation, but standards and samples must be extracted under same conditions. Standard addition calibration method (ii) for SPME involves addition of standards to samples which is, adding of known standards of target analytes to sample matrix which was initially a sample matrix with unknown target analyte concentration. Then a plot of responses for different analyte concentrations is developed and extrapolation of this plot of responses to zero determines the original concentration of target analytes. The main disadvantage of this techniques is it is very time taking process for sample preparation. There are also some advantages with this calibration method like sample matrix effects can be reduced a lot. This method is more useful with small number of samples of more complex matrix [25] [26] [27] [28]. The third calibration method is internal standard method. This
method involves addition of a compound which is different from analytes but well resolved for separation and also which can mimic the equilibrium of the target analyte. Calibration of the sample is achieved by developing a calibration plot considering the ratio of peak area of different concentrations of analytes to fixed concentration of internal standard. Internal standard method can compensate sample matrix effects and losses during sample preparation [29] [30] [31] [32]. The main disadvantage of this method it’s not easy to find suitable internal standard for complex samples.

As mentioned above equilibrium extraction is the most widely used quantification method for SPME technique in field sampling. Largely equilibrium method extraction is used as quantification method for SPME but sometimes exhaustive extraction is also used [33]. In exhaustive extraction method it is considered as amount extracted for target analyte from sample matrix is equal to the amount present in the sample matrix. Sometimes total amount of target analyte is not extracted from sample matrix. The mentioned equation 1 above for SPME can be modified as below for exhaustive extraction

\[ n \approx V_s C_0 \]

Equation: 3

The above equation can be explained when distribution coefficient (\(K_{fs}\)) is very large which makes \(K_{fs} V_f\) large then \(V_s\).

One of the application of exhaustive extraction is multiple extractions in which extraction is performed repeatedly for samples. The amount extracted can be calculated with only few extractions the relationship between total peak area and first peak area which can be represented as below
\[ A_T = \sum_{i=1}^{N} A_i = \frac{A_i}{1 - \beta} \]

**Equation: 4**

\( A_T \) - total peak area

\( A_i \) – first extraction peak area

\( \beta \) – Constant which can be calculated from slope of linear plot \( \ln A_i \) vs (i-1) obtained from few determinations. [34]

This method was used for solid and liquid samples [35] [36]. The advantage of this method is sample matrix effect is very minimal. Coming to the disadvantages of this method due to adsorption phenomenon fiber coating gets saturated by sample matrix components with multiple extractions which in turn invalidates the quantification [37] [38] [39].

As diffusion of analytes between sample matrix and fiber coating is the primary step in SPME extraction, diffusion coefficient based calibration methods for quantification of SPME are the most important quantification methods [40]. Figure 1.3 explains the importance of diffusion coefficient; from figure 3 it can be implied that when extraction time is longer than \( t_{95} \) (refer to figure 1.3) then it shows that extraction has reached equilibrium between sample matrix and fiber coating. When extraction time less than \( t_{50} \) (refer to figure 3) there is linear mass extraction by fiber coating. This shows that description of kinetic process of SPME explaining diffusion coefficient is crucial.

In diffusion based calibration method there are different types like cross flow model, interface model, and kinetic calibration for adsorption/absorption and desorption. In kinetic calibration method there are standard calibration and standard free calibration method and standard kinetic calibration method. All these calibration methods are based on Ficks first law.
Lastly, calibration of SPME was also performed by liquid injection method. Liquid injection calibration method is based on assumption that transferring of sample from liquid injection is equal to sample transfer from SPME. In practical mass transfer in liquid injection and SPME are effected by different factors. Efficiency of sample transfer by liquid injection will be different from SPME sample transfer if all the amount of analyte is not absolutely transferred [41].

There was continuous development in calibration process for quantification SPME. For example fiber retracted SPME devices were developed based on Ficks first law of diffusion for water and passive air sampling [42] [43] [44] [45]. Similarly an SPME internally cooled-fibre device was developed for exhaustive extraction [46] [47].

Figure 1.3: Typical extraction profile of SPME [40]
1.2.3 SPME Coupling to different analysis techniques

As mentioned earlier SPME can be coupled to different analysis techniques like gas chromatography (GC), high performance liquid chromatography -mass spectrometry (LC-MS) [48] [49] mass spectrometry [50] Coupling of SPME with GC or LC-MS was most widely investigated compared to coupling of SPME with mass spectrometry. In three ways SPME is coupled to mass spectrometry, they are (i) direct ionization of analytes on SPME (ii) surface desorption and ionization from SPME (iii) first desorption of analytes from SPME then ionization for mass spectrometry analysis [51]. A wide range of applications were seen using the hyphenation of SPME with mass spectrometry, like environmental [52] [53] [54] bio and food analysis [55] [56] [57] [58]. Similarly SPME coupled to GC was applied in different fields like food [59, 60], biological [61], cosmetic industry [62] etc. Analogously SPME coupled to HPLC was applied in fields like environamental [63, 64], biological [65, 66] and food [67].

1.2.4 Application of SPME for different kinds of matrices.

Complexity of the sample matrices was one of the reasons to develop new microextraction techniques which can provide high selectivity, and specificity. The wide application of SPME with different kind’s matrices implies the evolution of these techniques as one of the most accepted microextraction techniques. In environmental samples liquids like sea water [68-71],coastal water [72], transitional water [71], river water [73, 74],lake water etc were analyzed [75]. At the same time solid sampe matrices like soil, dust[76], plants [77] flowers,seed, pollen [78], were also analyzed by using SPME and CME. In biomedical applications, matrices like plasma [66, 79-83], urine[84-89], saliva [90], serum [91] or hair [92] analysis has been reported. Coming to food analysis, water [93], juice [75], tea [94] milk [95] and solid food including, cereal [96, 97] egg [98, 99],dried fruits [100] etc were analyzed by these microextraction techniques.
1.3 Capillary microextraciton or In-tube SPME

Two different extraction modes were elucidated for in-tube SPME, in one of them sample is passed continuously through the capillary in one direction. Another includes aspirating and dispensing sample which can be explained as draw and eject cycle mode. Different extraction modes suggest different configurations are involved in in-tube SPME. At the same time this also explains the versatility of the technique which can be coupled to a wide range of analysis procedures. Explained first mode that is flow through mode is the most widely used extraction mode due to its higher extraction efficiency compared to the draw and eject mode. A wide range of configurations were explored in literature to carry out these two extraction modes. Capillary microextraction (CME) is one of the configuration for flow through mode of in-tube SPME. In CME a sorbent coated capillary (fused silica capillary) is used as extracting devices. This capillary generally placed in place of sample loop in six-port injection valve. The term capillary microextraction was coined by Malik et al. [101]. In CME the extraction phase is inside the open tubular column (fused silica capillary) as coating, when sample is passed through this capillary the analytes are extracted by the coating due to intermolecular interactions between analytes in sample matrix and coating. Extracted analytes are desorbed by mobile phases (either solvents or gas depending on analytical instrument). Three configurations are possible with CME, open tubular, packed and monolith. CME can be coupled online to high performance liquid chromatography [102]. Major advantages of CME are easy automation, high mechanical stability (compared to syringe mode SPME device), time efficient, and capability for online hyphenation with analytical instruments. Different types of coatings were developed for CME for increasing its extraction efficiency, thermal and chemical stability. Conductive polymer coatings [103], sol-gel materials
monoliths [105], xerogels [106], are few of the most commonly used coatings in CME. Other coatings include molecularly imprinted polymers (MIP) [107], restricted access materials (RAM) [108]. MIP’s will provide selectivity of the analytes from a complex sample matrix. Restricted access materials are capable of excluding interfering macromolecules for analysis. MIP’s and RAM’s are more suitable for biological analytes like glycoproteins, enzymes, metabolites etc [78, 87, 91, 109, 110]. Immuno-affinity sorbents were developed for high selectivity like antibodies which are highly specific in nature ([85, 111]. Nanoparticle-deposited capillaries which provides high surface area for extraction were also developed.[74, 112, 113]. Sol-gel coatings were one of the more prominently used coatings for capillary microextraction [114]. Although a wide range of coatings for in-tube SPME are commercially available, still there is a need for development of custom made coatings for providing high extraction efficiency. Commercially available coatings has advantages like, different thickness, porosity, polarity are achievable. Disadvantages of commercially available in-tube SPME capillaries is low sample holding capacity which in-turn requires number of cycles for extraction process and also low selectivity. So there is still a lot of scope for development of new coating with high selectivity and efficiency.

1.3.1 Capillary microextraction (in-tube SPME) parameters.

The parameters that affect in-tube SPME are rate of flow, displacing volume and sample volume. Dimensions of the capillary, kind of capillary, and sorbent phase are also parameters needed to be considered for in-tube SPME. Sample type, pH of sample, nature of organic modifier, concentration of analytes, and salt content also considered as the parameters that affect in-tube SPME. The sorbent phase that is used for extraction should have stable union between capillary
tube and itself so that it is stable to the mobile phases used and at high temperatures [83]. The dimensions of the capillary tube affects sample volume loading, extraction and desorption process. For example, if capillary dimension or thickness of the sorbent phase is increased then peak tailing and broadening occurs [115]. This type of affect is more prominently seen when in-tube SPME is coupled to miniaturized techniques for analysis. Mostly commercially available in-tube SPME devices are used for reproducibility, but for specific analytes suitable sorbent phases were developed in literature.

Organic solvents used in the extraction process also plays significant role in extraction efficiency of CME. Sometimes analytes may get dissolved in organic solvents used in earlier steps of extraction ultimately this affects the efficiency of the extraction process [116]. Salt content generally increase the extraction efficiency but at the same time high concentrations will lead to blocking capillary. Type of ions in the salt and ionic strength that provides is also important for the efficiency of extraction process. These ions may compete with analytes for extraction sites on sorbent phase [117] [118]. Finally pH maintenance is important for the compounds which are susceptible for ionization. During selection of pH, stability of the compounds and sorbent phase needed to be considered [117].

Considering the steps involved in in-tube SPME process, a balance between time of extraction and efficiency should be maintained. In flow-through model when large volumes of sample are processed large amount of analytes are extracted but at the same time the time it can lead to longer time of analysis. Large amount of sample processing may also lead to block the capillaries due to the dirt in the sample matrix. In flow-through mode volume of solvent used for displacing the sample does also play important role and it should be optimized [119] [120]. In some cases a combination of derivatization step to microextraction is also needed for better selectivity,
sensitivity and to improve the separation. In these conditions the chosen derivatizing agents type, amount of derivatizing compound should be optimized [121] [78]. For a derivatization reaction there are other factors needed to be considered like pH of the medium, buffer concentration (if needed), and time of reaction. Finally, the most important parameters needed to be considered are type of capillary and sorbent phase, but depending on the requirements of analysis other parameters come into existence.

1.4 Sol-gel technology for SPME/CME

From above discussion it is understood that SPME is one of the most widely applied and accepted techniques for sample preparation due to its simplicity and high extraction efficiency compared to traditional solvent based extraction techniques. One of the most critical step in SPME is choosing a suitable coating for extraction process. Selection of the coating depends on many factors; factors related to target analytes can be explained as functional groups on target analytes which plays important role in interaction with coating. Depending upon the coupling of SPME with different analysis techniques thermal and chemical stability of the coating material, sensitivity, selectivity and reproducibility are other major factors in selection of the coating for extraction.

Some of predominantly applied commercially available polymer coatings in SPME are polydimethylsiloxane (PDMS) and polyacrylate, dispersions of solid adsorbents like carboxen and divinylbenzene in polymeric agglutinants. These commercially available coatings has some disadvantages depending on their nature, like swelling of the coating when exposed to organic solvents, necessity of operating at lower temperatures, low mechanical stability. If the coating is chemically bonded to fused silica capillary, it can overcome the above mentioned problems like low chemical and thermal stability. Therefore in an effort to overcome these problems Malik and co-workers [122] had applied sol-gel route for synthesizing coating on SPME fiber. The origin of
sol-gel technique can be traced back to mid-1800’s but it was applied in glass industry by Schott Company in Germany after a century [123]. Application of sol-gel technology for chromatographic stationary phases started in 1987 by Cortes et al. [124] Sol-gel technology is widely used in ceramic, nuclear field, and electrical industries [125]. Sol-gel technology offers a high degree of homogeneity at molecular level which can be attributed to the availability of highly purified precursors. Multicomponent hybrid materials can be developed with various shapes, sizes, and formats by using sol-gel route. Hybrid materials are developed from sol-gel route by using different organic ligands with sol-gel precursors. These hybrid materials can improve stability and extraction efficiency. In 1990’s by applying sol-gel route open tubular columns for liquid chromatography and capillary electrochromatography (CEC) were developed [126-128]. Major advantage of the sol-gel process is that the whole process can be controlled by analyst which leads to a proper design and production of coating materials.

Sol-gel process involves conversion of liquid ‘sol’ solution to solid ‘gel’ matrix. In general, for sol-gel process to take place the main reagents can be considered as (1) sol-gel precursor (generally metal alkoxides) (2) Organic solvent to disperse the precursor (3) catalyst: an acid or base or fluoride and (4) water. Figure 1.4 represent the chemical reactions that occur in sol-gel synthesis of silica based sorbent [129-133]. Sol-gel reaction can also be initiated by radiations like UV light [134] Two main steps involved in sol-gel process are (1) hydrolysis of precursors (2) polycondensation of hydrolyzed products along with the other sol-gel active materials present in the system. Condensed sol solution will develop into a three dimensional network which is called gel. A part of this three dimensional sol-gel network is used as coating materials for extraction or separation of analytes.
First time when sol-gel route was applied for coating SPME fiber by Malik et al., [122] it was created on the outer surface of the fiber by dipping bare end in sol solution. Sol solution used for dipping consisted of alkoxide based-precursor, hydroxyl terminated sol-gel active polymer, and a
surface-derivatizing reagent dissolved in suitable solvent. This type of coating had a porous structure and thickness of the coating was controlled by dipping time in sol solution. A brief description of preparation of sol-gel coated SPME fiber includes, (1) burning of outer polyimide coating on 15 cm piece of fused silica fiber with cigarette lighter, (2) cleaning the burnt fiber with methanol dipped kimwipe, (3) Then dipping the bare end of fiber in 0.1M NaOH for thirty minutes and then rinsing with deionized water and drying in air for another thirty minutes, (4) finally, the cleaned fiber was dipped in sol solution for coating. This coating process was repeated for 3 times with freshly prepared sol solution each time [122]. Sol-gel coated fiber was then thermally conditioned in GC injection port before using for SPME. To perform this Malik et al [122] had used specially designed syringe to install the sol-gel coated fiber on it and condition the coated fiber in the injection port of GC under hot helium gas flow. The coated fiber was coupled to GC (SPME-GC) and using homemade PDMS open tubular column environmentally important samples like alkenes, polycyclic aromatic hydrocarbons were analyzed.

Figure 1.5 demonstrates the thermal stability of the sol-gel based PDMS coated fiber for SPME-GC analysis of aliphatic alcohols, in which analytes were desorbed at high temperature. Such high thermal stability of the sol-gel coating can be explained due to the chemical bonding of sol-gel network to the fiber. The developed sol-gel coating though it was rinsed by organic solvents several times for cleanup purpose there was no change observed in SPME fiber extraction performance which displays high solvent stability of the sol-gel coating.

The porous structure of sol-gel bestows fast sorption-desorption of analytes during extraction and sample introduction. Mackenzie et al [135] reported that when sol-gel network is developed by tetralkoxysilanes as precursor, this will lead a compact structures which may produce cracks in pores during solvent evaporation. Sol-gel coatings with cracked pores will affect the
extraction efficiency. Equation which describes the capillary forces that leads to cracking of pores in sol-gel coatings or monolith during drying [136] is as follows

\[ P = \frac{2\gamma \cos \theta}{r} \]

**Equation: 5**

P= capillary pressure generated in the pore  
\( \gamma \)= surface tension of the pore liquid  
\( \theta \)= contact angle  
\( r \)= radius of the cylindrical pores  
The above equation explains that during drying process, the differential pressure between two different radii pores will effect on the wall separating two pores and when capillary thrust exceeds the tensile strength of the wall material cracks occur in between pores. There are several solutions for this problem, for example by adding drying controlling chemical additives like formamide in sol-gel reaction [137, 138] by using solvents with low surface tensions, [139] and supercritical drying [140]. Another solution is to develop open structures that can reduce that capillary strain generated during drying, this can be achieved by using alkyl or aryl derivatives of alkoxy silanes as precursors in sol-gel process [135]
Figure 1.5: Direct SPME-GC analysis of aliphatic alcohols from an aqueous sample matrix. Extraction conditions: Fiber: 200 µm; direct 30 minute extraction with stirring (no pH adjustment, no salting out); Injection conditions. Injection temperature: 250°C, Carrier gas: helium, Injection mode: split-splitless (first 5 minutes splitless followed by split with a ratio 100:1); GC conditions. Column: 10 m x 250 µm i.d; Stationary phase: sol-gel PDMS; Temperature programming: 40°C (5min) 6 °C min⁻¹, Detector. FID, 300°C. Peak identifications: (1) C₁₂ (2) C₁₄ (3) C₁₆ (4) C₁₈ straight chain aliphatic alcohols. [122]

Adaptability of sol-gel technology allows to develop surface bonded coating materials on unbreakable fiber materials like Ni-Ti, stainless steel [141], titanium [142] anodized aluminium wire [143]. Like most metal fibers the presence of protective oxide coating on aluminium and titanium fibers leads to the chemical bonding sol-gel sorbents on to the fiber. Sol-gel coatings on these unbreakable materials provides high mechanical stability when compared to the traditionally used
fused silica fibers for SPME coatings. In the process of conversion colloidal sol solution to gel, the growing sol-gel networks gets chemically bonded to the substrates surface via condensation reactions at sol-gel active sites on surface. Physiochemical properties of sol-gel coatings mainly depend upon factors like relative ratios of different components in sol solution, conditions in which reactions occur and also the post gelation conditions [144]. Factors that affect the gelation process are (1) type of precursor and precursor/water ratio,, (2) catalyst and its concentration (3) pH of sol solution, (4) type of solvent and its concentration, and (5) post-gelation and aging conditions.

1.4.1 Advances in Sol-gel based coatings for CME (in-tube SPME) and SPME

Sol-gel technology is playing a significant role in development of miniaturized sample preparation techniques. A wide range of sol-gel based sorbents with high selectivity, sensitivity and extraction efficiency were developed for environmental, food, biological and forensic applications. Here a brief description of some of them is being provided. Though primarily silica based sol-gel materials were prepared and applied in various fields, due to low pH stability of silica, application of different transitional metal alkoxides as sol-gel precursors came into existence. Transition metal alkoxides like titania [145-147] zirconia [148, 149] were applied as sol-gel precursors. Titania based sol-gel sorbents has shown better pH stability compared to traditional silica based sorbents.

Also sol-gel germainia triblock polymers has shown excellent chemical stability as sorbents in capillary microextraction [102, 150] A wide variety of organic ligands were chemically integrated in to sol-gel structures for better extraction efficiency. PDMS, and poly ethylene glycol and poly tetrahydrofuran are few of the organic ligands used in building sol-gel based organic-inorganic hybrids. Malik and co-workers were first research group to develop surface bonded PDMS based sorbent for SPME fiber [122] In process of developing selectivity, polarity in
sorbents different sol-gel active organic ligands were used in sol-gel reaction, like polyacrylate, poly vinylalcohol, crownethers, butylmethyl acrylate etc. [151-154] Sol-gel sorbent involving grafting of polyethylene glycol on multi walled carbon nanotubes provided polarity (presence of polyethylene glycol) along with large and also π-π interactions. These type of sorbents had provided high mechanical and chemical stability. Similarly a sol-gel sorbent with highly polar-nonpolar moieties were developed involving cyanopropyl and PDMS[155]. A novel benzyl terminated Dendron-based sol-gel coating was developed for extracting selective analytes like phenols, alcohols, polycyclic aromatic compounds by Kabir et al [156]. Figure 1.6 represent a dendrimer based sol-gel coating.
Ionic liquids which possess exclusive physiochemical properties like negligible vapor pressure, hydrophobicity, high thermal stability, tunable viscosity and also chemical functionality can be utilized for selectivity towards specific target analytes. These ionic liquids can be used as solvents, chemical additives for drying and also catalysts in sol-gel process [157]. Shearrow et al. had first
developed a sol-gel organic-inorganic hybrid for extracting non polar and moderately polar compounds using ionic liquid [157, 158]

Functional coatings were developed using organically modified functional precursors in sol solutions. Functionally modified sol-gel precursors like alkyl derivatized sol-gel precursors, 3-trimethoxy silyl propyl amine etc were used to develop sol-gel based coatings [159, 160]. Molecularly imprinted sol-gel sorbents were developed for SPME in process of developing a selective sorbent for extracting decabromidiphenyl ether (BDE)[161]. Similarly for determination of biomarkers of inborn metabolic errors, uracil and 5-fluorouracil templated molecularly imprinted polymers were developed [162]. Using amine based terminal groups, sol-gel SPME sorbents in SPME were developed for extraction of metals like Cu, Zn, Ni, Hg and Cd from biological samples[163]. Sol-gel based monolithic beds were also developed in CME by Malik et al. [164]. These monolithic beds has shown higher extraction efficiency when compared to open tubular coatings in CME. In recent literature, innovation of novel sol-gel extraction phases reflects in several patents awarded, [165-167] [168] [169] [143, 170].

Sol-gel calixerene, nanotube, fullerene based coatings were also developed to provide higher surface area of sorbent phase enhancing extraction capability of analytes. Li and coworkers had first developed sol-gel CD cavity (imparted β-cyclodextrins into sol-gel network) SPME fiber [171]. Calix[4]arene sol-gel SPME fibers were developed for determination of chlorophenols [172] in river water. Similarly calix[4]arene sol-gel fibers were used for determination of organochlorine pesticides in radish samples [173]. Sol-gel fullerene based coatings provided distinctive affinity for aromatic compound and also high thermal stability [174]. Pawliszyn and coworkers has introduced functionalized carbon nanotubes SPME fibers which provided high themal and solvent stability [175].
1.4.2 Characterization of sol-gel stationary phases.

A wide range of techniques were used in determination of properties of organic-inorganic hybrid sorbents created by sol-gel route. The techniques include Scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FT-IR), Transmission electron microscopy (TEM), Atomic force microscopy (AFM), nuclear magnetic resonance (NMR), X-ray photoelectron spectroscopy (XPS) and Thermal gravimetric analysis (TGA) etc. These techniques were used to determine the morphology of sol-gel based sorbents and various chemical bonds present in these materials. Morphology of sol-gel materials like surface characteristics and fine structure details were mostly studied by SEM. In SEM, sample surface is scanned by very fine electron beam which produces a three dimensional image with great depth of field. In general cross sectional SEM view of sol-gel materials coated on the fused silica capillary wall will provide information about structural characteristics, adherence of sol-gel materials to capillary, porosity of the sol-gel materials, distribution of the pores and integrity sol-gel materials.[133, 134, 176-186]. Figure 1.7 represents the SEM image of a sol-gel monolithic bed.
Figure 1.7: Scanning electron micrographs of a sol-gel C$_{18}$ monolithic columns (A) crossectional view (B) surface view (adapted from [177])

SEM can determine structural defects, uniformity of coating, coating thickness in open tubular columns. SEM can also determine the experimental parameter effect on structures of created sol-gel materials. In literature Zare’s group has clearly shown the effect of catalyst concentration on
developed sol-gel materials.[133] Tanaka had used longitudinal and cross sectional view in SEM to show the structural information of whole sol-gel material[186].

Similarly atomic force microscope (AFM) was also used to determine the topographical images of sol-gel materials[185]. Extended X-ray absorption was used to determine the near edge and fine structure of silica-titania sol-gel films [187]. Transmission electron microscopy was also used to determine the structural characteristics of silica-magnesium sol-gel materials[188]. Chemical bonding in sol-gel materials are determined by using techniques like Fourier transform-infrared spectroscopy (FTIR) [188-190] and Nuclear magnetic resonance (NMR) [191-193]. FTIR is one of the most prominently used spectroscopic technique in determination of the polymer bonds. This technique can also be used for determining the sol-gel progression by time. Toyoo’ka et al. had used attenuated total reflectance FT-IR hybrid technique to determine the progression of sol-gel reactions [194]. The typical absorptions of different chemical bond stretches provide the information regarding the chemical bonding in between organic ligands and sol-gel precursors. X-ray photoelectron spectroscopy (XPS) has been used to determine the esterification reaction between stearic acid and epoxy groups of glycidoxy-propyltrimethoxysilane [195]. Nuclear magnetic resonance was used by Rodriguez et al. to investigate species present in sol-solutions [196]. Chemical bonds in sol-gel materials have also been determined by mass spectrometry[197].

1.4.3 Applications of Sol-gel based sorbents in CME and SPME

Applications of a wide range of sol-gel CME or SPME sorbents were have been reported for fields like environmental, food, aroma, biomedical etc. Sol-gel sorbent made of crown ethers have been used for extraction phenols of water samples from a paper mill [198]. Sol-gel crown ethers were also used for the extraction of aromatic amines from waste water samples of a pharmaceutical factory[199]. A sol-gel PDMS based sorbent was used for extraction alkyl benzenes and ketones
In another research, sol-gel PDMS coating was used to extract a wide variety of compounds like alkanes, aniline derivatives, alcohols, phenolic compounds, and polycyclic aromatic hydrocarbons (PAHS) [122]. Similarly, sol-gel PDMDPS sorbent was used for extraction of polycyclic aromatic hydrocarbons, ketones, aldehydes, and dilute aqueous samples [149]. Hydroxy-terminated silicone oil fullerol has been used for extraction of less volatile organic compounds such as polychlorinated biphenyls, polyaromatic amines and PAHS [174]. Sol-gel hydroxy terminated silicone oil was used as sorbent for extraction organophosphorus pesticide residues in food [200]. Similarly, sol-gel based crown ether sorbents were used for extraction of organophosphorus pesticide residues from apple, water, apple juice and tomato [201]. Hydroxy terminated silicone oil, butylmethacrylate, methylnmethacrylate was used as sorbent for extraction of 2-chlorethyl sulfide from soil [151]. Functional group phenyl based sol-gel sorbents were used for extraction of organochlorine pesticides, trizine herbicide, estrogens, alkylphenols, and bisphenol [202]. For extraction of ephedrine and methamphetamine in human urine was achieved by using a sol-gel based β-cyclodextrin sorbents [203]. Similar sorbents were used for extraction of benzene, toluene, ethylbenzene, 2-octanone, benzaldehyde, acetophenone, dimethylphenol, and tridecane [204]. Sol-gel based crown ether sorbents were used in extraction of benzene, toluene, ethylbenzene, o-xylene, Chlorobenzene, and carcinogenic aryl amines from aqueous samples [197]. Carbowax 20M was used as stationary phase for extraction of BTEX from aqueous samples and compared with commercially available fibers [205]. N-Octyltriethoxy silane was used as stationary phase for determination of organometals [206]. Hydroxy terminated PDMS sorbents were used to extract aromatic hydrocarbons [207]. Sol-gel based calixerene sorbents were used to determine the propranolol enantiomers in urine [153, 208]. Polyvinyl alcohol and PDMS based sorbents were used for extraction of o-xylene, naphthalene, ethyl caprate, p-chlorotoluene
and parachlorinated biphenyls[154]. Sol-gel based sorbents involving calixerenes were used to extract chlorophenols from river water and soil[172]. Sorbents like hydroxyterminated silicone oil, and hydroxyl terminated PDMS were used to extract aroma compounds from beer and determination of gasoline residues from fire debris [142, 209]. Calixerene based sorbents were used for determination of BTEX, PAHS, and aromatic amines [210]. For detection of antiestrogens from biological matrices hydroxyterminated silicone oil sorbent was used [211].

In conclusion, sol-gel based capillary microextraction and solid phase microextraction are the most adaptable miniaturized extraction techniques for reproducing analysis with high selectivity and sensitivity. As these techniques involves solvent less process with high thermal and chemical stability they are considered to be most compatible with most of the analysis techniques. Here in this research sol-gel based niobia sorbents in capillary microextraction coupled to high performance liquid chromatography were developed. These sorbents were used to preconcentrate a wide variety of anlaytes like PAHS, alcohols, ketones, amine, organophosphorus pesticides and nucleotides.

1.5 References


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2. Chapter Two

Sol-gel Niobia - Polytetrahydrofuran coating in Capillary Microextraction Online coupled to High Performance Liquid Chromatography

2.1 INTRODUCTION

Solid phase micro extraction (SPME) is a solvent-free sample preparation technique. SPME is predominantly used in the fiber format (fiber-SPME). SPME fiber is prepared by coating the end segment of a small diameter solid rod (fiber) typically made of fused silica with a sorbent. To date, various sorbents have been reported for extracting organic and inorganic analytes from a wide variety of samples [1-5]. Though fiber-SPME is an environmentally friendly sample preparation technique, it has some significant disadvantages such as fragility of the fiber; susceptibility of the sorbent to scraping; scratching and damage during operation and storage and low sample capacity. To overcome these problems, in-tube SPME was developed [6] providing an extraction device in which sorbent is coated on the inner surface of a fused silica capillary. Here the wall of the capillary casing protects the sorbent from mechanical damage. The extraction in in-tube SPME is performed by passing the sample through the capillary. Though in-tube SPME have great features like higher extraction sensitivity and mechanical stability than fiber-SPME, these advantages cannot be fully translated into enhanced analytical performance by using a segment of GC column with physically coated thin stationary phase films to perform the extraction. Physically held sorbent coatings are
characterized by low thermal and solvent stabilities, whereas low coating thickness translates into reduced sample capacity. To overcome these problems Malik and co-workers have introduced sol-gel capillary microextraction (CME) [7]. In sol-gel CME the chemical bonding of the sorbent phase is quite versatile and provides thermal and solvent stability. Sol-gel technique can be used to prepare both thin and thick coatings bonded to their substrate.

Niobium, being group V ductile transition metal, may exist in different oxidation states like +5,+4,+3, but it is most commonly encountered as niobium (V) oxide. Niobium oxides and alkoxides are mostly used as catalyst in chemical and photochemical reactions [8-12]. In addition, alkoxides and oxides of niobium have a wide variety of applications like, preparing ceramic fibers [13], luminescent materials [14], pyro electric hybrids [15], bioactive coatings [16] and in the synthesis of new crystal structures in solid state chemistry [17]. Alkoxide sols of Niobium and silica have also been used to prepare micro porous molecular sieves [18]. As for biological applications, niobium pentoxide has been used for the enrichment of phosphoproteins and niobium derivatives were developed for active principle medicaments useful in treatment and prevention of carbohydrate and lipid metabolism disorders [19].

In the area of analytical applications, niobium doped titania powders, prepared by sol gel process, have been used for gas sensor applications[20]. Niobium doped titanium powders have also used in sensors for detecting dissolved oxygen [21] niobium pentoxide modified silica gel has been used for determining nickel in aqueous matrices by solid phase extraction coupled to flow injection system and flame atomic absorption spectrometry [22]. Carsek et al. had developed covalently bonded niobium (V) oxide on silica gel for the determination of zinc in biological samples by flame atomic absorption spectrometry [23]. Niobia modified-silica gel has been used as sorbent for the determination of lead [24] and chromium speciation [25] from environmental samples by
solid phase extraction coupled to flow injection system and flame atomic absorption spectrometry. Moreover, Carsek et al. had developed a new solid phase microextraction fiber by using niobium (V) oxide as sorbent on glass ceramic rod [26] to extract organic compounds in gaseous samples. In this research we developed novel niobia-based surface bonded organic–inorganic hybrid sol-gel coating. For this, niobium (V) ethoxide was used as the sol-gel precursor. Earlier, niobium pentaethoxide was used as precursor in sol-gel process for preparing niobium powders [27], thin films [28], organic and inorganic hybrids [29]. To the best of our knowledge this is the first report on the use of sol-gel based niobia-polyTHF sorbent in CME. To evaluate the extraction performance of this novel sol-gel based niobia sorbent in CME, silica-based sol-gel sorbents of analogous compositions were created.

2.2 Experimental

2.2.1 Equipment

Capillary microextraction coupled to high performance liquid chromatography (CME-HPLC) experiments on sol-gel niobia coated capillaries were performed using Waters model 2795 HPLC system equipped with photodiode array detector. Coupling of CME with HPLC was achieved by using 7725 Rheodyne six port injection valve. A Fisher model G-560 Vortex Genie (Fisher Scientific, Pittsburgh, PA, USA) was used for thorough mixing of sol solution ingredients. A Thermo IEC Micromax microcentrifuge (Needham Heights, MA, USA) was used for separation of precipitate from sol solution at 14000 rpm for 4 minutes. Home-made gas pressure operated purging device [30] was used to coat the fused silica capillary with sol solution and also to rinse the capillary with different solvents. Ultrapure water (18.0 MΩ) was obtained from Maxima pure water system (ELGA Maxima, England). Peek tubing (1/16 inch OD, 0.005 inch ID), Rheodyne fittings (PK 1/16 inch) purchased from Upchurch (Oak Harbor, WA, USA) was used to connect
fused silica capillary to rheodyne six port injection valve. An in-house built sample dispenser was used for CME [7]. An HP model 5790A GC oven was used for hydrothermal pretreatment and conditioning the sol-gel coated fused silica capillaries. Fourier transform infrared (FTIR) spectra for sol-gel niobia and silica sorbents were captured on a Spectrum Two model Perkin Elmer FTIR spectrometer. Hitachi model SU-70 scanning electron microscope (SEM) was used to generate SEM images of the sol-gel niobia coated capillaries.

2.2.2 Materials

Fused silica capillary was purchased from Polymicro Technologies (Phoenix, AZ, USA).

Chemicals: 9-anthracene methanol, phenanthrene, benzophenone, benzhydrol, anthraquinone, coumarin, flouranthene, perylene, were procured from Aldrich (Milwaukee, WI, USA). 2-Naphthol was purchased from Matheson, Coleman and Bell (Cincinnati, OH, USA).

Trifluoroacetic acid (99% pure) was purchased from Acros (Morris Plains, NJ, USA). HPLC grade methanol, methylene chloride and Naphthalene were purchased from Fisher scientific (Pittsburgh, PA, USA). Niobium (V) ethoxide and Tetraethoxy orthosilicate was purchased from Gelest (Morrisville, PA, USA). Polytetrahydrofuran-250 (polyTHF, Mol.wt: 225-275) was purchased from BASF corporation (Parsippany, NJ, USA).

2.2.3 Hydrothermal pretreatment of fused silica capillary

A 5-m (250 µm i.d) long piece of fused silica capillary was pretreated for cleaning its inner surface and to achieve enhanced density of surface silanol groups. The capillary was sequentially rinsed with methylene chloride, methanol and water (3 mL each). The capillary was then sealed at both ends by an oxy-acetylene torch and heated in GC oven at 350°C for 2 hrs. Subsequently the capillary was cooled down to room temperature and both ends were cut open with an alumina wafer. Finally, one end of the capillary was connected to the GC injection port and conditioned.
under helium purge (1 mL/min) by programming the GC oven temperature from 40˚C- 250˚C with a rate of 5˚C/minute. Capillary was held for 2 hrs at 250˚C.

2.2.4 Preparation of sol solutions

For preparation of niobia-polyTHF based sol solution, 50.0 mg of polytetrahydrofuran was weighed in a microcentrifuge tube, and to it 28.0 µL of methylene chloride was added. This mixture was vortexed for 5 minutes and left to sit for 12 hrs. 60.0 µL of niobium pentaethoxide was taken in another microcentrifuge tube, 20.0 µL of trifluoroacetic acid was added to it and vortexed for 2 minutes. This mixture was allowed to sit for 5 minutes to facilitate chelation with alkoxy groups on niobium. Contents of the two microcentrifuge tubes were combined with help of a micropipette and the mixture was vortexed for 4 minutes. Finally, niobia and polytetrahydrofuran containing microcentrifuge tube is centrifuged at 14000 rpm for 4 minutes. Then supernatant fluid was collected in to another micro centrifuge tube which was used for coating capillary. For pure inorganic sol-gel Nb2O5 sorbent 60 µL of niobium pentaethoxide and 20 µL of 99% trifluoroacetic acid were taken in microcentrifuge tube with the help of micropipette and vortexed for 2 minutes. Then the mixture was centrifuged at 14000 rpm for 4 minutes, later supernatant fluid was collected and used for coating capillary. Analogously for sol-gel silica-poly-THF sorbent was prepared by using tetraethoxy orthosilicate (60.0 µL) and 98% trifluoroacetic acid (20.0 µL), 50.0 mg of polytetrahydrofuran and 28.0 µL of methylene chloride. For pure inorganic sol-gel silica sorbent 60 µL of tetraethoxy orthosilicate and 20 µL of 98% of trifluoroacetic acid was taken. Gelation times for prepared sol solutions was close to 90 min.
2.2.5 Preparation of Surface bonded sol-gel coatings on inner walls of fused silica capillary

An 80-cm hydrothermally pretreated fused silica capillary was separately coated with the each of prepared sol solution. To accomplish this, sol solution after centrifugation was filled into fused silica capillary under nitrogen pressure (10 psi) by using filling/purging device (refer to Figure 2.1). Other end of the capillary was closed by rubber septum and sol solution was left in silica capillary for 30 minutes to facilitate sol-gel reactions and chemical bonding of growing sol-gel network with inner walls of fused silica capillary. After 30 minutes the rubber septum was removed to drain the excess sol solution and then the capillary was purged under nitrogen pressure (30 psi) for 60 minutes.
2.2.6 Conditioning of niobium-poly THF capillary

After coating the capillary, it was conditioned in a GC oven under helium flow (1mL/min). The temperature of the oven was programmed from 40°C-150°C at 0.5°C/minute. The capillary was held at 150°C for 5 hours. The capillary was then cooled to room temperature and further rinsed with a mixture of methylene chloride and methanol (1:1) followed by rinse with water. Then the capillary was rinsed with methanol so it can dry faster and flushed with nitrogen for couple of
minutes. The rinsed capillary was again conditioned in a GC oven analogously as mentioned above except the hold time was for 12hrs at 150˚ C.

2.2.7 Preparation of sol-gel sorbents for FTIR and TGA characterization

For FTIR and TGA analysis, sol-gel sorbents were prepared in 6 mm i.d. hydrothermally treated borosilicate glass tubes and freshly prepared sol solutions under identical set of conditions as were used for coating fused silica capillaries. Thermal treatment of these coatings was also carried out under identical conditions. The created sol-gel materials were scraped out with a stainless steel spatula and used for FTIR analysis.

2.2.8 Niobium-polyTHF coating in capillary microextraction coupled with high performance liquid chromatography

A 40-cm niobia polytetrahydrofuran coated fused silica capillary was installed as an external sampling loop on a six-port injection valve (Rheodyne 8125 model). It was accomplished with the help of 3 cm long piece of peek tubing, and plastic ferrules. The injection valve was coupled to model 2795 Waters HPLC with photodiode array detector. Aqueous samples containing target analytes were passed through the niobia polyTHF coated capillary from in-house built sample dispenser [7]. The injection valve was kept in load position (refer to Figure 2.2) during the extraction of analytes from aqueous samples by sol-gel niobia coated capillary. After reaching the extraction equilibrium the injection valve was switched to inject position (refer to Figure 2.3) to allow the mobile phase (methanol/water) to pass through the capillary and desorb the analytes transferring them to HPLC column (Waters C-18 column, 4.6 mm column diameter, 3.5µm particle size) for separation and photodiode array detection. Different compositions of mobile phase was used depending upon nature of the analyte extracted.
Figure 2.2: Schematic representation of Load position of CME coupled to HPLC
2.2.9. Evaluation of extraction performance of created sol-gel sorbents

To determine the onset of analyte sorption-desorption equilibrium between the sorbent phase and aqueous sample matrix extraction profiles were constructed using analytes of varying polarity. For this three analytes from different classes were selected i.e. phenanthrene (non-polar analyte), benzophenone (moderately polar) and benzyl alcohol (polar analyte). Aqueous samples of $2 \times 10^2 \mu g/L$ concentrations were prepared by dilution from 1000 part per million stock solutions. These samples were extracted by niobia-polyTHF and silica-polyTHF capillaries for different lengths of time (10, 30, 50, 70, 90, 110 minutes). Three replicate extractions were performed for each of these extraction cycles. The average peak areas of the extracted analytes were plotted against the time (in minutes) employed for extraction.
Calibration plots of analytes (phenanthrene, benzophenone, and benzyl alcohol) were plotted by performing the direct injections with 20 µL sample loop connected to a Rheodyne six port injection valve coupled to 2795 Waters HPLC. Precise concentrations of standard solutions that is $1 \times 10^2$ µg/L, $3 \times 10^2$ µg/L, $5 \times 10^2$ µg/L, $7 \times 10^2$ µg/L, $9 \times 10^2$ µg/L were injected for each analyte. Three replicates for each concentration were taken. The replicates peak area were averaged for each analyte and plotted against the corresponding concentrations. Figure 2.4 represents the calibration plots for phenanthrene, benzophenone, and benzyl alcohol. From calibration plot a linear relationship was determined between peak area and concentration.
Figure 2.4: Calibration plots for phenanthrene, benzophenone, and Benzhydrol, performed by direct injections. HPLC conditions: 75mm x 4.6mm I.D C-18 Waters column, mobile phase: 90/10 CH$_3$OH/H$_2$O for phenanthrene; 70/30 CH$_3$OH/H$_2$O for benzophenone; 80/20 CH$_3$OH/H$_2$O for 9-anthracene methanol; isocratic elution with a flow rate 1mL/min, photodiode array detection

To determine the reproducibility of developed method, five capillaries were coated and conditioned with niobia-polyTHF sol solutions under similar conditions as mentioned section 2.2.5 and 2.2.6. Then with these five capillaries each at a time microextractions (CME coupled to HPLC) were performed with target analytes (phenanthrene, benzophenone, and benzyl alcohol), three replicate extractions for each analyte and their peak areas relative standard deviation was taken.

2.2.10. Determination of Specific extraction (SE)

To compare the microextraction performance of the created sol-gel niobia sorbents recently introduced parameter, specific extraction (SE) [31] was considered. Specific extraction is defined as amount extracted per unit mass of sorbent.

$$SE = \frac{The \ mass \ of \ analyte \ extracted \ (\mu g)}{mass \ of \ sol \ gel \ sorbent \ (g)}$$
The mass of the sorbent was calculated from the weight difference (thoroughly cleaned and dried) between a coated and the corresponding uncoated capillary. The mass of analyte extracted was obtained from the mean of chromatographic peak areas of 3 replicate extractions and a calibration plot constructed by direct injections of standard solutions. Calibration plots were constructed by obtaining average peak area of 3 replicate measurements of direct injections of standard solutions with precisely known concentrations (0.1, 0.3, 0.5, 0.7, 0.9 ng/L). Then average peak area of three replicate measurements were plotted against the corresponding mass of the analyte in standard solutions and the best fit linear equation was obtained. Extracted amount of analytes were determined using the linear equation from calibration plots.

2.2.11. Determination of limit of detection (LOD) and run to run relative standard deviation (RSD)

Limit of Detection and Relative standard deviation was calculated by extracting the analytes with created sol-gel sorbents and analyzed by coupling the CME to HPLC. Aqueous of samples of desired analytes were prepared and microextractions were performed by sol-gel niobia-polyTHF, sol-gel niobia, sol-gel silica, and sol-gel silica-polyTHF sorbents. Limit of detection was calculated by considering the noise, peak height and concentration. Three replicate extractions were performed for each analyte with each sorbent. LOD was calculated by below formula;

\[
LOD = \frac{3 \times \text{noise} \times \text{concentration}}{\text{peak height}}
\]

For run to run relative standard deviation calculation, peak areas were considered. Three replicate extractions were performed for each analyte and average of peak areas were considered for relative standard deviation calculation.
2.3 Results and Discussion

Sol-gel sorbents in CME and fiber based SPME had not only provided enhanced chemical and thermal stability but also excellent extraction efficiency for a wide range of analytes in different fields like biomedical, environmental, food etc.

Poly-THF, also called as polytetramethylene oxide, is a hydroxyl-terminated polar material. A lot of research work was done on this linear polyether. As an organic component poly-THF has been applied in synthesizing organic-inorganic hybrid materials [32-34]. Sol-gel poly-THF was also used as bioactive bone repairing material [35]. Poly-THF has also been used as a sorbent in extraction and separation of small metal complexes [36]. Malik et al. [37, 38] has shown that as organic component in sol-gel based organic-inorganic hybrid sorbents for CME polyTHF can provide excellent extraction efficiency for various environmentally important compounds.

Examining the extraction performances of polyTHF (as organic component in sol-gel organic-inorganic hybrid) in extracting various types of analytes, led to further exploration of its extraction efficiency in CME along with niobia (as inorganic component in sol-gel organic-inorganic hybrid)

2.3.1. Synthesis of sol-gel sorbents

Figure 2.5 represents the schematic representation of sol-gel reactions that led to the evolution of sol-gel network. In the first step, a controlled hydrolysis of niobium pentaethoxide takes place, followed by polycondensation of hydrolyzed niobium pentaethoxide with poly-THF resulting in a 3-dimensional sol-gel network. Finally, the covalent bonding of the evolving sol-gel network with silanol groups in inner walls of fused silica capillary leads to the formation surface bonded sol-gel niobia-poly-THF coating.
Figure 2.5: Schematic representation of hydrolysis and polycondensation of niobium pentaethoxide and polyTHF in sol-gel process.
In general transitional metal alkoxides undergo sol-gel reactions at extremely high rates which results in precipitation of reaction products. Chelating agents are used to control the rate of hydrolysis of metal alkoxides, and to provide favorable conditions for chemical bonding of metal alkoxide with the organic component (organic ligand/polymer) of the sol solution. In this research we used trifluoroacetic acid as a chelating agent. Optimization of the chelating agent content in the sol solution is important to achieve the maximum loading of organic component in sol-gel materials. To figure out the optimum level of trifluoroacetic acid that need to be added to niobia-based sol-gel solutions, three different sol-gel niobia sorbents were prepared using different chelator to precursor ratios, keeping the organic component (poly-THF) concentration constant. These are; (i) Nb$_2$O$_5$ to TFA ratio ~0.5 (ii) Nb$_2$O$_5$ to TFA ratio ~1 (iii) Nb$_2$O$_5$ to TFA ratio ~1.5. These sol-gel materials were synthesized as mentioned in section 2.2.7 and thermogravimetric analysis was performed to determine the percent composition of organic component. From Figure 2.6 represents the TGA analysis of the above three sol-gel materials and figure 2.7 represents TGA analysis of poly-THF. From TGA analysis data it was found determined that maximum loading of organic component (22%) was in sol-gel sorbent (iii) and minimum loading of organic component (18%) was in sol-gel (i) sorbent.

These results were consistent with the findings of Livage et al [39] who established that when chelation ratio (chelator to precursor) exceeds 2, it can lead to excessive chelation resulting in hindrance to the hydrolysis of transition metal alkoxide precursors. Furthermore it was observed that pyrolysis temperature of polyTHF was kind of increased in case of sol-gel based organic-inorganic hybrid compared to pyrolysis temperature of free polyTHF (refer to figure 2.7). This increment can be attributed the chemical bonding of polyTHF to/within the sol-gel network.
Figure 2.6: Thermogravimetric analysis (TGA) of sol-gel niobia materials; Capillary two- Nb₂O₅ to TFA ratio ~0.5; Capillary three- Nb₂O₅ to TFA ratio ~1; Capillary four- Nb₂O₅ to TFA ratio ~1.5.
2.3.2 Characterization of the created niobia-based sol-gel sorbents.

To perform the FTIR characterization of developed sol-gel CME sorbents, niobia-based, these materials were synthesized in 6 mm i.d glass tubes as described in section 2.2.7. Figure 2.8 and 2.9 represents the FTIR spectra of sol-gel Nb$_2$O$_5$-poly-THF sorbent. Peak at 1089 cm$^{-1}$ (refer to figure 2.8) can be attributed to the chemical bonding Nb-O-C [40] in sol-gel niobia-poly-THF.
network. This peak can also be attributed to the chemical bonding of Nb-O-C in non-hydrolyzed precursor niobium pentaethoxide in sol-gel network.

As mentioned in section 2.3.1 chelating agents are significant to control the rate of sol-gel reactions (hydrolysis) of transition metal alkoxides. So exposing the non-hydrolyzed niobium pentaethoxide in sol-gel network to water for longer period of time will result in complete hydrolysis of niobium pentaethoxide. To hydrolyze the non-hydrolyzed niobium pentaethoxide in sol-gel network, water (~2 ml) was added to the glass tube consisting of synthesized sol-gel niobia-polyTHF material (refer to section 2.2.7 for synthesis procedure) with the help of micro pipette and then sonicated for 5hrs. Then the excess water was removed with the help of micropipette and then conditioned in GC oven for 12 hours (at 150°C). Later FTIR analysis was performed on the conditioned sol-gel niobia-poly-THF material (refer to figure 2.9). From figure 2.9 it is clearly evident that there is shrinkage of peak representing Nb-O-C (1094 cm\(^{-1}\)) which can be attributed to the hydrolysis of non-hydrolyzed niobium pentaethoxide and also confirming the presence of chemical bond (Nb-O-C) between niobia and poly-THF.

Figure 2.10 represents the SEM image of the surface bonded Nb_2O_5-poly-THF coating on the wall of CME capillary.
Figure 2.8: FTIR spectrum of sol-gel Nb$_2$O$_5$-poly-THF material

Figure 2.9: FTIR spectrum of Sol-gel Nb$_2$O$_5$-poly-THF material after further hydrolysis of the residual alkoxy groups in the sol-gel precursor that was only partially hydrolyzed during original synthesis.
Figure 2.10: SEM cross-sectional view of surface bonded sol-gel Nb$_2$O$_5$-poly-THF coating in a CME capillary
Figure 2.11: Extraction profiles of phenanthrene, benzophenone, and Benzhydrol, each with a concentration of $2 \times 10^2 \mu g/L$ for (a) sol-gel niobia-poly-THF sorbent and (b) sol-gel silica-polyTHF sorbent. HPLC conditions: 75mm x 4.6 mm I.D C-18 Waters column, mobile phase: 90/10 CH$_3$OH/H$_2$O for phenanthrene; 70/30 CH$_3$OH/H$_2$O for benzophenone; 80/20 CH$_3$OH/H$_2$O for 9-anthracene methanol; isocratic elution with a flow rate 1mL/min, photodiode array detection.
2.3.3 Evaluation of microextraction characteristics of sol-gel Nb$_2$O$_5$-poly-THF sorbent in CME.

Figure 2.11 represents the extraction profiles of phenanthrene, benzhydrol, and benzophenone, obtained on two sorbents: (a) sol-gel Nb$_2$O$_5$-poly-THF and (b) sol-gel SiO$_2$ – poly-THF. From figure 2.11 it is evident that within 30 minutes the analytes reached sorption-desorption equilibrium with the sol-gel sorbents and the sample matrix. Capillary to capillary reproducibility for sol-gel Nb$_2$O$_5$-poly-THF sorbent in CME was found to have RSD values (considering peak areas of extracted analytes) less than 5% (refer to section 2.2.9 for procedure). LOD values for all the analytes extracted by Nb$_2$O$_5$-poly-THF sorbent was found to be in ng/L and also RSD values were less than 5%.

2.3.4. Extraction of different classes of analytes by sol-gel Nb$_2$O$_5$-poly-THF sorbent.

The niobia-polyTHF coated capillary had provided exemplary extraction capabilities for non-polar (PAH’S) moderately polar (ketones), and polar (amines, alcohols) analytes. Most of these analytes can be carcinogenic, mutagenic, teratogenic and toxic environmental contaminants[7, 41]. The observed extraction capabilities can be attributed to the moderately polar structural features of polyTHF which makes it suitable for extraction of moderately polar and nonpolar analytes. Presence of Lewis acid sites on niobia makes it suitable for extraction of amines and alcohols (which represents the Lewis bases). Figures 2.12, 2.13, 2.14 and 2.15 represent the chromatograms of ketones, amines, PAH’s, and alcohols which were extracted by Nb$_2$O$_5$-poly-THF sorbent.
Figure 2.12: Ketones: Benzophenone ($2.7 \times 10^2$ µg/L), Anthraquinone ($1.6 \times 10^2$ µg/L) & Coumarin ($2.7 \times 10^2$ µg/L); Mobile phase: 75 methanol/25 water; Photodiode array detection
Figure 2.13: Amines: Caffeine (1.7 x 10^2 µg/L), m-Toluidine (1.5 x 10^2 µg/L) Diphenylamine (2.5 x 10^2 µg/L) & N-methyl aniline (2.0 x 10^2 µg/L) (Mobile phase: 75 methanol/25 water; Photodiode array detection)
Figure 2.14: PAHS: Naphthalene ($1.7 \times 10^2$ µg/L), Phenanthrene ($2 \times 10^2$ µg/L), Fluoranthene ($3 \times 10^2$ µg/L), & Perylene ($3.2 \times 10^2$ µg/L); Mobile phase: 90 methanol/10 water; Photodiode array detection
Figure 2.15: Alcohols: 2-Napthol (2.0 x 10^2 µg/L), Benzhydrol (1.6 x 10^2 µg/L) & 9-Anthracene methanol (1.0 x 10^2 µg/L); Mobile phase: 60 methanol/40 water; Photodiode array detection.

Extraction of different classes of analytes were performed by CME using four different sorbents (i) purely inorganic Nb_2O_5 sorbent (ii) Nb_2O_5-poly-THF sorbent (iii) purely inorganic SiO_2 sorbent (iv) SiO_2–poly-THF sorbent. Table 2.1 provides online CME-HPLC data of the extracted analytes by above four sorbents.
Table 2.1: Online CME-HPLC data for different groups of analytes extracted by four sorbents: (a) sol-gel Nb₂O₅ sorbent (b) sol-gel Nb₂O₅-poly-THF sorbent (c) sol-gel SiO₂ sorbent (d) sol-gel SiO₂-poly-THF sorbent.

Two types of molecular level interactions with analytes in sample matrix can be achieved with the prepared sol-gel sorbents that is (i) van der Waals interactions and (ii) Lewis acid-base interactions (depending on nature of the sorbent and/or the analyte). SE values representing an objective measure of extraction efficiency for the extracted analytes was used to characterize the extraction efficiencies of the created sol-gel sorbents. SE values of extracted analytes with sorbent (i) was observed to be 70 to 80% superior compared to sorbent (iii). This can be
attributed to the presence of Lewis acid sites on the niobia surface which are absent on silica surface. Presence of Lewis acid sites can lead to the Lewis acid-base interactions between the analytes (depending on nature of analyte) and the sol-gel sorbent. Similarly SE values of extracted analytes with sol-gel sorbent (ii) was 70 to 80% superior compared to sol-gel sorbent (iv), these results indicate the presence of same amount of organic polymer in both the sorbents. At the same time SE values of extracted analytes with sol-gel sorbent (ii) are 15 to 25 % higher than SE values obtained by sol-gel sorbent (i) which suggests the presence of polymer leading to higher extraction efficiency. Similar SE results were observed with sol-gel sorbents (iv) and (iii). Higher SE values of amines and alcohols compared to other groups of analytes can be explained by their Lewis acid-base interactions (as alcohols and amines can acts as Lewis bases) with sol-gel niobia sorbents.

2.4 Conclusions

A novel sol-gel niobia-poly-THF sorbent was created for CME coupled to HPLC. Extraction performance of this novel sol-gel niobia-based sorbent was evaluated by extracting various environmentally important analytes including PAH’S, ketones, amines, alcohols. Since silica based sorbents are the traditional sorbents used in microextraction techniques, a sol-gel based silica-poly-THF sorbent was also created and the extraction performance of novel sol-gel niobia-polyTHF was compared with that of sol-gel silica-poly-THF sorbent. It was observed that SE values for the extracted analytes on sol-gel Nb2O5-poly-THF sorbent were 70 to 80 % superior to SE values obtained for the same analytes on SiO2–poly-THF sorbent. Superior extraction performance of sol-gel Nb2O5-poly-THF sorbent can be explained by the presence of Lewis acid sites on niobia surface which are lacking on silica surface. Extraction performance of sol-gel niobia sorbent can conclude that niobia-based sorbents have immense potential for application as
in different microextraction techniques. Niobia based sorbents can be applied to solve analytical problems encountered in diversely important fields like environmental, biomedical, food etc.

2.5 References


3. Chapter three

Sol-gel based Niobia-Polytetrahydrofuran coating in capillary microextraction online coupled to high performance liquid chromatography for enrichment of organophosphorus pesticides

3.1 Introduction

Sample preparation is a crucial step in chemical analysis. Over the past few decades, a lot of research has been directed toward developing environmentally benign sample preparation techniques. One such technique is solid phase microextraction (SPME) which completely eliminates the use of hazardous organic solvent in sample preparation. Capillary microextraction [1] (CME also called in-tube SPME) one of the configurations of SPME was developed to overcome certain disadvantages of fiber SPME such as poor mechanical stability and low sample loading. The key component of a SPME or CME device that ultimately determines its analytical performance is the sorbent coating on the surface of the fiber/inner surface of the capillary. Conventional coating techniques applied for SPME sorbent coating had provided poor solvent and thermal stability. To overcome these problems Malik et al. [2] had introduced sol-gel technique for coating SPME fiber, which provides chemical bonding of coating to the surface of the fiber. The surface bonded coatings developed by using sol-gel technique provided good chemical and thermal stability.

Here we developed sol-gel based organo-inorganic hybrid niobia-polytetrahydrofuran (Nb$_2$O$_5$-polyTHF) sorbent for online enrichment of organophosphorus pesticides (OPP’S). OPP’S are one of the most toxic chemicals used in agriculture, neurotoxic chemical warfare agents etc.
They are widely used worldwide. Due to their high toxicity, monitoring trace levels of OPP’S in environmental and food samples is critically important for protection of environment and human health. Various extraction techniques were applied for analysis of OPP’S like, dispersive solid phase extraction [3], liquid-liquid extraction [4], stir-bar sorptive extraction [5], Liquid phase microextraction [6] solid phase microextraction (SPME) [7]. Silica [8], titania [9] based sorbents were used for extraction of OPP’S. Sol-gel based sorbents were used in enrichment of OPP’S [10-14]. Titania based sorbents were found to more effective in enrichment of OPP’S due to the presence of Lewis acid sites on surface of titania. These Lewis acid sites provides favorable interaction with the phosphate group which represents Lewis base. Niobia an oxide of group 5 transition metal expected to provide similar properties in enrichment of OPP’s due to the presence of Lewis acid sites.

Poly tetrahydrofuran (polyTHF) is a hydroxyl-terminated polar material. This linear polyether was used by Malik et al. [15, 16] as organic component in sol-gel organic-inorganic hybrid sorbents for extraction of various environmentally and biomedically important analytes. Excellent extraction efficiency provided by polyTHF in earlier research [15, 16] persuaded interest in exploring polyTHF as organic component in sol-gel organic-inorganic hybrid sorbent for enrichment of OPP’S. In this research we describe a sol-gel based Nb$_2$O$_5$-polyTHF sorbent for enrichment of OPP’S. To the best of our knowledge for first time, a sol-gel based Nb$_2$O$_5$-polyTHF sorbent is being used for enrichment of OPP’S.
3.2 Experimental

3.2.1 Equipment

Capillary microextraction coupled to high performance liquid chromatography (CME-HPLC) experiments on sol-gel niobia coated capillaries were performed using Waters model 2795 HPLC system equipped with photodiode array detector. Coupling of CME with HPLC was achieved by using 7725 Rheodyne six port injection valve. A Fisher model G-560 Vortex Genie (Fisher Scientific, Pittsburgh, PA, USA) was used for thorough mixing of sol solution ingredients. A Thermo IEC Micromax microcentrifuge (Needham Heights, MA, USA) was used for separation of precipitate from sol solution at 14000 rpm for 4 minutes. Home-made gas pressure operated purging device [17] was used to coat the fused silica capillary with sol solution and also to rinse the capillary with different solvents. Ultrapure water (18.0 MΩ) was obtained from Maxima pure water system (ELGA Maxima, England). Peek tubing (1/16 inch OD, 0.005 inch ID), Rheodyne fittings (PK 1/16 inch) purchased from Upchurch (Oak Harbor, WA, USA) was used to connect fused silica capillary to rheodyne six port injection valve. An in-house built sample dispenser was used for CME [18]. An HP model 5790A GC oven was used for hydrothermal pretreatment and conditioning the sol-gel coated fused silica capillaries. Fourier transform infrared (FTIR) spectra for sol-gel niobia and silica sorbents were captured on a Spectrum Two model Perkin Elmer FTIR spectrometer. Hitachi model SU-70 scanning electron microscope (SEM) was used to generate SEM images of the sol-gel niobia coated capillaries.
3.2.2 Materials

Fused silica capillary was purchased from Polymicro Technologies (Phoenix, AZ, USA). Chemicals: Trifluoroacetic acid (99% pure) was purchased from Acros (Morris Planes, NJ, USA). HPLC grade methanol, methylene chloride were purchased from Fisher scientific (Pittsburgh, PA, USA). Niobium (V) ethoxide and Tetraethoxy orthosilicate, Titanium isopropoxide were purchased from Gelest (Morrisville, PA, USA). Polytetrahydrofuran-250 (polyTHF, Mol.wt: 225-275) was purchased from BASF corporation (Parsippany, NJ, USA). Organophosphorus pesticides, bensulide, fenitrothion, and chlorfenvinphos were purchased from Sigma Aldrich (Milwaukee, WI, USA).

3.2.3 Hydrothermal pretreatment of fused silica capillary

A 5-m (250 µm i.d) long piece of fused silica capillary was pretreated for cleaning its inner surface and to achieve enhanced density of surface silanol groups. The capillary was sequentially rinsed with methylene chloride, methanol and water (3 mL each). The capillary was then sealed at both ends by an oxy-acetylene torch and heated in GC oven at 350˚C for 2 hrs. Subsequently the capillary was cooled down to room temperature and both ends were cut open with an alumina wafer. Finally, one end of the capillary was connected to the GC injection port and conditioned under helium purge (1 mL/min) by programming the GC oven temperature from 40˚C- 250˚C with a rate of 5˚C/minute. Capillary was held for 2 hrs at 250˚C.

3.2.4 Preparation of sol solutions

For preparation of niobia-polyTHF based sol solution, 50.0 mg of polytetrahydrofuran was weighed in a microcentrifuge tube, and to it 28.0 µL of methylene chloride was added. This mixture was vortexed for 5 minutes and left to sit for 12 hrs. 60.0 µL of niobium pentaethoxide
was taken in another microcentrifuge tube, 20.0 µL of trifluoroacetic acid was added to it and vortexed for 2 minutes. This mixture was allowed to sit for 5 minutes to facilitate chelation with alkoxy groups on niobium. Contents of the two microcentrifuge tubes were combined with help of a micropipette and the mixture was vortexed for 4 minutes. Finally, niobia and polytetrahydrofuran containing micro centrifuge tube is centrifuged at 14000 rpm for 4 minutes. Then supernatant fluid was collected in to another micro centrifuge tube which was used for coating capillary. For pure inorganic sol-gel Nb2O5 sorbent 60 µL of niobium pentaethoxide and 20 µL of 99% trifluoroacetic acid were taken in microcentrifuge tube with the help of micropipette and vortexed for 2 minutes. Then the mixture was centrifuged at 14000 rpm for 4 minutes, later supernatant fluid was collected and used for coating capillary. Similarly for sol-gel titania-polyTHF sorbent was prepared by using titanium isopropoxide (58.0 µL) and 50% trifluoroacetic acid (18 µL), 50 mg of polytetrahydrofuran and 28 µL of methylene chloride. For pure inorganic sol-gel titania sorbent 58.0 µL of titanium isopropoxide and 30.0 µL of 95% trifluoroacetic acid was used. Analogously for sol-gel silica-poly-THF sorbent was prepared by using tetraethoxy orthosilicate (60.0 µL) and 98% trifluoroacetic acid (20.0 µL), 50.0 mg of polytetrahydrofuran and 28.0 µL of methylene chloride. For pure inorganic sol-gel silica sorbent 60.0 µL of tetraethoxy orthosilicate and 20.0 µL of 98% of trifluoroacetic acid was taken. Gelation times for prepared sol solutions was close to 90 min.

3.2.5 Preparation of Surface bonded sol-gel coatings on inner walls of fused silica capillary
An 80-cm hydrothermally pretreated fused silica capillary was separately coated with the each of prepared sol solution. To accomplish this, sol solution after centrifugation was filled into fused silica capillary under nitrogen pressure (10 psi) by using filling/purging device. Other end of the capillary was closed by rubber septum and sol solution was left in silica capillary for 30 minutes
to facilitate sol-gel reactions and chemical bonding of growing sol-gel network with inner walls of fused silica capillary. After 30 minutes the rubber septum was removed to drain the excess sol solution and then the capillary was purged under nitrogen pressure (30 psi) for 60 minutes.

3.2.6 Conditioning of sol-gel coated capillaries

After coating the capillary, it was conditioned in a GC oven under helium flow (1mL/min). The temperature of the oven was programmed from 40°C-150°C at 0.5°C/minute. The capillary was held at 150°C for 5 hours. The capillary was then cooled to room temperature and further rinsed with a mixture of methylene chloride and methanol (1:1) followed by rinse with water. Then the capillary was rinsed with methanol so it can dry faster and flushed with nitrogen for couple of minutes. The rinsed capillary was again conditioned in a GC oven analogously as mentioned above except the hold time was for 12hrs at 150°C.

3.2.7 Preparation of sol-gel sorbents for FTIR characterization

For FTIR analysis, sol-gel sorbents were prepared in 6 mm i.d. hydrothermally treated borosilicate glass tubes and freshly prepared sol solutions under identical set of conditions as were used for coating fused silica capillaries. Thermal treatment of these coatings was also carried out under identical conditions. The created sol-gel materials were scraped out with a stainless steel spatula and used for FTIR analysis.

3.2.8 Gravimetric evaluation of coating volume, mass, and density

The weight of a thoroughly dried sol-gel coating created on the inner walls of a fused silica capillary was determined gravimetrically. For this, initial weight of a thoroughly cleaned and dried capillary (250 μm i. d. x 2 m) was subtracted from the weight of the same piece of capillary containing a thoroughly cleaned, dried and thermally conditioned sol-gel surface-bonded coating on its inner surface. The resulting weight difference provided the weight of sol-gel coating on the
2-meter long coated capillary. By using this data, weight of the coating in a 40 cm capillary segment used for CME was calculated.

The volume of the sol-gel coating was determined as follows. The filling/purging device was placed in thermostat. A 2-meter uncoated capillary (sealed on both ends with Restek capillary column glass caps) was weighed at 17°C before and after it was filled with dichloromethane (DCM) using the filling/purging device. The 2-meter capillary was then coated with sol-gel sorbent, conditioned, and dried as described earlier and then weighed. The sol-gel coated dry capillary was then filled with DCM at 17°C as described and carefully sealed at both ends using glass caps. The weight of the DCM-filled coated capillary was taken. The volume of DCM in the uncoated capillary and the sol-gel coated capillaries were calculated by dividing the corresponding DCM weight with the specific gravity of DCM at 17°C (1.328 g/mL [19]). The difference in the volumes of DCM obtained from these measurements gives the volume sol-gel coating created in a 2-meter capillary segment. The volume of the sol-gel coating in a 40-cm segment of the capillary used in CME was calculated from this data. The density of the coated sol-gel sorbent was determined by dividing the weight of the coated sorbent by its volume.

3.2.9. Online coupling of CME to HPLC

A 40-cm sol-gel coated fused silica capillary was installed as an external sampling loop on a six-port injection valve (Rheodyne 8125 model). It was accomplished with the help of 3 cm long piece of peek tubing, and plastic ferrules. The injection valve was coupled to model 2795 Waters HPLC with photodiode array detector. Aqueous samples containing target analytes were passed through the sol-gel coated capillary from in-house built sample dispenser [18]. The injection valve was kept in load position during the extraction of analytes from aqueous samples by sol-gel coated capillary. After reaching the extraction equilibrium the injection valve was switched to inject
position to allow the mobile phase (methanol/water) to pass through the capillary and desorb the analytes transferring them to HPLC column (Waters C-18 column, 4.6 mm column diameter, 3.5µm particle size) for separation and UV detection.

3.2.10. Evaluation of microextraction performances of created sol-gel sorbents

To determine the onset of analyte sorption-desorption equilibrium between the sorbent phase and aqueous sample matrix extraction profiles were constructed using OPP’S. For this aqueous samples of three pesticides with $2 \times 10^2 \mu g/L$ concentrations were prepared by dilution from 1000 part per million stock solutions. These samples were extracted by niobia-polyTHF, titania-polyTHF and silica-polyTHF capillaries for different lengths of time (10, 20, 30, 40, 50, 60 minutes). Three replicate extractions were performed for each of these extraction cycles. The average peak areas of the extracted OPP’S were plotted against the time (in minutes) employed for extraction.

Calibration plots of OPP’S (bensulide, fenitrothion and chlorfenvinphos) were plotted by performing the direct injections with 20 µL sample loop connected to a Rheodyne six port injection valve coupled to 2795 Waters HPLC. Precise concentrations of standard solutions that is $2 \times 10^2 \mu g/L$, $4 \times 10^2 \mu g/L$, $6 \times 10^2 \mu g/L$, $8 \times 10^2 \mu g/L$, $10 \times 10^2 \mu g/L$ were injected for each pesticide. Three replicates for each concentration were taken. The replicates peak area were averaged for each pesticide and plotted against the corresponding concentrations. Figure 3.1 represents the calibration plots for bensulide, fenitrothion and chlorfenvinphos. From calibration plot a linear relationship was determined between peak area and concentration.
Figure 3.1: Calibration plots for Bensulide, Fenitrothion, and Chlorfenvinphos, performed by direct injections. HPLC conditions: 75mm x 4.6mm I.D C-18 Waters column, mobile phase: 75 methanol (0.1 % TFA) / 25 water (0.1% TFA) isocratic elution with a flow rate 1mL/min, UV detection.

### 3.2.11. Determination of Specific extraction (SE)

To compare the microextraction performance of the created sol-gel niobia sorbents recently introduced parameter, specific extraction (SE) [20] was considered. Specific extraction is defined as amount extracted per unit mass of sorbent.

\[
SE = \frac{The \ mass \ of \ analyte \ extracted \ (\mu g)}{mass \ of \ sol \ gel \ sorbent \ (g)}
\]

The mass of the sorbent was calculated from the difference of the weight of thoroughly cleaned and dried coated and uncoated capillary. The mass of anlayte extracted was obtained from the mean of chromatographic peak areas of 3 replicate extractions and a calibration plot.
constructed by direct injections of standard solutions. Calibration plots were constructed by
obtaining average peak area of 3 replicate measurements of direct injections of standard solutions
with precisely known concentrations (0.2, 0.4, 0.6, 0.8, 1.0 ng/L). Then average peak area of three
replicate measurements were plotted against the corresponding mass of the analyte in standard
solutions and the best fit linear equation was obtained. Using the linear equation from calibration
plots extracted amount of analytes were observed.

3.2.12. Determination of Desorption efficiency (DE %)

Desorption efficiency (DE) % [20] was determined to evaluate the completeness of desorption of
the extracted analytes from sol-gel CME sorbent. Each analyte was injected into the HPLC column
by using a 40-cm piece of deactivated fused silica capillary as the external sampling loop. Using
 calibration plots, the obtained peak area was converted into corresponding analyte mass extracted.
Each sample containing 200 ng of analyte was passed through coated capillary for 30 minutes to
attain extraction equilibrium and the liquid exiting from the capillary was collected. Mass of
analyte in the exited liquid was calculated by performing a direct injection of this collected liquid
into a HPLC system. Amount of the analyte extracted was calculated by subtracting the amount of
analyte in the exited liquid from the original amount of analyte in sample volume that passed
through the coated capillary for extraction. Desorbed amount of the analyte was obtained from the
HPLC peak area obtained through desorption of the extracted analyte using the HPLC mobile
phase. DE was calculated using the following equation [20]:

\[
Desorption\ efficiency\ (\%) = \frac{\text{Amount\ desorbed}}{\text{Amount\ extracted}} \times 100
\]

3.3 Results and Discussion

Sol-gel process provides a unique and simple approach in modifying the chemical composition
and morphology of coatings. Sol-gel based sorbents show high thermal and chemical stability.
Malik et al [21-24] had created different sol-gel based sorbents in CME for enrichment of environmentally and biologically important analytes.

Here in this research, application of sol-gel method in creating novel niobia-polyTHF based sorbents had provided exemplary extraction efficiency for enrichment of OPP’S.

### 3.3.1 Synthesis and characterization of sol-gel sorbents

A typical sol-gel reaction involves hydrolytic polycondensation reactions of sol-gel precursors resulting in colloidal system (the sol). A three-dimensional liquid filled network will be formed subsequently from the sol (the gel) [25]. Here in this work, niobia-based sol-gel precursor niobium pentaethoxide, and hydroxyl terminated polyTHF (sol-gel active polymer) were used to create organic-inorganic hybrid sol-gel sorbents. Trifluoroacetic acid was used as chelating agent (chelating agent controls the hydrolysis of metal alkoxides). For satisfactory level mixing of all components in sol solution, methylene chloride was used as solvent.

Controlled hydrolysis of niobium pentaethoxide in the presence of trifluoroacetic acid results in hydrolyzed products of the precursor which undergoes subsequent polycondensation resulting in niobia-based sol-gel network. During this process, sol-gel active terminal hydroxyl groups of polymer polytetrahydrofuran will have an opportunity to condense into growing sol-gel matrix.

The patches of growing sol-gel network in vicinity of silanol groups on inner walls of fused silica capillary form chemical bonding resulting in surface bonded coating.

Sol-gel coated capillaries were thermally conditioned at low temperature in GC oven under continuous flow of nitrogen. Thermal conditioning of coated capillaries will accelerate the condensation reactions in the sol-gel coating.
Figure 3.2 represents the SEM image of surface bonded sol-gel Nb$_2$O$_5$-polyTHF on inner walls of fused silica capillary. Coating thickness was found to be ~1.66 micrometer. By using this data volume and density of coating in 40 cm capillary was calculated.

Figure 3.2: SEM cross-sectional view of the surface bonded sol-gel Nb$_2$O$_5$-poly-THF coating in CME capillary
FTIR analysis was performed for developed sol-gel sorbents to determine the chemical bonding of sol-gel precursors with polyTHF. Figure 3.3 represents the FTIR spectra of sol-gel Nb$_2$O$_5$ – polyTHF, sol-gel TiO$_2$-polyTHF and sol-gel SiO$_2$-polyTHF sorbents. Sol-gel materials for FTIR analysis were synthesized as mentioned in section 3.2.7. Peak at 1094 cm$^{-1}$ in figure 3.3 indicates the chemical bonding of Nb-O-C [26] in sol-gel Nb$_2$O$_5$ –polyTHF network. Peak at 1065 cm$^{-1}$ represents the chemical bonding of Ti-O-C [27] in sol-gel TiO$_2$ –polyTHF network. Similarly peak at 1135 cm$^{-1}$ represents the chemical bonding of Si-O-C [28] in sol-gel SiO$_2$-polyTHF network.
Gravimetric analysis of sol-gel Nb₂O₅-polyTHF sorbent has revealed the volume of the coating in a 40-cm fused silica capillary was found to be 0.55 µL. This volume obtained by gravimetric analysis was comparable to the volume calculated by considering the coating thickness obtained by SEM (~ 0.50 µL). Densities of the created sol-gel Nb₂O₅-polyTHF was calculated by using this volume and mass of sorbent in 40 cm fused silica capillary (mass of sol-gel Nb₂O₅-polyTHF sorbent in fused silica capillary was found to be 1.8 mg) as 3.2 gm/cm³. Obtained density values for created sol-gel Nb₂O₅-polyTHF sorbent was found to be comparable to the densities of niobium pentoxide materials studied earlier (4.6 gm/cm³ to 5.3 gm/cm³) [29]. Low density of
sol-gel Nb$_2$O$_5$-polyTHF sorbent compared to literature value can be attributed to the presence of polymer polyTHF and porous structure of the sol-gel network.

3.3.2. Evaluation of microextraction characteristics of created sol-gel sorbents.

To determine the sorption-desorption equilibrium attained by the analyte between sol-gel sorbent and sample matrix extraction profiles were plotted. Figures 3.4, 3.5 and 3.6 represent the extraction profiles of chlorfenvinphos, bensulide, fenitrothion respectively, obtained by sol-gel Nb$_2$O$_5$-polyTHF, sol-gel TiO$_2$-polyTHF and sol-gel SiO$_2$-polyTHF sorbents. The onset of plateau the peak area vs extraction time plot indicates the analyte equilibrium time between the sample matrix and sol-gel sorbents. From figures 3.4, 3.5 and 3.6 we can conclude that within 30 minutes organophosphorus pesticides had reached sorption-desorption equilibrium with sol-gel Nb$_2$O$_5$-polyTHF and sol-gel TiO$_2$-polyTHF sorbents whereas with sol-gel SiO$_2$-polyTHF sorbent within 40 minutes organophosphorus pesticides had reached sorption-desorption equilibrium.

In CME-HPLC analysis of organophosphorus pesticides, peak areas were achieved RSD values less than 5%.
Figure 3.4: Extraction profiles of Chlorfenvinphos with a concentration of $2 \times 10^2 \mu g/L$ for sol-gel niobia-poly-THF sorbent, sol-gel titania-polyTHF sorbent and sol-gel silica-polyTHF sorbent. HPLC conditions: 75mm x 4.6 mm I.D C-18 Waters column, mobile phase: 75/25 CH$_3$OH/H$_2$O with 0.1% TFA in both the solvents.
Figure 3.5: Extraction profiles of Bensulide with a concentration of $2 \times 10^2 \mu g/L$ for sol-gel niobia-poly-THF sorbent, sol-gel titania-polyTHF sorbent and sol-gel silica-polyTHF sorbent. HPLC conditions: 75mm x 4.6 mm I.D C -18 Waters column, mobile phase: 75/25 CH$_3$OH/H$_2$O with 0.1% TFA in both the solvents.
Figure 3.6: Extraction profiles of Fenitrothion with a concentration of $2 \times 10^2$ µg/L for sol-gel niobia-poly-THF sorbent, sol-gel titania-polyTHF sorbent and sol-gel silica-polyTHF sorbent. HPLC conditions: 75mm x 4.6 mm I.D C-18 Waters column, mobile phase: 75/25 CH$_3$OH/H$_2$O with 0.1% TFA in both the solvents.

3.3.3 Evaluation of pH stability of the created sol-gel Nb$_2$O$_5$-polyTHF sorbent

The sol-gel Nb$_2$O$_5$-polyTHF sorbent has demonstrated excellent pH stability unlike silica based materials which are unstable under alkaline [30] and acidic [31] conditions. Sol-gel Nb$_2$O$_5$-polyTHF coated capillary was exposed to 1M HCl solution (pH ~0.0) for 18 hours (using home-made purging device) and then washed with excess water and then used for CME-HPLC analysis of organophosphorus pesticides. Obtained chromatograms (refer to figure 3.7) has demonstrated that extraction efficiency of sol-gel Nb$_2$O$_5$-polyTHF coating was not affected. Analogously sol-gel Nb$_2$O$_5$-polyTHF coating was also exposed to 0.01M NaOH (pH ~12) for 18 hrs and CME-HPLC analysis for organophosphorus pesticides was performed. Sol-gel Nb$_2$O$_5$-polyTHF coating was survived with this extreme alkaline conditions also. These pH stability results were
consistent with the pH stability studies studied on other sol-gel based transitional metal coatings [16, 22, 32].

Figure 3.7: CME-HPLC-UV analysis of organophosphorus pesticides using sol-gel Nb₂Os-polyTHF coated capillary (a) before (b) after exposure to 1.0M HCl for 18 hrs (c) after exposure to 0.01 M NaOH for 18 hrs. HPLC conditions: 75mm x 4.6 mm I.D C-18 Waters column, mobile phase: 75/25 CH₃OH/H₂O with 0.1% TFA in both the solvents, UV: 230nm; Bensulide (2.5 x 10² µg/L), Fenitrothion (3.5 x 10² µg/L) and Chlortefrinphos (2.5 x 10² µg/L).
3.3.4 Extraction of organophosphorus pesticides by sol-gel sorbents

Extraction of organophosphorus pesticides was performed by six different sorbents; (i) sol-gel inorganic Nb$_2$O$_5$ sorbent (ii) sol-gel Nb$_2$O$_5$-polyTHF sorbent (iii) sol-gel inorganic TiO$_2$ sorbent (iv) sol-gel TiO$_2$–polyTHF sorbent (v) sol-gel inorganic SiO$_2$ sorbent and (vi) sol-gel SiO$_2$–polyTHF sorbent. To compare the CME performance of created sol-gel Nb$_2$O$_5$-polyTHF with state-of-the-art titania based, using same procedure sol-gel TiO$_2$–polyTHF sorbent was created. Analogously SiO$_2$–polyTHF sorbent was created to compare the CME performance of sol-gel Nb$_2$O$_5$-polyTHF sorbent with traditional silica based sorbents.

Extraction performances of the created sol-gel sorbents can be explained by two types of interactions that exists between analyte (organophosphorus pesticide) and sol-gel sorbents, that are van der Waals interactions and Lewis acid-base interactions. Specific extraction is a specific measurement for extraction efficiency of the created sol-gel sorbents. Table 3.1 represents the CME-HPLC data of organophosphorus pesticides achieved by sol-gel inorganic sorbents. Sorbent (i) provides 70 to 80 % higher SE values for organophosphorus pesticides compared to sorbent (v). This superior extraction performance of sorbent (i) can be explained by presence of Lewis acid sites on surface of niobia (which lacks on silica surface [33]) results in Lewis acid-base interactions with the analytes. Also sorbent (i) provides 40 to 50 % higher SE values than sorbent (iii) and this can be explained by presence of bronsted acid sites [33] (which are absent on titania surface) on niobia surface along with Lewis acid sites. Figure 3.8 represents the chromatograms of organophosphorus pesticides obtained by sol-gel Nb$_2$O$_5$-polyTHF, sol-gel TiO$_2$–polyTHF and sol-gel SiO$_2$–polyTHF sorbents.
Table 3.1: Online CME-HPLC data for organophosphorus pesticides extracted by pure inorganic sol-gel sorbents (a) Nb$_2$O$_5$ (b) TiO$_2$ (c) SiO$_2$ sorbents.

<table>
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<th></th>
<th>Nb$_2$O$_5$</th>
<th>Run to Run RSD (%)</th>
<th>LOD (ng/L)</th>
<th>SE (%)</th>
<th>DE (%)</th>
<th>TiO$_2$</th>
<th>Run to Run RSD (%)</th>
<th>LOD (ng/L)</th>
<th>SE (%)</th>
<th>DE (%)</th>
<th>SiO$_2$</th>
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Table 3.2: Online CME-HPLC data of organophosphorus pesticides extracted by sol-gel organic-inorganic hybrid sorbents: (a) Nb$_2$O$_5$-polyTHF (b) TiO$_2$-polyTHF (c) SiO$_2$-polyTHF.
Figure 3.8: CME-HPLC chromatogram of aqueous samples of mixtures OPP’S by sol-gel sorbents: (a) Nb$_2$O$_5$-polyTHF (b) sol-gel TiO$_2$-polyTHF and (c) sol-gel SiO$_2$-polyTHF. HPLC Conditions: Column- Agilent eclipse XDB-C$_{18}$ (5µm, 4.6 x 150mm); Waters HPLC and UV detection at 230nm; Mobile phase: 75/25 methanol/ water with 0.1% TFA in both the solvents; HPLC peaks: 1. Fenitrothion (2 x 10$^2$ µg/L) 2. Bensulide (4 x 10$^2$ µg/L) 3. Chlorfenvinphos - 2 x 10$^2$ µg/L.

Table 3.2 represents CME-HPLC data of organophosphorus pesticides obtained by sol-gel Nb$_2$O$_5$-polyTHF, sol-gel TiO$_2$ – polyTHF and sol-gel SiO$_2$ –polyTHF sorbents. Incorporating
polyTHF in to these inorganic sol-gel sorbents in equal concentrations has provided 12 to 16 % superior SE values for organophosphorus pesticides for each of the sol-gel sorbents (sorbents (ii), (iv) and (vi)) compared to pure inorganic sol-gel sorbents (sorbent (i), (iii), and (v)). This also concludes that this 12 to 16 % superiority is due to the presence of polymer polyTHF.

Desorption efficiency (a measure of completeness desorption of extracted analytes) for niobia based sol-gel sorbents were found to be 95 to 96%. Desorption efficiency for titania and silica based sol-gel sorbents were found to be 90 and 94% respectively. The lower DE of titania based sorbents can be explained by high Lewis acidic strength [34] of Lewis acid sites on titania surface compared to niobia surface, which makes difficult for the analytes to desorb.

3.4. Conclusions

A novel niobia-based sorbent was designed to provide efficient online enrichment of organophosphorus pesticides. To compare the CME performance of created sol-gel niobia based sorbents with state-of-the-art and traditional sorbents for enrichment of organophosphorus pesticides, sol-gel titania based sol-gel silica based sorbents were created following similar procedures. Sol-gel niobia based sorbent has provided 40 to 50 % superior extraction efficiency over sol-gel titania based sorbent and 70 to 80 % superior extraction efficiency over sol-gel silica based sorbents. Excellent pH stability provided by sol-gel Nb$_2$O$_5$-polyTHF sorbent can serve as effective tool in environmental, biomedical analyses including proteomics. DE values provided by sol-gel niobia based sorbent (compared to sol-gel titania based sorbents) concludes that niobia based sorbents not only provides effective extraction efficiency but also desorption efficiency which is utmost important in sample enrichment process.
3.5 References


4. Chapter four
Sol-gel niobia sorbent with a positively charged octadecyl ligand providing enhanced enrichment of nucleotides and organophosphorus pesticides in capillary microextraction for online HPLC analysis

4.1 Introduction

One of the most widely used coating procedures in miniaturized extraction techniques is based on sol-gel chemistry [1, 2]. Advantages of the sol-gel coating technique include ability to provide direct chemical bonding to the substrate, coatings with high porous structure, thermal, chemical and solvent stability of the created sorbent coatings. These advantages of sol-gel coating techniques paved the way for its application in solid phase microextraction (SPME) [3] popularly used in a variety of analytical fields including biomedical [4], environmental [5], food [6], etc. The introduction of sol-gel coatings in SPME by Malik et al. [7] played a significant role in further expansion of the analytical scope of SPME and other microextraction techniques. Capillary microextraction (CME) [8] also known as in-tube SPME [9] is the capillary variant of SPME. The introduction of sol-gel coatings in CME by Malik et al. [8] was instrumental in overcoming a number of disadvantages encountered in traditional fiber-based SPME. The lack of direct chemical bonding of traditionally coated in-tube SPME sorbents to capillary walls is the root cause for
inadequate coating stability and has been the main hurdle to direct hyphenation of CME to liquid-phase separation techniques like HPLC. The introduction of sol-gel coatings in CME provided an effective solution to these problems. Growing applications of sol-gel CME involving enrichment of wide variety of analytes from different areas of science and industry may be attributed to the advantages arising from direct chemical bonding of sol-gel coatings to capillary walls [10-12].

OPCs are of great importance in a wide range of areas including agriculture, food, health, environment, biology, defense, etc. OPPs are widely used worldwide. However, due to their toxicity, monitoring trace levels of OPPs in food and environmental samples is critically important for the protection of human health and environment. Sample preparation is a key step in this regard. Various sample preparation techniques have been reported for the analysis of trace levels of OPPs from diverse sample matrices. These include solid-phase extraction (SPE) [13], SPME [14], liquid-liquid microextraction [15], dispersive liquid-liquid extraction [16], Liquid phase microextraction [17], solvent bar microextraction[18], stir bar sorptive extraction [19] . Silica [20] and titania [21] based sorbents are most widely used in contemporary microextraction techniques designed for enrichment of OPCs.

Sol-gel sorbents have been used for the enrichment of different OPCs including OPPs [22] and nucleotides [21]. Silica based sol-gel sorbents with crown ether ligands [22] have been reported for the enrichment of OPPs. Titania based sorbents have proved to be highly effective in the enrichment of OPCs [23, 24]. The presence of Lewis acid sites on titania provides favorable interaction with the phosphate group representing a Lewis base. Saito et al. [25] reported a titania-C_{18} sorbent for the extraction of OPCs.

Niobia, being an oxide of a group 5 metal, can be expected to provide analogous advantages in the enrichment OPCs thanks to the presence of Lewis acid sites on the surface.
Ficarro et al. [26] explored the use of niobium oxide for the extraction of phosphopeptides and reported a significant divergence in the selectivity of the Nb₂O₅ particles compared to TiO₂. Lin et al. [27] had developed niobium oxide coated magnetic nanoparticles for the enrichment of phosphopeptides. From existing literature it is quite evident that niobia-based sorbents having Lewis acid sites possess affinity for extraction of OPCs. This extraction affinity can be further enhanced by simultaneously exploiting two other types of molecular level interactions - electrostatic and van der Waals interactions. We are not aware of any study involving niobia-based sorbents where all of the above mentioned interactions have been simultaneously exploited in microextraction of OPCs.

In this research we describe a niobia-based organic-inorganic hybrid sol-gel sorbent carrying a covalently attached C₁₈ ligand with a positive charge (Nb₂O₅-C₁₈(+ve)). Such a sorbent is expected to provide enhanced extraction of OPCs by simultaneously exploiting Lewis acid-base, van der Waals, and electrostatic interactions.

4.2. Experimental

4.2.1 Materials and Methods

CME-HPLC experiments were performed using sol-gel microextraction capillaries and a Waters model 2795 HPLC system equipped with a UV/Vis detector. Coupling of CME with HPLC was achieved using a Rheodyne model 7725 six-port injection valve. A Fisher model G-560 vortex was used for thorough mixing of sol solution ingredients. A Thermo IEC Micromax microcentrifuge was used for separation of precipitate (if any) from sol solutions. An in-lab designed gas pressure-operated filling/purging device [28] was used to fill the fused silica capillary with sol solution, expel the unreacted/unused portion of sol solution from the coated capillary and also to rinse/purge the capillary with different solvents/gases. Fused silica capillary was purchased from Polymicro
Technologies. Ultrapure water (18.0 MΩ) was obtained from Maxima nanopure water system. An in-house-built sample dispenser [8] was used for online CME. PEEK tubing (1/16 inch OD and 0.005 inch ID) and Rheodyne fittings (PK, 1/16 inches) were purchased from Upchurch and to connect fused silica capillary to Rheodyne six port injection valve. An HP model 5790A GC oven was used for hydrothermal pretreatment and conditioning the sol-gel coated fused silica capillaries. Fourier transform infrared (FTIR) spectra for sol-gel niobia and titania sorbents were captured on a Spectrum Two model Perkin Elmer FTIR spectrometer. Hitachi model SU-70 scanning electron microscope (SEM) was used to generate SEM images of the sol-gel niobia coated capillaries. Trifluoroacetic acid (TFA-99% purity) was purchased from Acros. HPLC grade methanol, methylene chloride were purchased from Fisher scientific. Niobium (V) ethoxide and 60 % (wt/wt) methanolic solution of C_{18} (+ve) and n-Octadecyltrimethoxysilane (95%) was purchased from Gelest. Deoxycytidine monophosphate, guanosine monophosphate, bensulide, fenitrothion, chlorphenvinphos, sodium phosphate monobasic monohydrate, and disodium hydrogen phosphate were purchased from Sigma-Aldrich.

4.2.2 Preparation of sol solutions

Niobium pentaethoxide (50.0 µL, 1.9 x 10^{-4} moles) and TFA (18.0 µL, 2.3 x 10^{-4} moles) were taken in a microcentrifuge tube and vortexed for 5 min and allowed to sit for 10 minutes to facilitate chelation of alkoxy groups with TFA. In another microcentrifuge tube, 100.0 µL (1 x 10^{-4} moles) of C_{18} (+ve) and 20.0 µL (2.6 x 10^{-4} moles) of 98% TFA were vortexed for 5 min. Using a micropipette the later solution was transferred to the microcentrifuge tube containing chelated niobium alkoxide precursor solution, and the mixture was vortexed for 4 min. The resulting mixture was centrifuged at 14000 rpm for 4 min to remove the precipitates (if any). The supernatant was then transferred to another clean micro centrifuge tube and was used for coating.
pretreated fused silica capillary. Similarly, sol solutions for positively charged titania sorbents were prepared by using titanium isopropoxide (58.0 µL 1.9 x 10^{-4} moles) and 50% TFA (18.0 µL, 2.3 x 10^{-4} moles), 100.0 µL (1 x 10^{-4} moles) of C_{18} (+ve) and 20.0 µL (2.6 x 10^{-4} moles) of 98% TFA. The coating solution for sol-gel niobia sorbent with electrically neutral octadecyl ligand was prepared in an analogous way. In this case, niobium pentaethoxide was used as the sol-gel precursor and octadecyltrimethoxysilane was used as the co-precursor bearing C_{18} ligand. The sol solution for purely inorganic Nb_{2}O_{5} coating was prepared as follows: niobium (V) ethoxide (50.0 µL) and 99% TFA (18.0 µL) (molar ratio of 1.9:2.3) vortexed in a microcentrifuge tube for 5 minutes and centrifuged. Gelation times of prepared the sol solutions were close to 90 minutes.

### 4.2.3 Preparation of surface bonded sol-gel coatings on inner walls of fused silica capillary

An 80-cm segment of a hydrothermally pretreated [8] fused silica capillary was separately coated with each of the prepared sol solutions. To accomplish this, the capillary was filled with the sol solution using the filling/purging device under nitrogen pressure (at 10 psi). The exit end of the capillary was then sealed with a rubber septum and the sol solution was left in capillary for 30 minutes to facilitate sol-gel reactions and chemical bonding of the growing sol-gel network with silanol groups on inner walls of fused silica capillary. After this, the rubber septum was removed, the unbonded/unused sol solution was drained, and the capillary was purged under nitrogen pressure (20 psi) for 60 min. The capillary was further thermally conditioned in a GC oven under helium purge (1 mL/min) by programming the temperature from 40°C to 150°C at 0.5°C/min. The capillary was held at 150°C for 5 hours. It was then cooled to room temperature and subsequently rinsed with a mixture of methylene chloride and methanol (1:1 v/v) followed by rinse with deionized water. The capillary was then rinsed with 5 mL of methanol and purged with nitrogen for 40 min. The capillary was conditioned again in a GC oven using the same temperature
programming. The capillary was held at 150°C for 3 hours. Following the above method, five capillaries were coated with each sol solution.

4.2.4 Preparation of sol-gel niobia and titania sorbent samples for FTIR characterization.

For FTIR analysis, sol-gel sorbents were prepared in 6 mm i.d. hydrothermally treated borosilicate glass tubes and freshly prepared sol solutions under identical set of conditions as were used for coating fused silica capillaries. Thermal treatment of these coatings was also carried out under identical conditions. The created sol-gel materials were scraped out with a stainless steel spatula and used for FTIR analysis.

4.2.5 Gravimetric evaluation of coating mass, volume, and density.

The weight of a thoroughly dried sol-gel coating created on the inner walls of a fused silica capillary was determined gravimetrically. For this, initial weight of a thoroughly cleaned and dried capillary (250 µm i. d. x 2 m) was subtracted from the weight of the same piece of capillary containing a thoroughly cleaned, dried and thermally conditioned sol-gel surface-bonded coating on its inner surface. The resulting weight difference provided the weight of sol-gel coating on the 2-meter long coated capillary. By using this data, weight of the coating in a 40 cm capillary segment used for CME was calculated.

The volume of the sol-gel coating was determined as follows. The filling/purging device was placed in thermostat. A 2-meter uncoated capillary (sealed on both ends with Restek capillary column glass caps) was weighed at 17°C before and after it was filled with dichloromethane (DCM) using the filling/purging device. The 2-meter capillary was then coated with sol-gel sorbent, conditioned, and dried as described earlier and then weighed. The sol-gel coated dry capillary was then filled with DCM at 17°C as described and carefully sealed at both ends using glass caps. The weight of the DCM-filled coated capillary was taken. The volume of DCM in the
uncoated capillary and the sol-gel coated capillaries were calculated by dividing the corresponding DCM weight with the specific gravity of DCM at 17°C (1.328 g/mL [29]). The difference in the volumes of DCM obtained from these measurements gives the volume sol-gel coating created in a 2-meter capillary segment. The volume of the sol-gel coating in a 40-cm segment of the capillary used in CME was calculated from this data. The density of the coated sol-gel sorbent was determined by dividing the weight of the coated sorbent by its volume.

4.2.6 **Online coupling sample extraction and analysis by CME-HPLC.**

Online coupling of CME to HPLC was achieved as described earlier by Malik and coworkers [30]. Briefly, a Rheodyne six port injection valve was used to couple CME to HPLC by replacing the sampling loop on six port injection valve with a 40-cm sol-gel CME. Using the different positions of injection valve (load and injection positions) the aqueous samples of OPCs were extracted and then desorbed by HPLC mobile phase to the C18 Column.

4.2.7 **Characterization of CME performances via specific extraction (SE)**

To compare the microextraction performances of the created sol-gel niobia sorbents, a recently introduced parameter, specific extraction (SE), was employed. SE is defined as follows: [31].

$$SE = \frac{The \ mass \ of \ analyte \ extracted \ (\mu g)}{mass \ of \ sol \ gel \ sorbent \ (g)}$$

The mass of the sorbent was determined gravimetrically as described in section 2.5. The mass of anlayte extracted was obtained from the mean of chromatographic peak areas from 3 replicate extractions and a calibration plot constructed by direct injections of analyte standard solutions.

4.2.8 **Determination of Desorption efficiency (DE %)**

Desorption efficiency (DE) % [31] was determined to evaluate the completeness of desorption of
the extracted analytes from sol-gel CME sorbent. Each analyte was injected into the HPLC column by using a 40-cm piece of deactivated fused silica capillary as the external sampling loop. Using calibration plots, the obtained peak area was converted into corresponding analyte mass extracted. Each sample containing 250 ng of analyte was passed through coated capillary for 30 minutes to attain extraction equilibrium and the liquid exiting from the capillary was collected. Mass of analyte in the exited liquid was calculated by performing a direct injection of this collected liquid into a HPLC system. Amount of the analyte extracted was calculated by subtracting the amount of analyte in the exited liquid from the original amount of analyte in sample volume that passed through the coated capillary for extraction. Desorbed amount of the analyte was obtained from the HPLC peak area obtained through desorption of the extracted analyte using the HPLC mobile phase. DE was calculated using the following equation [31]:

\[
Desorption\,\,efficiency\,\,\,(\%) = \frac{Amount\,\,desorbed}{Amount\,\,extracted} \times 100
\]

4.3 Results and Discussion

Sol-gel technology is widely used to prepare SPME fibers and CME capillaries for diverse applications [32, 33] because of high thermal and chemical stabilities of the created sorbents. A wide variety of surface bonded sol-gel coatings were developed by Malik et al. [34, 35] for SPME and CME.

Malik et al. [36] had used silica-based sorbent with C\textsubscript{18} (+ve) for enrichment of amino acids in capillary electrophoresis exploiting electrostatic interactions. In the present work, sol-gel chemistry allowed us to create a sorbent effectively integrating the electrostatic interaction, van der Waals interactions and Lewis acid-base interactions between the sorbent components and OPCs.
4.3.1 Synthesis of sol-gel sorbents

In this work, we developed sol-gel niobia sorbents using the hydrolytic sol-gel approach. Figure 4.1 represents the hydrolysis of sol-gel niobium pentaethoxide precursor and C\textsubscript{18} (+ve) (co-precursor) and condensation of sol-gel-active chemical species in sol solution. The patches of sol-gel network growing in the vicinity of capillary walls are in a position to form chemical bonds with the surface silanol groups residing on capillary inner walls, leading to the formation of a positively charged surface-bonded sol-gel niobia coating.
Hydrolysis of niobia precursor and organic C_{18} ligand

\[
\text{H}_3\text{C(H}_2\text{C)}_{17}-\text{N}-(\text{H}_2\text{C})_3\text{Si}-\text{OCH}_3 + n\text{H}_2\text{O} \\
\text{H}_3\text{C(H}_2\text{C)}_{17}-\text{N}-(\text{H}_2\text{C})_3\text{Si} + n\text{H}_2\text{O}
\]

Polycondensation between hydrolyzed Niobia and Organic C_{18} ligand

\[
\text{H}_3\text{C(H}_2\text{C)}_{17}-\text{N}-(\text{H}_2\text{C})_3\text{Si} + \left[C_2\text{H}_5\text{O} \right]_{5-n}\text{Nb} \left[\text{OH} \right]_n + n\text{H}_2\text{O}
\]

Figure 4.1: Schematic representation of hydrolysis and polycondensation of niobium pentaethoxide and organic ligand in sol-gel process

Transition metal alkoxide-based precursors are characterized by extremely high rates of sol-gel reactions that lead to instantaneous precipitation of the resulting products [37], making it difficult to control the reactions. To overcome this difficulty carboxylic acids or diketones are used as chelating agents to slow down the hydrolysis reaction. In this research we used TFA as a
chelating agent. In the presence of a chelating agent, hydrolysis reaction rate of a metal alkoxide can be reduced to a desired level providing a tool for reaction rate control for the desired synthesis. In this work, chelation ratio $x$ (chelator (TFA) to precursor (niobium pentaethoxide) molar ratio) was maintained at $\sim$1. This is consistent with the findings of Livage et al [37] who established that $1 \leq x < 2$ can provide a moderate to slow metal alkoxide reactivity in hydrolysis. Any value of $x$ more than 2 can lead to excessive chelation, hindering the hydrolysis of transition metal alkoxides precursors.

Sol-gel coated capillaries were thermally conditioned in a GC oven for accelerated completion of condensation reactions within the sol-gel coating. Conditioning under continuous flow of nitrogen helped in drying of sol-gel coating. Low temperature programming rates ($\sim$ 0.5°C/min) generated less stress on sol-gel porous structure during evaporation of solvents. The use of a moderate upper conditioning temperature (150°C) was conducive to the creation of an amorphous structure with minimal crystalline regions [38].

### 4.3.2 Characterization of sol-gel sorbents

Figure 3.2 represents FTIR spectra of (a) Nb$_2$O$_5$-C$_{18}$ (+ve) and (b) TiO$_2$-C$_{18}$ (+ve) sorbents. In Figure 4.2(a), the peak at 951 cm$^{-1}$ can be attributed to Nb-O-Si bonds in the niobia-silica sol-gel network formed through condensation with the hydrolyzed C$_{18}$ (+ve)[39]. Peak at 575 cm$^{-1}$ can be attributed to Nb-O stretch [40] from sol-gel niobia network. From Figure 4.2(b), the peak at 915 cm$^{-1}$ [41] can be attributed to Ti-O-Si bonds in the titania-silica hybrid sol-gel network formed in an analogous way.

Figure 4.3 shows an SEM image of surface bonded Nb$_2$O$_5$-C$_{18}$ (+ve) coating on the wall of a CME capillary. The coating thickness was estimated $\sim$ 2.2 µm. By using this data, volume and
density of coating in 40 cm capillary was calculated.

Figure 4.2: FTIR spectrum obtained on a samples of sol-gel sorbents: (a) Nb$_2$O$_5$-C$_{18}$ (+ve) ligand and (b) sol-gel TiO$_2$-C$_{18}$ (+ve) ligand.
Figure 4.3: SEM image showing the cross sectional view of a fused silica capillary with a positively charged sol-gel niobia coating bonded to the capillary inner surface.

The gravimetric analysis of the Nb$_2$O$_5$-C$_{18}$ (+ve) sorbent revealed that the volume of the coating in a 40-cm capillary segment (used in this work for CME) was ~0.85 µL. This volume calculated by gravimetric analysis was comparable to the volume of the coating (0.9 µL) calculated from coating thickness obtained by SEM. (Figure 3.3). Through gravimetric analysis, volumes of Nb$_2$O$_5$-C$_{18}$ (+ve) and Nb$_2$O$_5$-C$_{18}$ sorbents (each in 40 cm coated fused silica capillary) were estimated at 0.6 µL and 0.72 µL respectively. Densities of created niobia-based sol-gel sorbents were calculated using the above volumes and coating masses (in 40-cm capillary: Nb$_2$O$_5$-C$_{18}$ (+ve) sorbent ~ 2.1 mg, Nb$_2$O$_5$-C$_{18}$ sorbent ~ 3.2 mg, inorganic-Nb$_2$O$_5$ ~ 3.4 mg). Densities of the created niobia based sol-gel sorbents were found to be 3.8 gm/cm$^3$ for Nb$_2$O$_5$-C$_{18}$ (+ve), 3.5 gm/cm$^3$ for...
Nb$_2$O$_5$-C$_{18}$, and 4.8 gm/cm$^3$ for Nb$_2$O$_5$, respectively. The calculated densities were found to be comparable with the density of niobium pentoxide materials studied earlier which was in a range of 4.6 to 5.3 gm/cm$^3$ [42]. Lower densities of Nb$_2$O$_5$–C$_{18}$ (+ve) and Nb$_2$O$_5$–C$_{18}$ sorbents compared to the literature values can be attributed to the presence of organic ligands as well as to the porosity of the compared materials. To our knowledge this is the first report on the densities of such CME sorbents.

### 4.3.3 Evaluation of microextraction characteristics of sol-gel niobia sorbents in CME.

For evaluation of the time taken to reach analyte sorption-desorption equilibrium between sol-gel sorbent coating and sample matrix, extraction profiles (Figure 4) were constructed using CME-HPLC data. The onset of the plateau the peak area vs. extraction time plot corresponds to the analyte equilibrium time between the sample matrix and the Nb$_2$O$_5$-C$_{18}$ (+ve) sorbent. As can be seen in Figure 4.4, OPP’S and nucleotides had reached equilibrium within 30 minutes.
Figure 4.4: Extraction profiles OPCs: (a) organophosphorus pesticides (aqueous samples with 200 ng/L were used); (b) Nucleotides (aqueous samples with 200 ng/L were used) using positively charged sol-gel niobia with covalently bonded Octadecyl C$_{18}$ ligand sorbent with similar chromatographic conditions as in Figures 5 and 6.

In CME-HPLC analysis of OPCs, peak area were achieved RSD values less than 5 %. To evaluate the developed CME method, 5 capillaries were coated (Nb$_2$O$_5$-C$_{18}$ (+-ve)) analogously and extractions performances for OPCs were evaluated. Peak area RSD values were less than 5 % (capillary to capillary reproducibility.
4.3.4 Extraction of organophosphorus pesticides and nucleotides using niobia based sol-gel sorbents in CME

Extraction of pesticides and nucleotides were performed using four different sol-gel sorbents; (i) $\text{Nb}_2\text{O}_5$ (ii) $\text{Nb}_2\text{O}_5 - \text{C}_{18}$, (iii) $\text{Nb}_2\text{O}_5 + \text{C}_{18}$ (+ve) and (iv) $\text{TiO}_2 - \text{C}_{18}$ (+ve). These analytes contain phosphate group carrying a negative charge. It can be assumed that enhanced extraction of such analytes can be achieved by using a sorbent that contains Lewis acid sites as well as a positively charged moiety. Extraction of these OPCs can be further enhanced through exploitation of van der Waals interactions of these analytes with the sorbents. To that end, we have designed the $\text{Nb}_2\text{O}_5 - \text{C}_{18}$ (+ve) sorbent capable of providing all three of the above-mentioned interactions: (a) electrostatic interactions of the analyte phosphate groups with the positive charge on the sorbent, (b) Lewis acid-base interaction between analyte phosphate group (Lewis base) with the Lewis acid sites on niobia and (c) van der Waals interactions between various moieties the sorbent and the analyte. The SE [31] values presented in Table 4.1 show that coating (iii) has clearly provided 50 to 60 % higher compared to coating (i). This enhanced extraction performance of sorbent (iii) compared to sorbent (i) is due to the ability of sorbent (iii) to provide two additional types of molecular level interactions, (a) electrostatic interactions between the positive charge on the sorbent and the phosphate group on the OPCs and (b) molecular level interactions provided by the bonded ligand) that are unavailable in extraction with sorbent (i). The extraction performance of sorbent (ii) is primarily governed by van der Waals interactions along with Lewis acid-base interactions. Table 4.1 also shows that sorbent (iii) provides 20 to 40 % higher SE values for OPCs compared with sorbent (ii). This difference in SE can be attributed to the electrostatic interactions between the positive charge on sorbent (iii) and negative charge on the phosphate group on organophosphate analytes. The lack of electric charge in sorbent (ii) precludes its ability to provide
such electrostatic interactions with OPCs. Both sorbent (i) and (iii) are capable of providing Lewis acid-base interactions.

To compare CME performance of the \( \text{Nb}_2\text{O}_5-\text{C}_{18} (+\text{ve}) \) sorbent to that of the state of the art titania based sorbent, we used the same sol-gel procedure to create a \( \text{TiO}_2-\text{C}_{18} (+\text{ve}) \) sorbent for CME. As it is evident from Table 3.1 SE values for nucleotides and OPPs obtained on \( \text{Nb}_2\text{O}_5-\text{C}_{18} (+\text{ve}) \) were a 40-50% higher compared to SE values obtained on its titania-based counterpart. This superior extraction performance of sol-gel niobia sorbent can be attributed to the surface characteristics of niobium (V) oxide providing bronsted acid sites along with Lewis acid sites, which were spectroscopically undiscernible on titania surface as was established by Tamura \textit{et al.} [43]. Figures 4.5 and 4.6, represent the chromatograms of OPPs and nucleotides obtained by CME-HPLC on \( \text{Nb}_2\text{O}_5.\text{C}_{18} (+\text{ve}) \) and \( \text{TiO}_2.\text{C}_{18} (+\text{ve}) \) sorbents, and their corresponding SE values are presented in Table 4.1.
Figure 4.5: CME-HPLC chromatogram of aqueous samples of mixtures OPP’S by sol-gel sorbents: (a) Nb$_2$O$_5$-C$_{18}$ (+ve) ligand and (b) sol-gel TiO$_2$-C$_{18}$ (+ve) ligand. Conditions: Column- Agilent eclipse XDB-C$_{18}$ (5µm, 4.6 x 150mm); Waters HPLC and UV detection at 230nm; Mobile phase: 75/25 methanol/ water with 0.1% TFA in both the solvents; HPLC peaks: 1. Fenitrothion (2 x 10$^2$ µg/L) 2. Bensulide (4 x 10$^2$ µg/L) 3. Chlorfenvinphos - 2 x 10$^2$ µg/L.
Figure 4.6: CME-HPLC chromatogram of aqueous samples of mixtures nucleotides by sol-gel sorbents: (a) Nb$_2$O$_5$-C$_{18}$ (+ve) ligand and (b) sol-gel TiO$_2$-C$_{18}$ (+ve) ligand. Conditions: Column- Agilent eclipse XDB-C$_{18}$ (5µm, 4.6 x 150mm); Waters HPLC and UV detection at 254nm; Mobile phase: 95% 20mM phosphate buffer (pH 6) and 5% ethanol; HPLC peaks: 1. Deoxycytidine monophosphate (2 x 10$^2$ µg/L) 2. Guanosine Mono phosphate (2 x 10$^2$ µg/L);
Table 4.1: Online CME-HPLC data for nucleotides and pesticides extracted on four different sol-gel sorbents: (a) sol-gel niobia sorbent, (b) electrically neutral sol-gel niobia sorbent carrying a C₁₈ ligand, (C) sol-gel niobia sorbent carrying positively charged C₁₈ ligand, and (d) sol-gel titania sorbent carrying positively charged C₁₈ ligand.

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<th>Nb₂O₅-C₁₈</th>
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<th>TiO₂-C₁₈ (+ve)</th>
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Desorption efficiency for all three niobia-based sol-gel sorbents was found to be in between 94-96% (including Nb$_2$O$_5$-C$_{18}$ (+ve)). Desorption efficiency of TiO$_2$-C$_{18}$ (+ve) was found to be ~90%. This higher desorption efficiency provided by Nb$_2$O$_5$-C$_{18}$ (+ve) compared to TiO$_2$-C$_{18}$ (+ve) can be attributed to the higher strength of surface acidity on titania compared to niobia, which makes it more difficult to desorb extracted OPCs from TiO$_2$-C$_{18}$ (+ve) coating [44]. Limit of detection was in ng/l concentration for all analytes.

### 4.4 Conclusion

A niobia-based sorbent was designed for CME to provide efficient online enrichment of organophosphate analytes by simultaneously exploiting three different types’ molecular level interactions: Lewis acid-base-, van der Waals-, and electrostatic interactions. Sol-gel chemistry was effectively utilized to synthesize such a sorbent with the following structural and functional features: (a) Nb$_2$O$_5$ – an inorganic component with Lewis acid sites capable of providing Lewis acid-base interactions with the analyte phosphate group, a Lewis base; (b) an octadecyl ligand capable of providing various types of van der Waals interactions with OPCs; and (c) a positively charged quaternary amine group which is capable of undergoing electrostatic interaction with the negatively charged phosphate groups on organophosphate analytes. Since titania-based sorbents are recognized as the state-of-the-art extraction media for OPCs, the CME performance of the newly developed sol-gel niobia sorbent (Nb$_2$O$_5$.C$_{18}$ (+ve)) was compared with that of an analogously synthesized titania-based sorbent with the same structural characteristics (TiO$_2$.C$_{18}$ (+ve)). Online CME-HPLC analysis of OPCs using these sorbents revealed that Nb$_2$O$_5$.C$_{18}$ (+ve) provided 40-50% higher extraction efficiency ((SE) over (TiO$_2$.C$_{18}$ (+ve)). This enhanced extraction capability of Nb$_2$O$_5$.C$_{18}$ (+ve) for OPCs may be accounted for by the presence of both Lewis- and Bronsted acid sites on this niobia-based sorbent but essential lack of Bronsted acid
sites on the titania-based sorbent. During online CME-HPLC analysis of OPPs and nucleotides, Nb$_2$O$_5$C$_{18}$ (+ve) also provided superior desorption efficiency over (TiO$_2$C$_{18}$ (+ve)): 96% vs. 90%. This can be explained by the fact that titania presents a stronger Lewis acid than niobia, making it easier for the HPLC mobile phase to desorb organophosphate analytes (Lewis bases) from the weaker Lewis acid sites present on the niobia-based sorbent.

4.5 References


Appendix

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