DEVELOPMENT OF SOL-GEL IMMOBILIZED SORBENT FOR CAPILLARY MICROEXTRACTION

BY

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A Dissertation Presented to the

DEANSHIP OF GRADUATE STUDIES

KING FAHD UNIVERSITY OF PETROLEUM & MINERALS

DHAHRAN, SAUDI ARABIA

In Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

CHEMISTRY

April, 2018

KING FAHD UNIVERSITY OF PETROLEUM & MINERALS

DHAHRAN- 31261, SAUDI ARABIA

DEANSHIP OF GRADUATE STUDIES

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Dedicated to my beloved father Prof. Ch. Muhammad Khalid (Late) who is the only motivation for pursuing Ph.D.

Dedicated to my beloved family for the prayers and support

ACKNOWLEDGMENTS

All praises and thanks to Allah Azwajjal, Who directed me towards the best possibilities and blessed me the courage to complete the tasks. All respect and honour for Prophet Muhammad (Peace be Upon Him) who empowered the mankind to know the creator and to comprehend the way of life.

I am thankful to King Fahd University of Petroleum and Minerals for not only providing me scholarship for my Ph.D. studies but also giving me a chance to visit the most respectful places i.e., Haramain. I would like to extend my gratitude towards the whole administration of KFUPM that all of them have provided their best expertise whenever asked for. From the bottom of my heart, I acknowledge the principled guidance and expert advice of chairman, Department of Chemistry. I would gladly present my sincere gratitude to my esteemed, learned, courteous and valued research supervisor Dr. Khalid Alhooshani, who trusted in my abilities and always supported me through all the bad or good patches in research and personal life. He did his scientific contributions for this work and pointed out the scholarly merits and demerits of this work. Furthermore, thanks to my Co-advisor Dr. Abdallah Abulkibash and Ph.D. committee members Dr. Anvarhusein A. Isab, Dr. Basheer Chanbasha and Dr. Asrof Ali for their extended recommendations whenever needed. I would admire the world-class respected faculty members and lab fellow including Abdulkadir Tanimu and Saheed Ganiyu Adewale who always remain available for all sort of support.

Finally, I emotionally present my appreciations to the individuals who mean a lot to me, my Mother and Father for their love, care and sacrifice. I would never be able to pay back the love and kindness lavished upon by my parents. It's my fortune to appreciatively acknowledge the support of my brothers, sisters, nephews and nieces for their continued love, support, and understanding during my pursuit of Ph.D. degree. I recognize my daughter and wife with gratitude for showing forbearance during my research work and enduring my ignorance. I believe myself the luckiest individual in the world to have such an adorable and caring family with unconditional support.

SHEHZADA MUHAMMAD SAJID JILLANI

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LIST OF ABBREVIATIONS

CME	:	Capillary Microextraction
HPLC	:	High performance liquid chromatography
GC	:	Gas chromatography
RSD	:	Relative standard deviation
LOD	:	Limit of detection
LOQ	:	Limit of quantification
UV	:	ultra violet
DAD	:	Diode array detector
BHEA	:	[bis(hydroxyethyl)amine] terminated polydimethylsiloxane
BPU	:	bis(trimethoxysilylpropyl) urea
XPS	:	X-ray photoelectron spectroscopy
FE-SEM	:	Field emission scanning electron microscope
SE	:	Secondary electron
BSE	:	Back scattered electron
EDS	:	Energy dispersive X-ray spectrometer
ID	:	Internal diameter
TFA	:	Trifluoroacetic acid

BEs	:	Binding energies
PDMS	:	Polydimethylsiloxanes
BHEA-Y	:	[bis(hydroxyethyl)amine] terminated polydimethylsiloxane - Yttria
PAHs	:	Polyaromatic hydrocarbons
YMEO	:	Yttrium methoxyethoxide
C to C	:	Capillary to capillary
4-CN	:	4-chloro-1-naphthol
PDMS/Ge	:	Polydimethylsiloxanes/germania
SBSE	:	Stir-bar sorptive extraction
SPME	:	Solid phase microextraction
OH-PDMS	:	Hydroxy-terminated polydimethylsiloxane
TMOG	:	Tetramethoxygermane
ACN	:	Acetonitrile

ABSTRACT

Full Name : [Shehzada Muhammad Sajid Jillani]

Thesis Title : [DEVELOPMENT OF SOL-GEL IMMOBILIZED SORBENT FOR CAPILLARY MICROEXTRACTION]

Major Field : [Chemistry]

Date of Degree : [April 2018]

This work is to develop sol-gel based sorbent materials for capillary microextraction. Three sorbent materials were synthesized including urea functionalized-[bis(hydroxyethyl)amine] terminated polydimethylsiloxane (BHEA-BPU), yttria surfaced-[Bis (hydroxyethyl) amine] terminated polydimethylsiloxanes (BHEA-Y), and germania-based polydimethylsiloxane (PDMS/Ge). The former two coatings were used for capillary microextraction and germania based coating used for stir-bar sorptive extraction. The characterization all the materials was successfully done by using X-ray photoelectron spectroscopy, thermogravimetric analysis, field emission scanning electron microscope, and energy dispersive X-ray spectrometer. BHEA-BPU and BHEA-Y coated capillaries were used for online extraction and high-performance liquid chromatographic analysis of amides, phenols, alcohols, ketones, aldehydes, and polyaromatic hydrocarbons. The PDMS/Ge based coating on the stir bar presented high preconcentration for the detection of 4-chloro-1-naphthol. All the extraction devices have proved their excellent potential by providing reliable, reproducible, and sensitive data in-terms of %RSD, LODs, and linearity. Moreover, real sample analysis is provided along-with extraction recovery and reproducibility. BHEA-BPU coating showed excellent overall sensitivity in terms of lower detection limits (S/N = 3) for the analytes (0.10 ng mL^{-1} to 14.29 ng mL^{-1}) with acceptable

reproducibility that is less than 12.0 %RSD (n = 3). Moreover, the capillary to capillary reproducibility of the analysis was also tested by changing the capillary of the same size. This provided excellent %RSD of less than 10.0 % (n = 3). BHEA-Y coating produced detection limits ranging from 0.18 ng mL^{-1} to 7.35 ng mL^{-1} (S/N = 3) with relative standard deviation (RSD) between 0.6% to 6.8% (n=3). The capillary preparation and coating method reproducibility was also considered by capillary to capillary extraction analysis with acceptable RSD between 4.1% to 9.9%. The PDMS/Ge coated stir bars showed a good preparation reproducibility of 1.7% (n=3) for one batch and 3.5% (n=3) for different batches. Under optimized experimental conditions, the method showed linearity in the range of 0.4-800 ng mL^{-1} with R^2 = 0.9992 and limit of detection (S/N=3) as 0.034 ng mL^{-1}. 4-chloro-1-naphthol was also extracted from wastewater, pool water, and human urine and showed relative recoveries between 87.1 - 97.2% with acceptable relative standard deviation i.e. 4-11%.

ملخص الرسالة

الاسم الكامل: شهزاده محمد ساجد جيلاني

عنوان الرسالة: تطوير مواد ماصّة غير متنقلة بتقنية SOL-GEL للاستخلاص الميكروي الشعري

التخصص: كيمياء

تاريخ الدرجة العلمية: أبريل ٢٠١٨

هذا العمل يهدف لتطوير مواد ماصّة بتقنية SOL-GEL للاستخلاص الميكروي الشعري. تم تصنيع ثلاثة مركبات مختلفة لهذا الغرض وتتضمن:

1) Urea functionalized-[bis(hydroxyethyl)amine] terminated polydimethylsiloxane (BHEA-BPU).
2) Yttria surfaced-[Bis (hydroxyethyl) amine] terminated polydimethylsiloxanes (BHEA-Y).
3) Germania-based polydimethylsiloxane (PDMS/Ge).

تم استعمال المركبين الأول والثاني كطلاء في الاستخلاص الميكروي الشعري، فيما تم استخدام المركب الثالث في الاستخلاص بتقنية Stir-bar Sorptive Extraction. خصائص جميع المواد تم تحديدها باستعمال:

1) X-ray photoelectron spectroscopy.
2) Thermogravimetric analysis.
3) Field emission scanning electron microscope.
4) Energy dispersive X-ray spectrometer.

تم استعمال BHEA-BPU and BHEA-Y coated capillaries في online extraction وhigh-performance liquid chromatographic analysis للأميدات، الفينولات، الكحول، الكيتونات، الألدهيدات، والهيدروكربونات الأروماتية المتعددة.

أظهر PDMS/Ge based coating تركيز مسبق عالي في Stir bar عند الكشف عن 4-chloro-1-naphthol. أثبتت جميع أجهزة الاستخراج إمكانياتها الممتازة من خلال توفير بيانات موثوقة، قابلة للتكرار، ودقيقة من حيث معامل الاختلاف، حدود الكشف، والعلاقة الخطية. بالإضافة لذلك، يتم توفير تحليل العينات الحقيقية مع استعادة المستخلصات وإعادة التكرار. أظهرت BHEA-BPU coating حساسية عامة ممتازة بالنسبة لمعدلات الكشف المنخفضة (S/N = 3) للحلائل ($0.10\ ng\ mL^{-1}$ to $14.29\ ng\ mL^{-1}$) مع قابلية تكرار مقبولة بمعامل اختلاف أقل من 12.0% (n = 3).

كذلك، تم أيضًا اختبار The capillary to capillary reproducibility للتحاليل عن طريق تغيير الحجم. وقد أظهر ذلك معامل اختلاف ممتاز أقل من 10.0% (n = 3).

وبينت BHEA-Y coating حدود كشف تتراوح من 0.18 ng mL^{-1} لـ 7.35 ng mL^{-1} (S/N = 3) مع معامل اختلاف بين 0.6% لـ 6.8% (n = 3).

وقد تم الأخذ بالحسبان قابلية تكرار عملية إعداد وطلاء الـ Capillary باستخدام تحليل Capillary to capillary extraction مع معامل اختلاف مقبول بين 4.1% لـ 9.9%. وأبدت PDMS/Ge coated stir bars قابلية تكرار جيدة بـ 1.7% (n = 3) لدفعة واحدة و3.5% (n = 3) لدفعات مختلفة. تحت الظروف التجريبية المحسنة، أظهرت المنهجية علاقة خطية في نطاق 0.4-800 ng mL^{-1} و R^2 = 0.9992 وحد كشف (S/N=3) بمقدار 0.034 ng mL^{-1}.

تم استخراج 4-chloro-1-naphthol من مياه الصرف الصحي، مياه المسابح، والبول البشري وأظهرت استردادات نسبية بين 97.2% - 87.1 مع معامل اختلاف مقبول بـ 11%-4.

CHAPTER 1

INTRODUCTION

1.1. Sample preparation:

One complete analysis for a certain analyte in complex matrix may have many steps and the sample preparation is one important step. All the steps are critical and should be related to have reliable and reproducible results. The sample preparation step has some objectives and their importance. Since, it is a general issue of most of the analytical instruments that real samples cannot be injected or analyzed directly, therefore, one of the objective for sample preparation is to isolate the analyte of interest from the complex matrix. Moreover, the sensitivity of the most analytical instruments has limitations, therefore, the sample preparation helps in preconcentration of the analyte and the expected sensitivity may be achieved. Sample preparation may have done by extraction procedures following the clean-up steps to avoid the dirty and un-explored nature of the real sample. There is a need of all the analysis steps to be error free and automated. However, ideally it is considered that those analysis are more reliable that can reduce human involvement and help in onsite investigation. Therefore, miniaturization has taken place in extraction devices that further leads towards onsite analysis and combining the sampling and sample preparation method. There are many extraction methods, involving multi-steps and time consumption and taking 80 % of the analysis time but the trend is now to avoid these shortcomings [1].

1.2. Extraction techniques:

Extraction techniques may be classified in many ways e.g., fundamental principle, state of the extracting phase and sample introduction method. Fundamentally, extraction could be exhaustive or non-exhaustive, where for the exhaustive removal, the sample introduction may be in the form of batch or a continuous flow [2]. However, continuous flow is preferred over batch for being efficient mass transfer [3]. On the other hand, the non-exhaustive approach is based in equilibrium and pre-equilibrium-based technique. Pre-equilibrium-based non-exhaustive technique is the one where the sample matrix is not passed through the sorbent until it achieves equilibrium [4], this happens where the acceptable levels of sensitivity and reproducibility are not required. However, the equilibrium based non-exhaustive technique is one where small amount of extraction phase is used as compared to sample volume. Solvent microextraction [5], solid phase microextraction (SPME) [6] and gaseous headspace techniques [7] are the example related to this category of extraction.

It was a real milestone in sample preparation when SPME was introduced for the first time in 1987 [8]. This technique has reduced the use of organic solvents along-with miniaturization and automation for sample preparation step. The success of this technique has brought a real shift in sample preparation procedures as previously exhaustive mechanism techniques were dominating. There is no use of hazardous organic solvents, therefore, the SPME and its various types became important for the environment. This technique combined sampling, extraction and enrichment of the analyte of interest. These advantages made SPME famous and it was very much used with various instruments including high-performance liquid chromatography (HPLC) [9,10], capillary

electrophoresis [11], supercritical fluid chromatography[12], inductively coupled plasma with mass spectrometry [13] and optical emission spectrometry [14].

Later, many formats of SPME were developed and emphasized for their own uniqueness and attributes [15–17]. Fig 1.1. presents the various types of SPME including fiber SPME, Thin-film SPME, Stir-bar sorptive extraction and in-tube SPME etc.

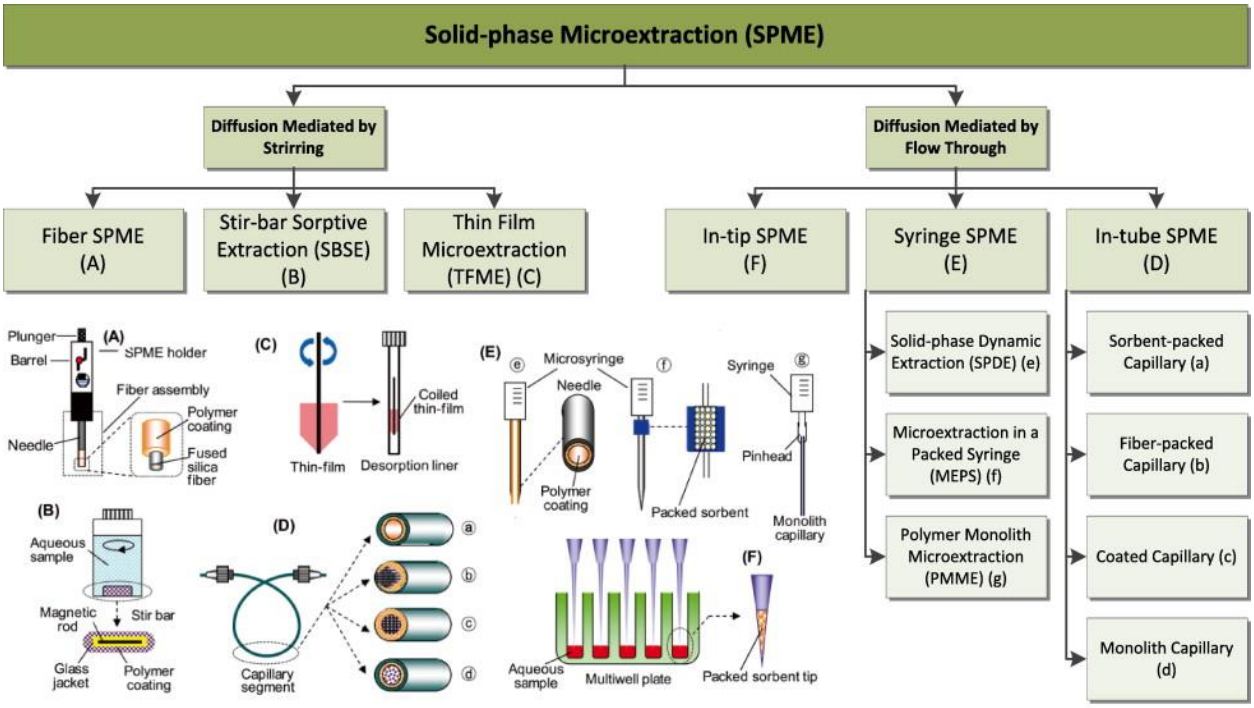


Fig 1. 1. Types of SPME and the graphical presentation taken from [17].

1.3. Stir-bar sorptive extraction (SBSE):

SBSE is a solvent free sample preparation method that is based in sorptive extraction. The sorptive extraction is basically to extract the analyte from its matrix into an immiscible liquid phase [18]. The famous sorptive extracting phase is a polymer called polydimethylsiloxane (PDMS), which is commercially available. Stability of the coating material is very important and PDMS is already employed as coating in gas chromatography[19,20]. After the introduction of SBSE in 1999 [21], it proved an improved and better sample preparation technique. These stir bars may have less than 1 mm thick coating. This technique progressed very well and being applied for various analytes including organochlorine pesticides [22], volatile aromatic [23,24], polyaromatic hydrocarbons [25–32], polychlorinated biphenyl [33], halogenated solvents[23,24], pyrethroid pesticides[34], chlorophenols [35–37], estrogens [38], organotin compounds [39], odorous compounds [40,41] and alkylphenols[25,39,42,43]. SBSE has not only shown its applicability for environmental pollutants but also proved a reliable technique for the extraction of target analytes related to biomedical applications. Various analytes have been extracted from biological samples including fatty acids, phenols, nicotine, terpenes, steroids, drugs of abuse, sesquiterpenes [44], prescription drugs [45], caffeine [46], 1-hydroxypyrene [44,47], phenolic xenoestrogens [37,48–51], benzodiapenes and barbiturates [52], metabolites of caffeine [53], PCBs [54], tuberclosteric acid [55] and di(2-ethylhexyl)phthalate [44].

Typically, SBSE procedure is to add the coated stir-bar inside the appropriate amount of sample containing the target analyte either for direct immersion and headspace as shown in Fig 1.2. It is then stirred for the extraction time that is decided by parameters such as

sample volume, stirring speed and stir-bar size. Optimization of these parameters lead to optimize the extraction time. The optimization of the parameters is selected by achieving the equilibrium conditions, where any further change in that parameters may not result in any increase to the response. The stir-bar is taken out of the sample, dried on a lint free tissue paper, and introduced to the desorption system. Various types of desorption procedure are used including thermal desorption and liquid desorption.

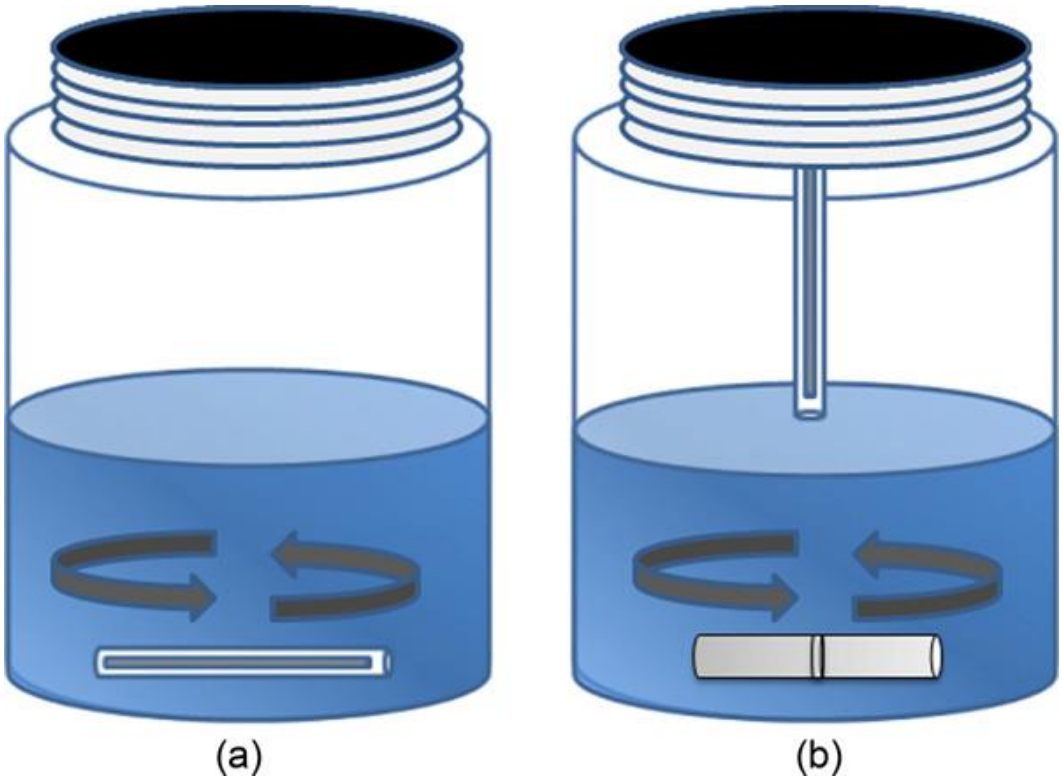


Fig 1. 2. Extraction for SBSE (a) direct immersion, and (b) headspace. (taken from[56])

1.4. Capillary Microextraction (CME) or In-tube SPME:

After the introduction of SPME [57] [58], the fiber SPME was the first one that was developed and got recognition. Several limitations of fiber SPME lead to find more ways that help to promote the similar concept of microextraction. Capillary microextraction is one of the technique that developed along-with other developing techniques including in-needle, in-tip, stir-bar sorptive extraction. The capillary microextraction brought many advantages like easy to hyphenate, less parameters to optimize, time effective, and online hyphenation with most of the instruments enable it to reduce the chances of analyte during various parameters. As shown in Fig 1.3., there are three different types of capillaries used for extraction i.e., open tubular, packed capillary and monolithic capillary. The open tubular capillary is the one where sorbent is wall coated inside the capillary [59]. For this gaseous or liquid sample containing the analytes may be used for extraction and thermal desorption in gas chromatographic inlet. Many volatile organic compounds and polyaromatic hydrocarbons were extracted and analyzed using GC detector [60]. CME is also referred as in-tube SPME and sample solution may be passed through open capillary for extraction and later solvent desorption takes place while it is coupled to high performance liquid chromatography (HPLC) [9]. The online hyphenation of the capillary to the manual injection port of the HPLC is also shown in Fig 1.4.

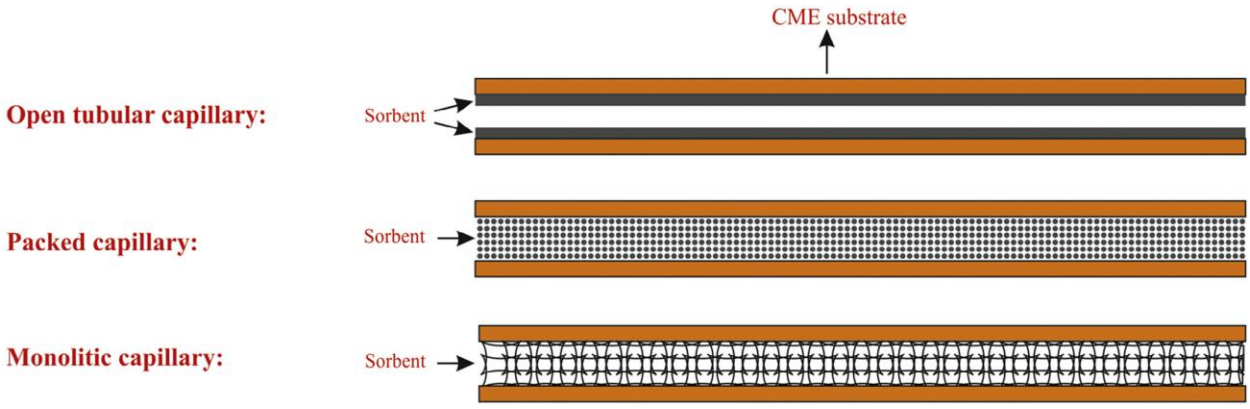


Fig 1. 3. Type of capillaries used in capillary microextraction. (taken from [61])

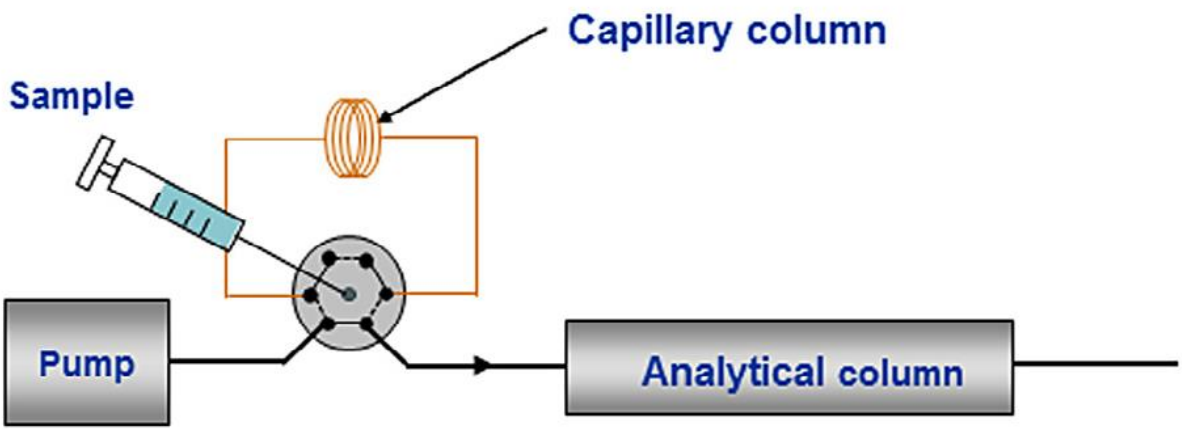


Fig 1. 4. Online hyphenation of capillary the manual injector of HPLC. (taken from [62])

Various types of coatings have been prepared based on the material and methodology. However, coatings may be classified as conductive polymeric coatings, sol-gel based coating, monolithic coatings, xerogels based coatings, and molecularly imprinted coatings for CME application. The major focus of the titled work is to develop the sol-gel based coating for microextraction.

1.5. Sol-gel chemistry and its applications

There is a common method in the inorganic chemistry of polymerization to synthesize oxides and avoid harsh reaction conditions. Sol-gel chemistry involves the introduction of thermally labile groups in the polymeric network. Those groups may include biological, bioorganic, macromolecules, organic and coordination compounds. The most useful, stable, and cheap reagent known is the silicate precursor in the sol-gel chemistry. The sol-gel active precursor usually in the presence of water and catalyst, encloses the solvent and form a layer of its material that may stick to the wall. This type of material undergoes many states like solution, colloid and solid [63]. Generally, it could be stated that a precursor undergoes hydrolysis and condensation reactions to achieve gel as shown in Fig 1.5.

There were many shortcomings in the conventional CME including low sample capacity, and lower sensitivity. However, the utilization of sol-gel chemistry in CME [64] has resolved the stated problems. Sol-gel reactions not only help to have the reasonable size of coating to enhance sample capacity and sensitivity but also provide high sensitivities. The sol-gel has also provided a chemical linkage between the polymeric network and the capillary wall and overcame the issues like solvent and thermal stability of coated material in conventional CME when hyphenated to GC of HPLC. While hyphenating to HPLC there is a constant flow of mobile phases and solvents though the capillary that used to make the

life of the conventional coating short. However, the sol-gel coatings have shown excellent solvent and thermal stabilities. For coating the sol-gel based coating in side the capillary, typically, a sol solution is designed in way that it gets gel after 2-3 hours and that sol solution is kept inside the capillary for varied time based on the coating thickness for certain application. A representative sol solution may contain, sol-gel active precursor, sol-gel active polymer, catalyst, solvent, surface deactivation reagent.

Keeping in mind, the nature of dendrimers being a branched macromolecule that is better than linear polymer, a benzyl terminated with dendron coating was developed for CME [65]. The coating presented excellent thermal and solvent stabilities and produced nice analytical data for alcohols, phenols, ketones, aldehydes and PAHs. Many other materials that have shown the stabilities and improved enrichment may include poly-tertahydrofuran [66], titania [67], 3-mercaptopropyltrimethoxysilane [68], polydimethyldiphenylsiloxanes [69], octdecyltrimethoxysilane [70], and 3-mercaptopropyltrimethoxysilane with polyethylene glycol [71]. Each new sorbent material adds versatility to the technique. However, the silica-based sorbent materials were found not much stable for more than 8.0 pH. Therefore, the need to establish a coating that can survive higher pH conditions. Then the application of non-silica-based sorbents were explored. Hence, surface like titania [72], germania [73]and zirconia [74,75] based coating were introduced and proved that these coating perform nicely in extreme pH environments. There is still need of more new surfaces to introduce to enhance the preconcentration and sensitivity.

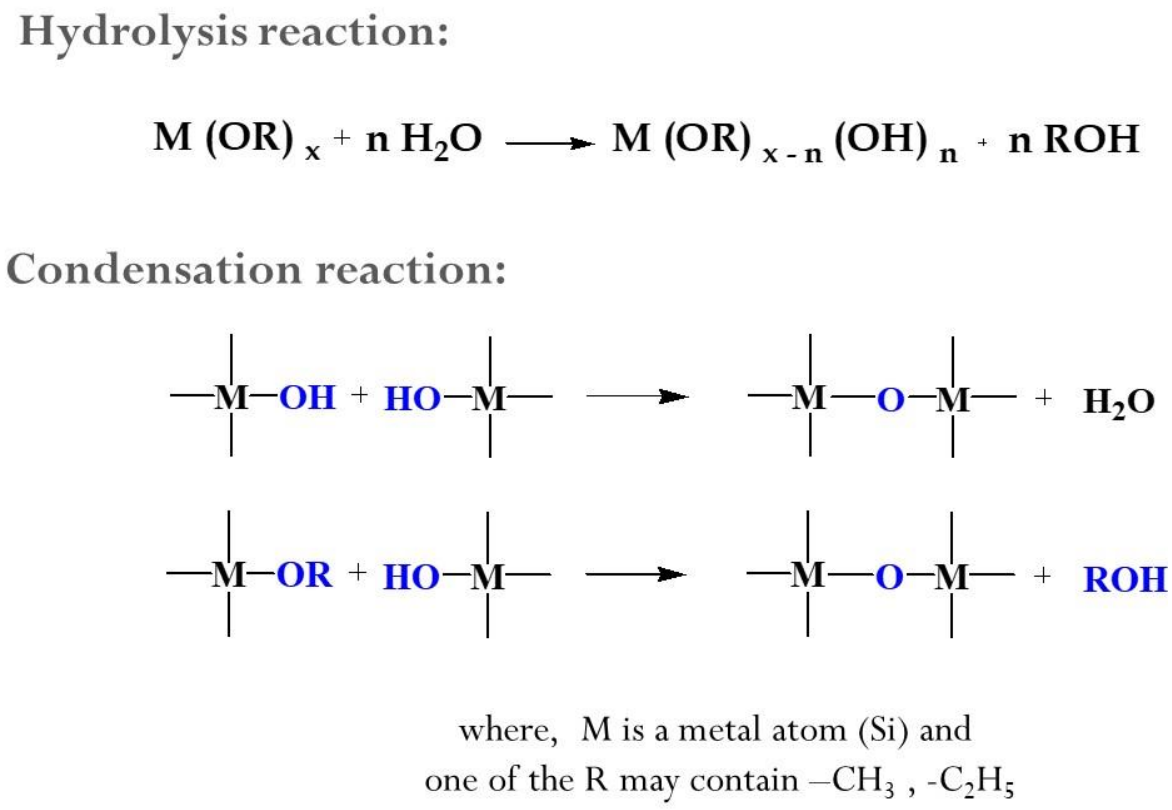


Fig 1. 5. General presentation of sol-gel reaction (hydrolysis and condensation reaction).

CHAPTER 2

Urea functionalized surface-bonded sol-gel coating for on-line hyphenation of capillary microextraction with high-performance liquid chromatography

2.1. Introduction

A solvent-free sample preparation technique, solid-phase microextraction, was developed by coating fused silica capillary on the outer surface with a polymer[9,57,58]. The analytes of interest were preconcentrated on the small coated external surface of the fused silica capillary and subsequently injected into GC system for analysis. This procedure is commonly known as fiber SPME, and it has many shortcomings. These problems include low sample capacity, problems with immobilizing thick coatings, issues with coatings in thermal and solvent variations [76], technical complications for hyphenation with liquid chromatographic techniques[77,78], and susceptibility of the coated surface to mechanical damage [79,80].

In contrast, in-tube solid phase microextraction [9,81] or capillary microextraction (CME)[64], provided ease of hyphenation of the microextraction technique to liquid chromatographic techniques. This hyphenation was more attractive for the analysis of thermally liable compounds[82] that are typically unable to be analyzed using gas chromatographic techniques. The major disadvantage of using the previously mentioned

fiber SPME technique was is that mechanical damage can occur to the coated surface during analysis. In the case of CME, this problem was avoided because the wall coated GC capillary columns contain the preconcentrated analytes inside the capillary column which then need to be desorbed into a mobile phase for HPLC analysis. However, instead of being chemically bonded, the wall coating inside the capillary is just a thin layer which poses some disadvantages for CME to be used in HPLC analysis. These disadvantages include limited sorption; poor stability for solvent; and thermal and pH stabilities. Since, a variety of mobile phases were used for the HPLC application, CME with thin layer wall coating is not a suitable technique[83].

To help counteract these aforementioned shortcomings of HPLC analyses being used for CME, sol-gel chemistry can be utilized in the coating process[76] for solid phase microextraction[64]. This process significantly helps to minimize the effect of solvent and thermal stabilities of the coating. The sol-gel chemistry was the process of chemical bonding of the polymer coating inside the capillary. As a result, various functional groups are immobilized into the polymeric network for better extraction and improved sensitivity. Various silica[84–86] and non-silica based[72,87,88] coatings were used for better features and enhanced extraction. Apart from the development of various new techniques in the field of solid-phase microextraction, capillary microextraction has its own uniqueness and advantages of being an online hyphenation[89] with HPLC. Capillary microextraction has a simplified procedure and not many variables to optimize; therefore, it is more precise than other techniques where the various parameters are set for just one complete analysis. However, there is a need to explore more new surfaces and materials[71,90–93] to make this technique more versatile.

To the best of our knowledge we presented the first synthesis of sol-gel [bis(hydroxyethyl)amine] terminated polydimethylsiloxane - bis(trimethoxysilylpropyl) urea (BHEA-BPU) surface immobilized coating for capillary microextraction. This material showed extraction sensitivity for compounds having various polarities that ranged from non-polar to highly polar. The selected analytes for online CME-HPLC analysis are well-established environmental pollutants. All are reported for toxicity and persistence in environment including PAHs, Alcohols [94], Aldehydes, Ketones, Amides [95,96] and Phenols [97].

2.2. Experimental

2.2.1. Equipment

Experiments were performed on HPLC system (Agilent Technologies, USA) equipped with a quaternary pump (G1311B/C), a DAD (G4212B), an auto-sampler (G1329B), and Chemstation software. An Agilent 1260 infinity isocratic pump (G13103B) was also used as a sample flow system. The column used for separation was Agilent ZORBAX Eclipse XDB C-18 (5µm, 4.6mm id x 250 mm). For the preparation and homogenized mixing of BHEA-BPU sol, Thermofisher Scientific MaxiMix Vortex mixer was used (model M16715). The precipitates of the sol solution were separated by using Sorvall™ Legend™ micro17 microcentrifuge. X-ray photoelectron spectroscopy (XPS) analysis of the BHEA-BPU coating was conducted on *Thermo Scientific ESCALAB 250Xi* (PHI 5000 Versa Probe II, ULVAC-PHI Inc., UK) to determine the bonding state and chemical composition of the coating material.

Before analysis, a chunk of polymer sample was mounted on carbon tape and subjected to high vacuum to remove impurities or moisture adsorbed on the sample.

Thermal stability and decomposition of BHEA-BPU coating was observed by thermogravimetry analysis (SDT Q600, V20.9 Build 20, thermal analyzer, USA) under nitrogen (N_2) environment from 30 to 600 ^oC with constant heating rate of 10 ^oC min^{-1}. The morphological information of the BHEA-BPU coated in capillary fused silica was examined by field emission scanning electron microscope (FE-SEM) from TESCAN, LYRA 3 Czech Republic, using secondary electron (SE) and back scattered electron (BSE) mode at an accelerating voltage of 30 kV and equipped with energy dispersive X-ray spectrometer (EDS, Oxford Inc.) detector for elemental analysis.

2.2.2. Chemicals and materials

Fused silica capillary (320 µm I.D. for coating and 250 µm I.D. for sample flow system after deactivating the inner surface) was purchased from Polymicro Technologies USA. Bis(trimethoxysilylpropyl) Urea (BPU) and [Bis(Hydroxyethyl)Amine] Terminated Polydimethylsiloxanes (BHEA) were purchased from Gelest, USA. Trifluoroacetic acid (TFA), 4-bromoacetanilide, N-methyl-1-naphthylacetamide, benzanilide, 2,3-dichlorophenol, 2,4-dichlorophenol, 2,4,6-trichloropehnol, 2-benzyl-4-chlorophenol, pentachlorophenol, 4-tert-octylphenol, 2-naphthol, 1-naphthol, diphenylcarbinol, 4-methoxyacetophenone, 4-hydroxybenzophenone, 2-hydroxy-2-phenylacetophenone, propiophenone, benzophenone, benzil, 4-chlorobenzophenone, 4-hydroxy-3-methoxybenzaldehyde, 5-nitrosalisaldehyde, 4-chlorobenzaldehyde, 5-bromobenzaldehyde, biphenyl, fluorene, phenanthrene, and acenaphthene were purchased from Sigma-Aldrich, USA.

2.2.3. Preparation of sol solution

The sol solution was prepared by vortexing a sol-gel active polymer BHEA 100 µL into 200 µL ethanol in a microcentrifuge tube for 30 s. The sol-gel active precursor BPU 20 µL was added to the reaction mixture with 30 s vortexing. Moreover, the chelating agent TFA 8.0 µL and water 5.3 µL were added to the mixture and mixed very well. Vortexing continued for 2 min. Then the reaction mixture was centrifuged at 13000 rpm for 10 min and the top clear layer was decanted into another microcentrifuge tube for the coating to the inside the fused silica microextraction capillary.

2.2.4. Preparation of sol-gel BHEA-BPU coated microextraction capillary

A 3.0 m long fused silica capillary (320 µm i.d.) was rinsed with methanol and dichloromethane and pretreated with 1.0 M NaOH solution, where NaOH solution was kept inside the capillary for 10 hours by closing the both ends of the capillary and flushed later. The capillary was rinsed with 0.1 M HCl to neutralize any NaOH present and later rinsed with water for confirmed cleaning. All these rinsing and etching procedures were done under helium pressure using an in-house built gas pressure-operated capillary filling device. The capillary was then kept inside the GC oven for drying at 250 ^{O}C for overnight under helium flow and later took out from GC and installed to in-house built gas pressure-operated capillary filling device for rinsing with methanol and dichloromethane to remove any impurity and cleaning. Furthermore, the heat treatment to 300 ^{O}C for 2 hours was done using GC oven under helium pressure for drying.

A 1.0 m long piece of the pretreated capillary was used for sol-gel coating using gas pressure operated purging device. The sol solution was purged to the capillary and kept

inside the capillary for 15 min to enhance the on-surface reaction of the sol coating. The unreacted sol solution was expelled out of the capillary using helium gas pressure, and the helium flow was continued for 15 additional min. This coated capillary was then subjected to post-treatment [72] using the GC oven to make the sol-gel material more porous and cleaned.

2.2.5. Capillary microextraction (CME) and online CME-HPLC analysis

The CME-HPLC analysis is presented in Fig 2.1., where there is a sample flow system, manual injection port, and an HPLC system. The analytical column was pre-equilibrated with the mobile phase and kept ready for manual injection. A 40 cm long sol-gel BHEA-BPU coated capillary was fixed in place of the sample loop in the manual injection port. The injection port was switched to “load” mode and an aqueous sample having the analytes of interest was pre-concentrated in the sol-gel BHEA-BPU coated capillary with a constant flow of 1.0 mL/min using the isocratic pump. The deionized water was later flushed through the sol-gel BHEA-BPU coated capillary to remove the sample matrix in the capillary loop. The injection port was then switched to “inject” mode for the desorption of the extracted analytes from sol-gel BHEA-BPU capillary to the analytical column. The analytical column separated the analytes based on the interaction between mobile phase and stationary phase and detected with a UV detector.

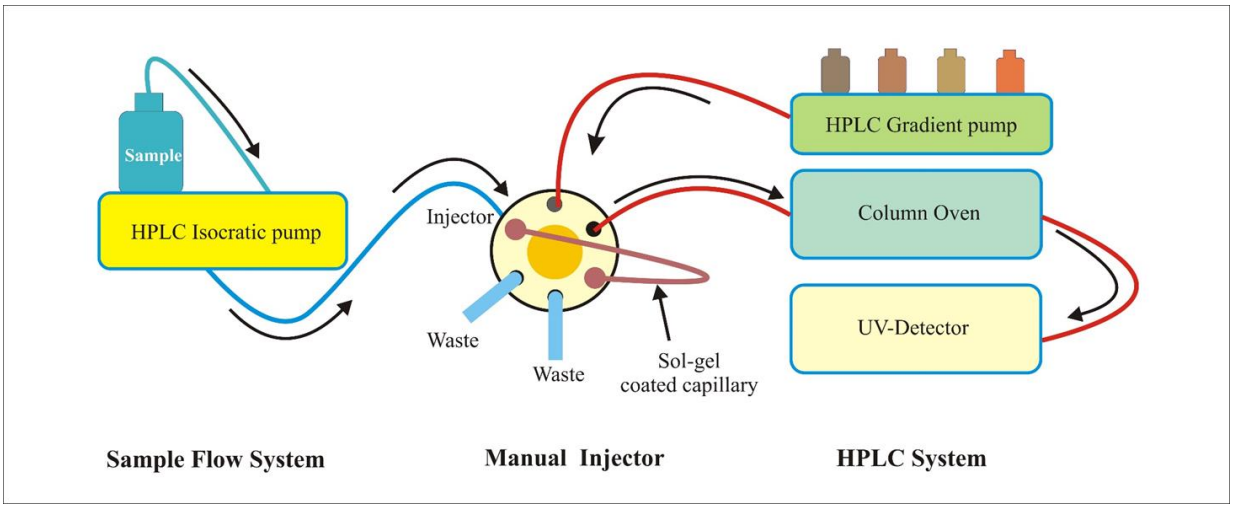


Fig 2. 1. Representation of the setup; CME-HPLC online analysis

2.2.6. Enrichment factor

The enrichment factors for all the analytes were also presented. The enrichment factor[98] was calculated by dividing the peak area of the extracted analyte to the peak of standard analyte. The peak area for the analyte from the standard solution was obtained by injecting 20 µL of the standard solution to the HPLC manual port without any extraction involved.

2.2.7. Real sample analysis using BHEA-BPU coated capillary

A similar run that was designed to present the capillary to capillary extraction was also used to demonstrate the applicability of BHEA-BPU coated capillary in real sample. Each member from the different organic class of compounds was selected as a representative of varying nature and polarity. Real samples were collected from three different sources that is wastewater, sea water and swimming pool water. Samples were filtered using filter paper (pore size 0.45 µm) before the online CME-HPLC analysis. To evaluate the recovery from the samples, peak areas obtained from the analysis of the standard solutions were compared with the peak areas of the spiked samples in the real water samples. Spiking was done at 100, 300 and 500 ng mL^{-1} and each run was repeated thrice (n = 3).

2.3. Results and discussion

The aim of this work was to develop a novel urea functionalized surface bonded sol-gel coating for capillary microextraction in hyphenation with high-performance liquid chromatography. The special features of sol-gel chemistry made a surface bonded BHEA-BPU coating on the inner walls of the fused capillaries and to impregnate desired functional

groups like urea and amino in capillary microextraction. In comparison to other coating techniques, sol-gel is a one step process to create the surface coating and chemical linking.

2.3.1. Creation of sol-gel based BHEA-BPU coating

The sol gel precursor Bis(trimethoxysilylpropyl) Urea (BPU) underwent controlled polycondensation reactions to form the colloidal system called sol and this sol further form the three-dimensional structure that is called gel. [Bis(Hydroxyethyl)Amine] Terminated Polydimethylsiloxanes (BHEA) act as sol-gel active polymer and ethanol acts as a solvent to dissolve the contents in the sol solution. The trifluoroacetic acid (TFA) acts as a chelating agent for the gelation of the sol-gel active precursor. The hydrolyzed reactive species further undergo polycondensation reactions to produce the urea functionalized three-dimensional network.

The portions of BHEA sol-gel active polymer get condensed with the silanol groups of the inner side of the fused silica capillary and produce a surface bonded polymer with sol-gel active precursor BPU network over the surface as some urea functionalized moieties. This condensation is followed by heat treatment to produce cross-linking and porosity. The chelating agent, TFA, controls the gelation process by decelerating the process of BHEA-BPU coating. The sol-gel process for the creation of coating on the inner surface of fused silica capillaries involves (i) hydrolysis of the Bis(trimethoxysilylpropyl) Urea precursor, (ii) polycondensation of the BPU precursor to form a sol-gel network after being hydrolyzed, (iii) chemical reaction of the sol-gel active polymer BHEA with BPU sol-gel network, (iv) chemical immobilization of the sol-gel material to the silanol groups on the inner surface of fused silica capillaries. These steps result in making a urea functionalized sol-gel surface bonded coating for microextraction. Fig 2.2. shows the hydrolysis and

polycondensation of the BPU precursor and Fig 2.3. displays the final coating material network immobilized inside the capillary wall.

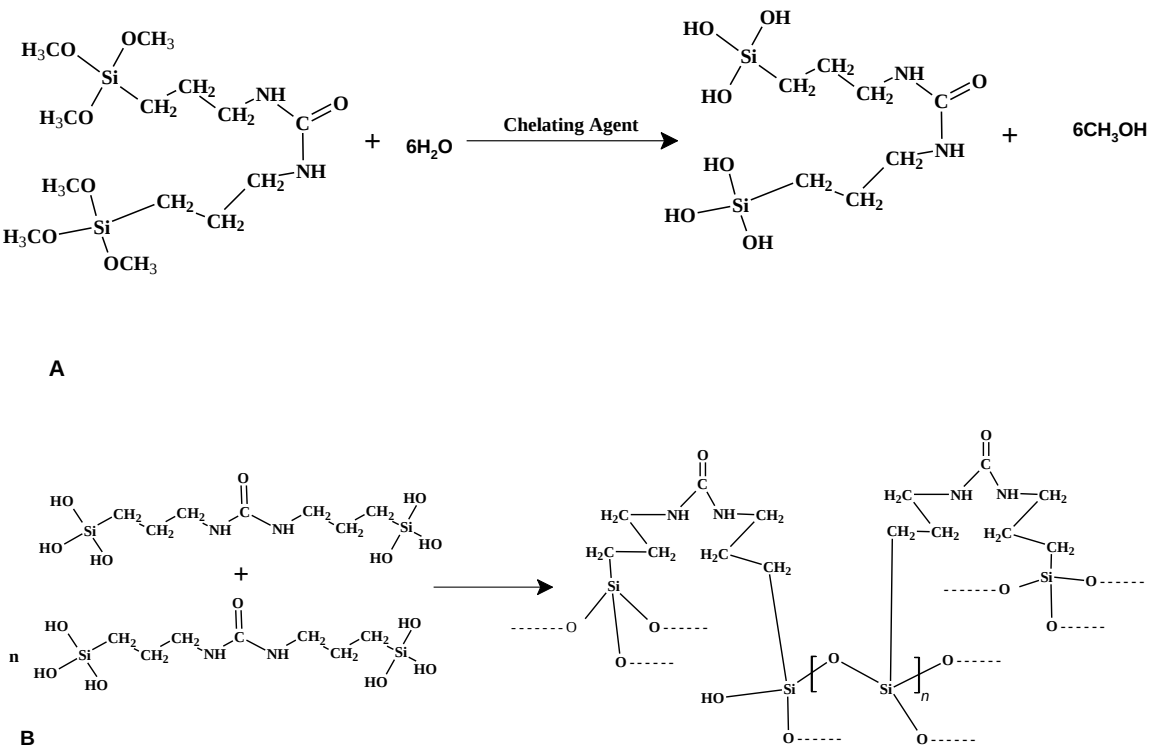


Fig 2. 2. (A) Hydrolysis of BPU precursor and (B) Polycondensation of hydrolyzed BPU

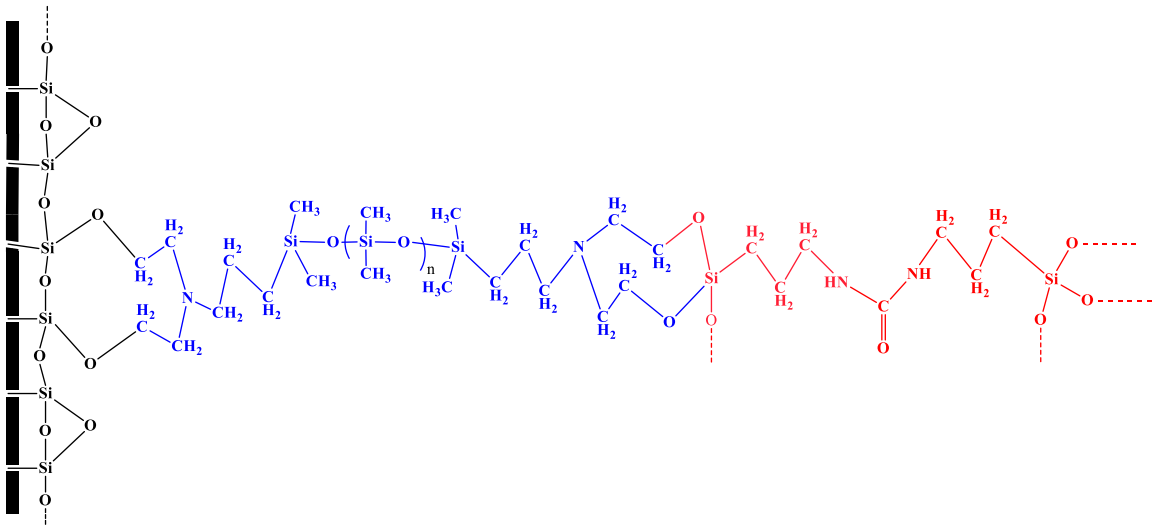


Fig 2. 3. Final structure of BPU-BHEA coating

2.3.2. Characterization of sol-gel BHEA-BPU coating for CME

XPS analysis of BHEA-BPU polymer synthesized was analyzed to gain insight into the structural arrangement of the main components. Fig 2.4. and the Table 2.1. present the binding energies (BEs) and the atomic weight (%) of the C, N, Si, and O, respectively. The carbon (C 1s) found at 282.7 and 284.9 eV binding energies (BEs), represent the main content or backbone of the polymer (45%) as observed from the monomers. These two C 1s forms represent the carbon largely bonded with semi-metallic element (282.7 eV) at 98.8% and the carbon in graphitic form (284.9 eV) at 1.13% [99]. The oxidation state of nitrogen revealed two forms of nitrogen at 397.4 and 396.3 eV, corresponding to N 1s in nitride-form in two different environments. The observed BEs of O 1s at 530.46 and 530.98 eV correspond to the presence of carbonates (C=O) and Si-O oxygens of the constituents that formed the polymer, respectively. The evidence of Si 2p bonded with carbon and oxygen atoms was found at BEs of 99.84 and 100.73 eV, respectively. The observation of polymer's main composition with respect to atomic weight-% is in good agreement with the proposed structure and confirms the successful polymerization reactions for uniform capillary coating applications.

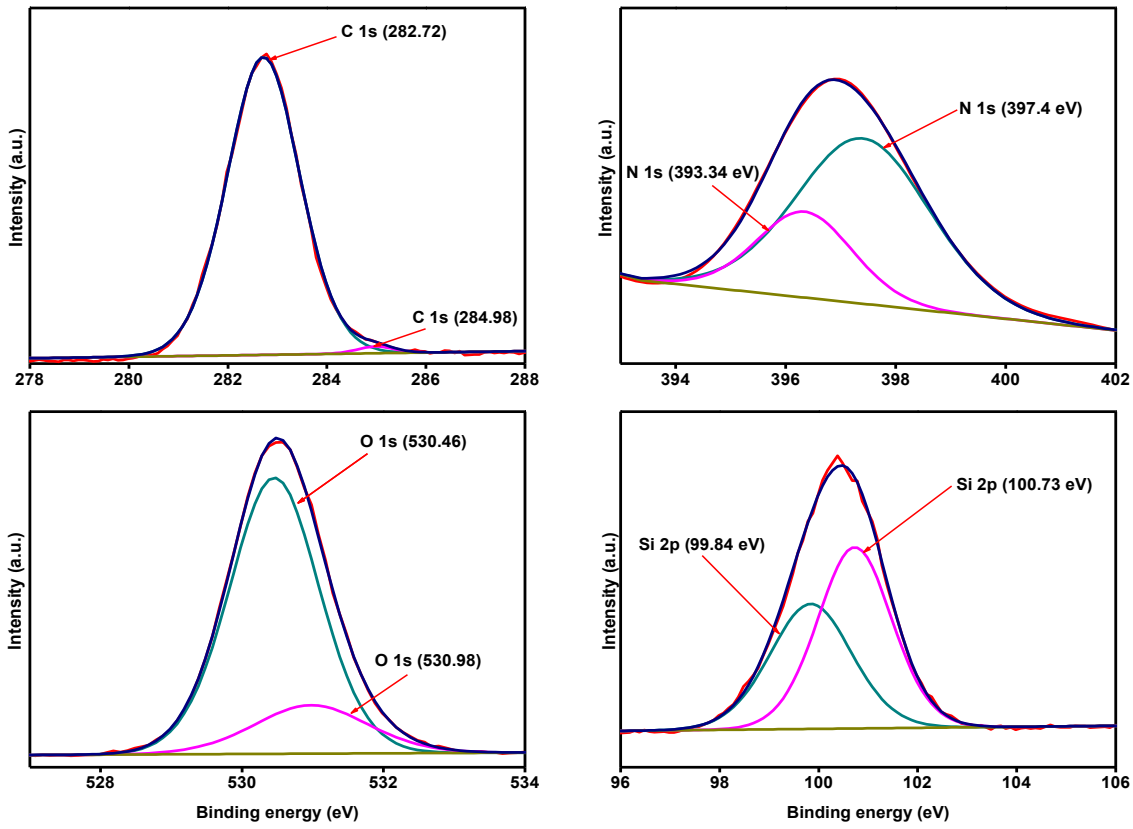


Fig 2. 4. XPS analysis of as-synthesized BHEA-BPU polymer before capillary coating in fused-silica showing different bonding states of C, N, O and Si.

Table 2. 1. The bonding states and atomic weight (%) of polymer composition by XPS

Name	Peak BE	Atomic %
C1s	282.72	45.3
C1s	284.98	0.52
N1s	397.4	0.42
N1s	396.34	0.15
O1s	530.46	21.81
O1s	530.98	4.84
Si2p	99.84	11.66
Si2p	100.73	15.3

Thermal and structural stability of BHEA-BPU polymer was obtained to understand the broad applicability of the material under heating/thermal environmental. A gradual loss of weight (approx. 15%) was observed from room temperature (30 ^{o}C) to above 250 ^{o}C in the first phase transition. This observation could be attributed to the loss of adsorbed water and other impurities associated with the sol-gel prepared polymer. Furthermore, a gradual weight loss estimated to be 15% was observed until 450 ^{o}C in second phase transition, which could be attributed to gradual decomposition of organic material network (backbone) in the polymer. After 450 ^{o}C, there is an exponential drop in the weight loss of the polymer material up to 600 ^{o}C and the estimated weight loss is about 90%. This phase transition indicates the complete decomposition of polymer and formation of carbon/soot [100,101]. Therefore, the thermal stability and practical working temperature of as-developed polymer will be within the range of 0 – 300 ^{o}C without significant decomposition as shown in Fig 2.5.

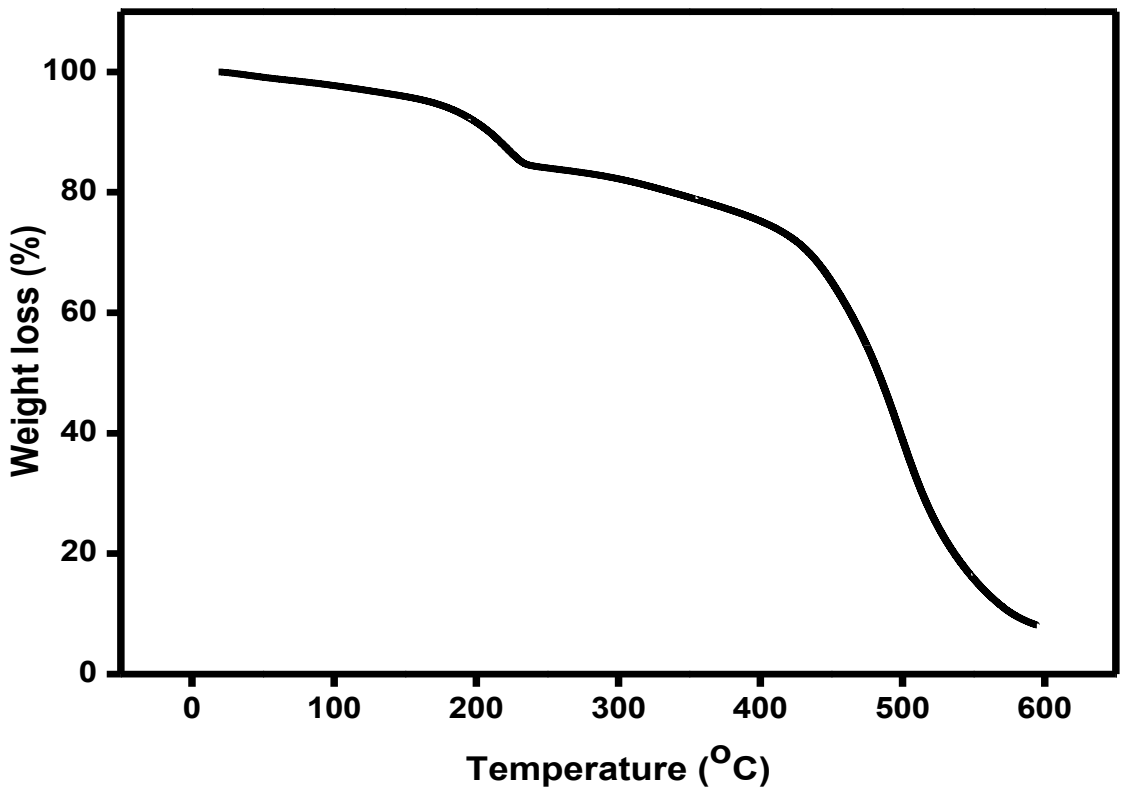


Fig 2. 5. Thermogravimetric analysis of as-synthesized BHEA-BPU polymer before capillary coating in fused-silica capillary.

The successful capillary coating operation of BHEA-BPU polymer prepared by sol-gel method for mirco-extraction applications is observed by scanning electron microscope (SEM) at high resolution. As shown in Fig 2.6., the BHEA-BPU polymer was uniformly deposited/coated inside the fused silica of 320 µm i.d. with estimated 2.5 µm thickness. In addition, the coating morphology reveals no cracks or discontinuity of polymer material within the coated fused silica, and this will offer better accessibility and high sorption capacity of extractant during micro-extraction operation. Furthermore, the energy dispersive spectroscopy (EDS) was used to confirm and complement the elemental compositions of the polymer as observed by XPS, and there exist a good correlation between the obtained elemental atomic weight (%) by EDS and the theoretical calculation from the monomers, as shown in Fig 2.7.(inset).

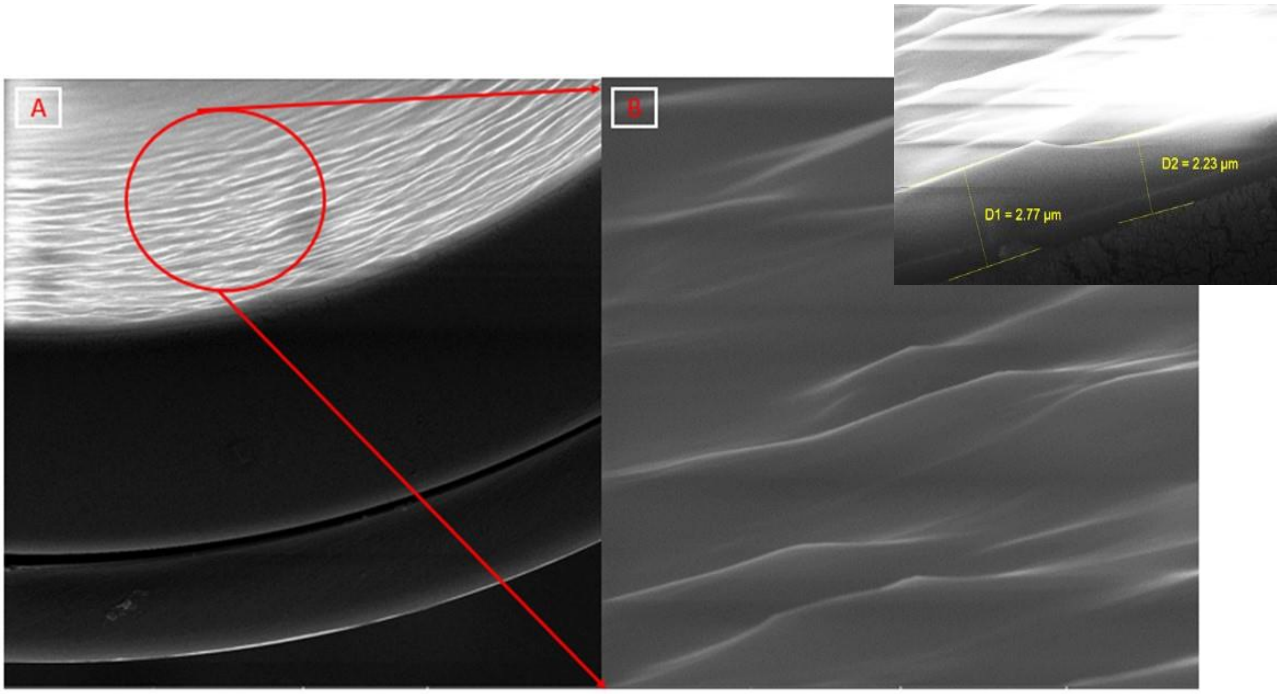


Fig 2. 6. SEM analysis of BHEA-BPU coating inside the fused silica capillary at low (A) and high (B) magnifications. The inset showing in (B) the thickness of polymer coated in capillary fused-silica

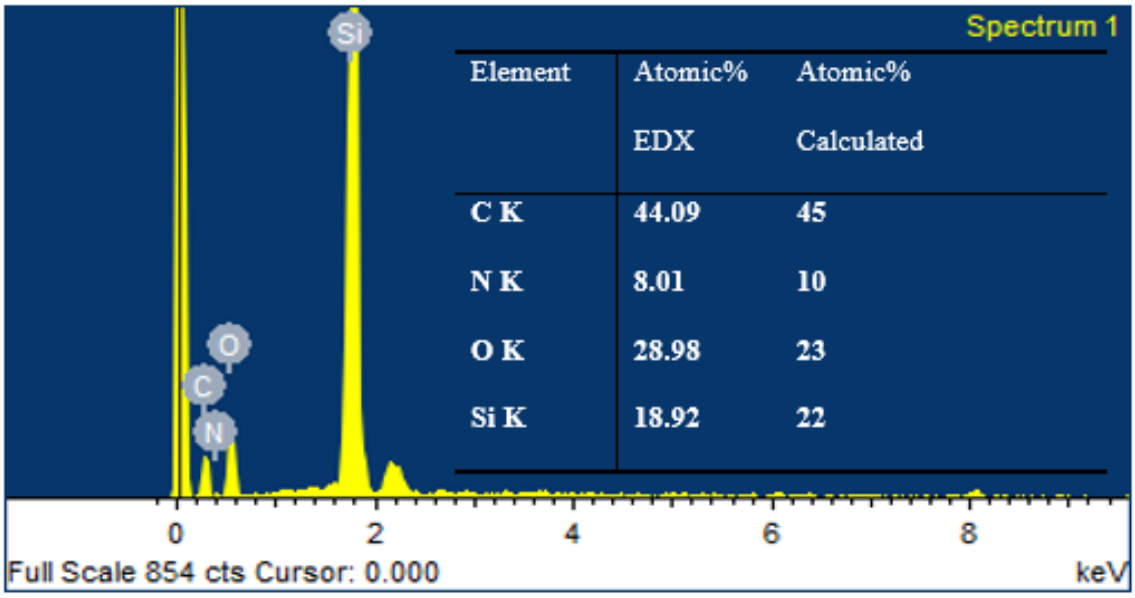


Fig 2. 7. EDS analysis of BHEA-BPU polymer coated in capillary fused-silica with inset representing atomic weight (%)

2.3.3. Online CME-HPLC analysis using sol-gel coated BHEA-BPU capillary

The sol-gel BHEA-BPU coated capillary demonstrated excellent extraction abilities for various class of compounds, ranging from non-polar to highly polar compounds. The various classes include the polyaromatic hydrocarbons, aldehydes, ketones, alcohols, phenols, and amides. The BHEA-BPU coated capillary was equally suitable for a range in polarities of various analytes. Extraction of non-polar analytes was due to polydimethylsiloxanes (PDMS) moiety in the BHEA sol-gel active polymer, and polar analytes were extracted efficiently due to functional groups like amine and urea in precursor BPU and polymer BHEA respectively. The online CME-HPLC analysis of three amides is shown in Fig 2.8. Amides are considered as a polar functional group and the BHEA-BPU coating inside the fused silica has amine functional groups that make the coating a perfect extracting tool for amides. As shown in Table 2.2., the CME-HPLC analysis showed low detection limits ranging between 5.21-11.90 ng mL^{-1} (S/N=3) and reliable %RSD (less than 10%) where n=3.

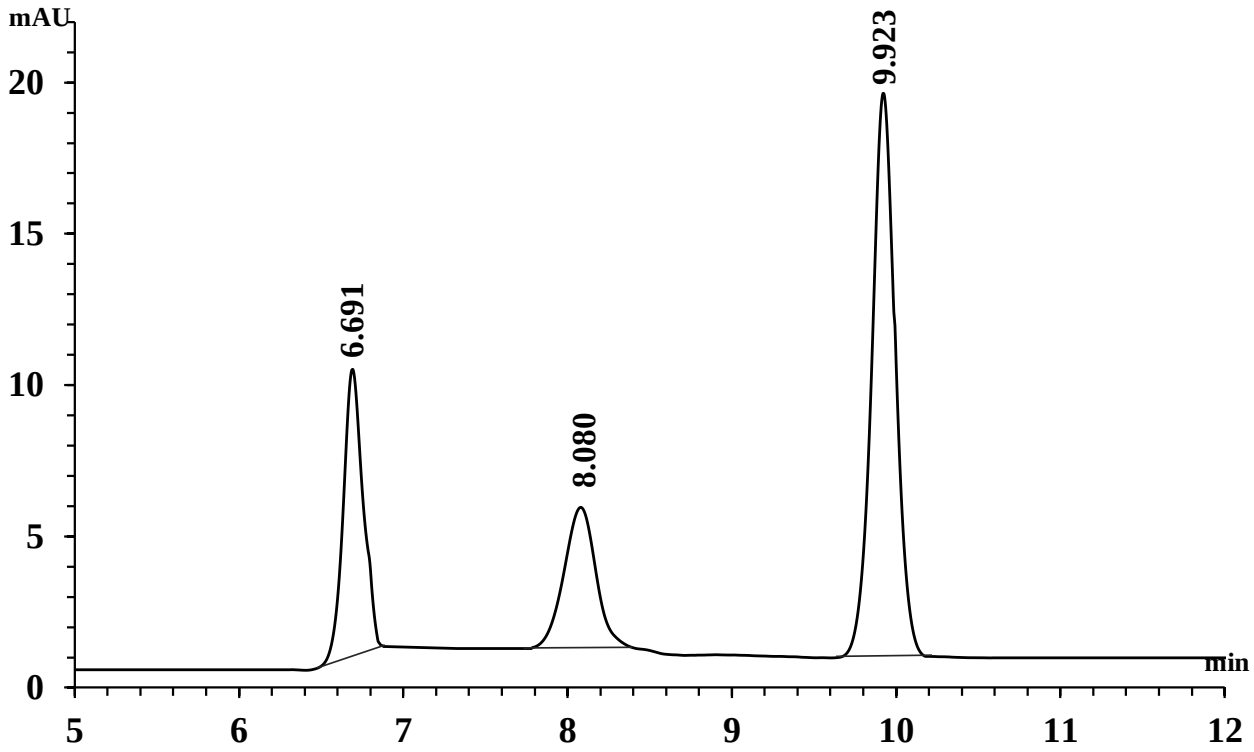


Fig 2. 8. Capillary microextraction-HPLC analysis of Amides. Extraction conditions: 40 cm × 0.32 mm i.d. sol-gel BPU-BHEA- coated capillary; extraction time: 30 min: HPLC conditions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 µm dp). Gradient elution from 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH to 70% ACN till 20 min and 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH till 30 min; runtime 30 min; 0.8 mL min^{-1} flow rate, UV detection at 230 nm, ambient temperature. Peaks: (t_R = 6.691) 4-bromoacetanilide (50 ng mL^{-1}), (t_R = 8.080) n-methyl-1-naphthylacetamide (50 ng mL^{-1}), (t_R = 9.923) benzanilide (50 ng mL^{-1}).

Table 2. 2. Peak area reproducibility and detection limits for Amides, phenols, alcohols, ketones, aldehydes, and PAHs in CME-HPLC using a sol-gel BPU-BHEA coated microextraction capillary [a]

Chemical class Analyte name	Peak area reproducibility (n=3)		Detection limit (ng mL^{-1}) (S/N=3)	Enrichment factors
	Mean peak area (mAU)	RSD (%)		
Amides				
4-bromoacetanilide	138.4	4.6	7.25	41.4
N-methyl-1-naphthylacetamide	68.5	9.5	11.90	25.2
Benzanilide	206.1	3.2	5.21	57.6
Phenols				
2,3-dichlorophenol	26.3	3.6	12.50	24.0
2,4-dichlorophenol	54.8	2.0	9.43	31.8
2,4,6-trichloropehnol	21.1	6.0	4.55	55.0
2-benzyl-4-chlorophenol	30.1	1.4	1.92	36.4
Pentachlorophenol	48.2	1.8	6.41	39.0
4-tertoctylphenol	47.6	5.2	6.94	36.0
Alcohols				
2-naphthol	77.3	4.9	1.45	172.5
1-naphthol	33.5	4.9	2.38	105.0
Diphenylcarbinol	85.2	2.8	1.39	180.0
Ketones				
4-methoxyacetophenone	29.4	6.7	10.75	27.9
4-hydroxybenzophenone	47.3	2.9	8.93	33.6

2-Hydroxy-2-phenylacetophenone	20.9	2.6	13.66	22.0
Propiophenone	40.2	1.6	9.62	31.2
Benzophenone	145.4	2.1	2.46	122.2
Benzil	167.1	2.8	2.14	140.2
4-chlorobenzophenone	351.3	2.6	1.70	176.2
Aldehydes				
4-Hydroxy-3-methoxybenzaldehyde	20.2	11.3	9.09	33.0
5-Nitrosalisaldehyde	140.8	5.3	2.88	104.2
4-chlorobenzaldehyde	9.3	11.8	14.29	21.0
5-bromobenzaldehyde	55.6	8.0	5.40	55.6
Polyaromatic Hydrocarbons				
Biphenyl	167.2	1.9	0.31	193.5
Fluorene	134.4	3.0	0.40	150.0
Phenanthrene	717.8	2.5	0.10	240.0
Anthracene	213.3	4.5	0.21	171.4

[a] Extraction conditions: 40 cm × 0.32 mm i.d. sol-gel BPU-BHEA- coated capillary; extraction time: 30 min: HPLC conditions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 μm d_p).
For amides, phenols, alcohols, ketones, and aldehydes: gradient elution from 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH to 70% ACN for 20 minutes and 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH till 30 minutes; runtime 30 minutes; 0.8 mL min^{-1} flow rate, UV detection at 230 nm (amides, alcohols and aldehydes), 280 nm (phenols), 254 nm (ketones)
For PAHs: isocratic elution from 80:20 (v/v) ACN:Water for 15 min; 0.8 mL min^{-1} flow rate; UV detection at 254 nm.

Fig 2.9. represents the CME-HPLC analysis of highly polar phenols. For the extraction experiment, mostly chlorinated phenols were selected to enhance the polarity of phenols and prove the extraction capability of the BHEA-BPU coated capillary. Fig 2.9. shows all 6 phenols in a run with concentration ranges from 10 ng mL^{-1} to 50 ng mL^{-1} for various phenols. However, the sol-gel BHEA-BPU coated capillary showed extraordinary %RSD less than 6.0 (n = 3) with detection limits ranging between 1.92 ng mL^{-1}- 12.50 ng mL^{-1} (S/N=3) as shown in Table 2.2. These low detection limits and efficient extraction of polar moieties may be explained by the polar groups (amine group in BHEA and urea group in BPU) in the sol-gel BHEA-BPU coated capillary.

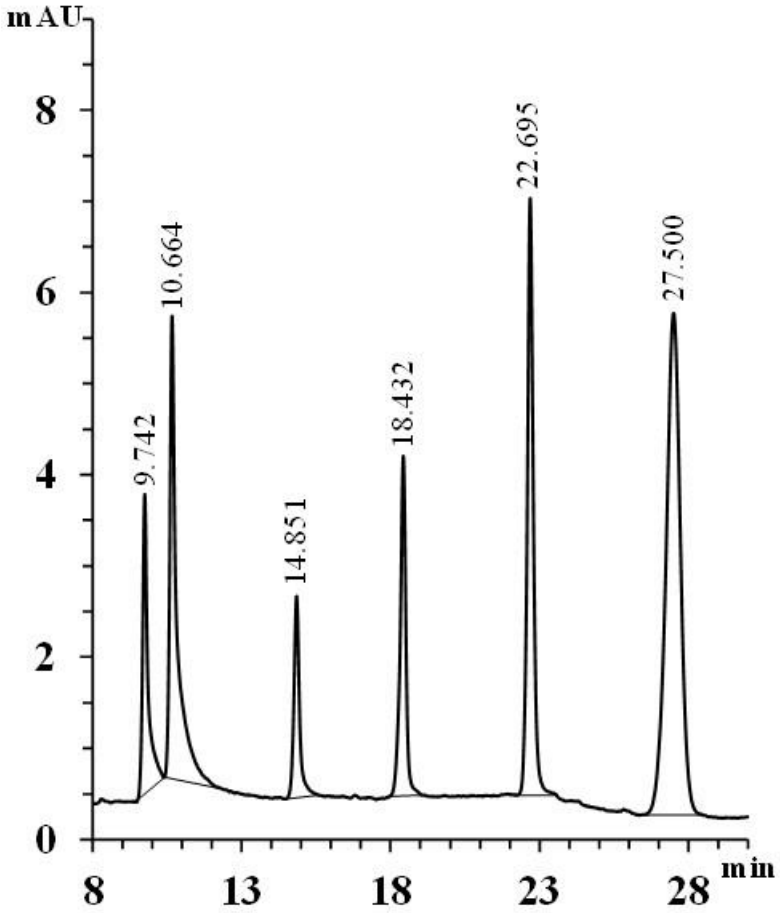


Fig 2. 9. Capillary microextraction-HPLC analysis of Phenols. Extraction conditions: 40 cm × 0.32 mm i.d. sol-gel BPU-BHEA- coated capillary; extraction time: 30 min: HPLC conditions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 µm dp). Gradient elution from 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH to 70% ACN till 20 min and 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH till 30 min; runtime 30 min; 0.8 mL min^{-1} flow rate, UV detection at 280 nm, ambient temperature. Peaks: (t_R = 9.742) 2,3-dichlorophenol (50 ng mL^{-1}), (t_R = 10.664) 2,4-dichlorophenol (50 ng mL^{-1}), (t_R = 14.581) 2,4,6-trichlorophenol (25 ng mL^{-1}), (t_R = 18.432) 2-benzyl-4-chlorophenol (10 ng mL^{-1}), (t_R = 22.695) pentachlorophenol (25 ng mL^{-1}), (t_R = 27.500) 4-tert-octylphenol (25 ng mL^{-1}).

The alcohols are considered less polar than phenols but still have polarities on the higher side of the organic functional groups. A few alcohols were also tested for the online CME-HPLC analysis as shown in Fig 2.10. The extraction process was much enhanced as compared to phenols because the selected alcohols have multiple benzene rings that make them suitable for enhanced interaction with the capillary coating. Since capillary coating is designed to have both polar and non-polar groups, the benzene rings were attracted towards non-polar groups like polydimethylsiloxane in BHEA polymer and the alcohol functionalized part interacted with amine functionalities in the coating. These interactions resulted in lower detection limits between 1.39-2.38 ng mL^{-1} (S/N=3) and %RSD as less than 5 (n=3).

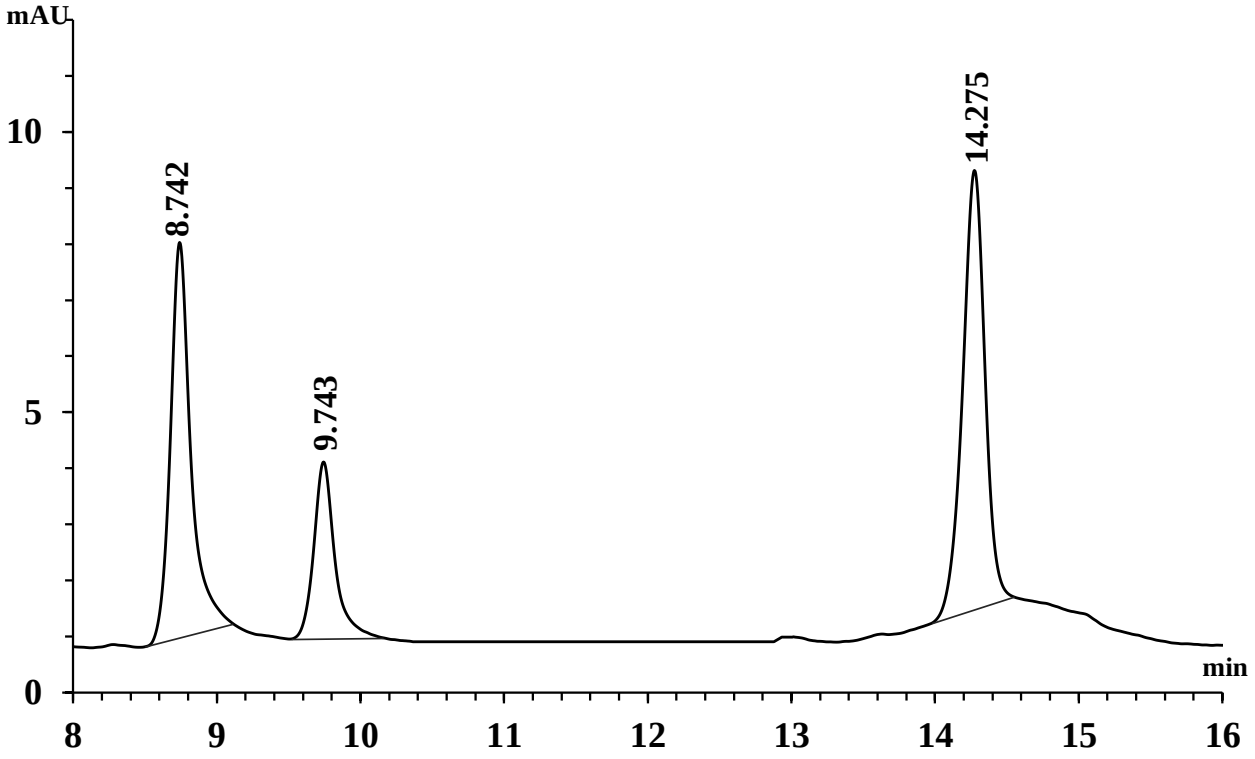


Fig 2. 10. Capillary microextraction-HPLC analysis of Alcohols. Extraction conditions: 40 cm × 0.32 mm i.d. sol-gel BPU-BHEA- coated capillary; extraction time: 30 min: HPLC conditions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 µm dp). Gradient elution from 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH to 70% ACN till 20 min and 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH till 30 min; runtime 30 min; 0.8 mL min^{-1} flow rate, UV detection at 230 nm, ambient temperature. Peaks: (t_R = 8.742) 2-naphthol (10 ng mL^{-1}), (t_R = 9.743) 1-naphthol (10 ng mL^{-1}), (t_R = 14.275) diphenylcarbinol (10 ng mL^{-1}).

Fig 2.11. illustrates the online CME-HPLC analysis of moderately polar ketones. For this purpose, seven members of this class was selected including, 4-methoxyacetophenone, 4-hydroxybenzophenone, 2-Hydroxy-2-phenylacetophenone, propiophenone, benzophenone, benzil. The sol-gel BHEA-BPU coated capillary showed excellent extraction efficiencies towards moderately polar analytes with limit of detections 1.70 ng mL^{-1} – 13.66 ng mL^{-1} and the reproducibility of the extraction process was within 7% (n = 3) which is clearly represented in Table 2.2. The similar type of extraction interactions was shown by aldehydes due to similar and comparable polarities of aldehydes and ketones.

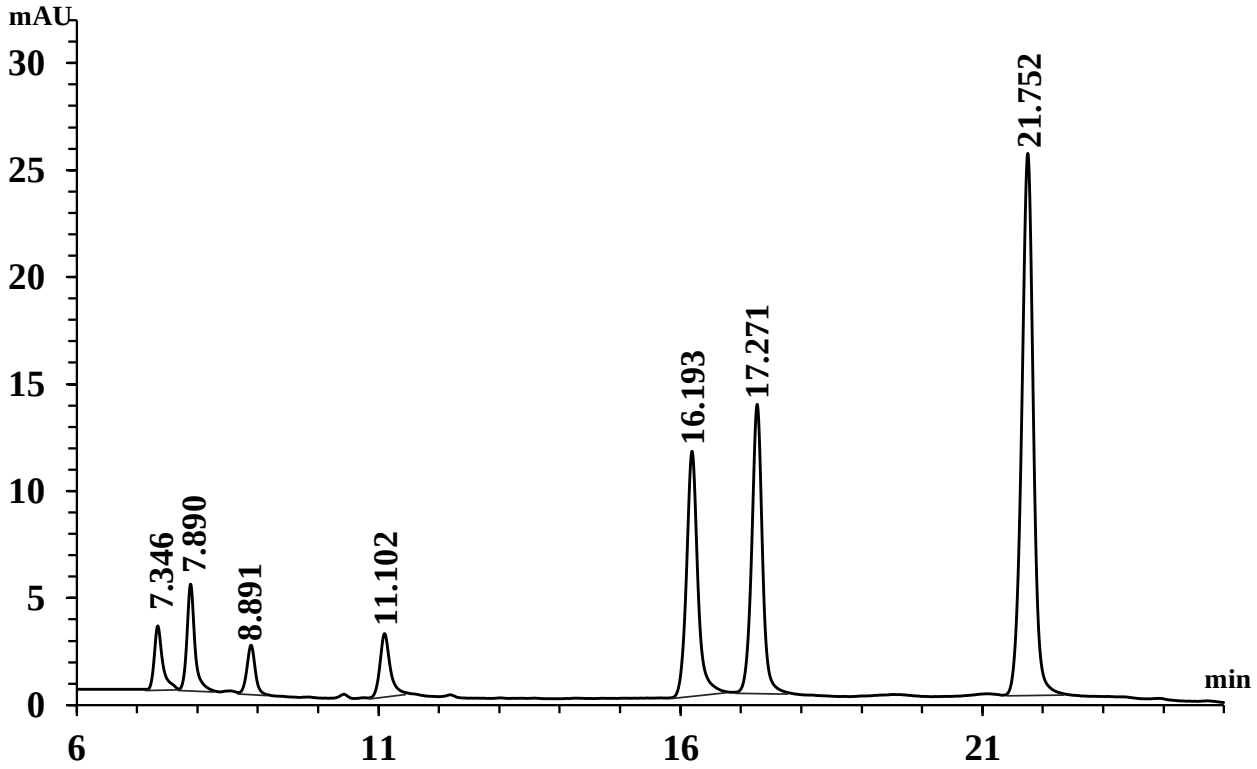


Fig 2. 11. Capillary microextraction-HPLC analysis of Ketones. Extraction conditions: 40 cm × 0.32 mm i.d. sol-gel BPU-BHEA- coated capillary; extraction time: 30 min: HPLC conditions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 µm dp). Gradient elution from 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH to 70% ACN till 20 min and 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH till 30 min; runtime 30 min; 0.8 mL min^{-1} flow rate, UV detection at 254 nm, ambient temperature. Peaks: (t_R = 7.346) 4-methoxyacetophenone (50 ng mL^{-1}), (t_R = 7.890) 4-hydroxybenzophenone (50 ng mL^{-1}), (t_R = 8.891) 2-Hydroxy-2-phenylacetophenone (50 ng mL^{-1}), (t_R = 11.102) propiophenone (50 ng mL^{-1}), (t_R = 16.193) benzophenone (50 ng mL^{-1}), (t_R = 17.271) benzil (50 ng mL^{-1}), (t_R = 21.752) 4-chlorobenzophenone (50 ng mL^{-1}).

Fig 2.12. shows the CME-HPLC analysis of four selected aldehydes at lower concentrations. The analysis was very efficient for all the compounds, presenting %RSD less than 12% (n=3) and LOD ranging between 5.40 ng mL^{-1} to 14.29 ng mL^{-1} (S/N=3).

The online CME-HPLC analysis of polyaromatic hydrocarbons as a non-polar representative class using sol-gel BHEA-BPU coated capillary is shown in Fig 2.13. For the extraction procedure, four members of this class were selected including biphenyl, fluorene, phenanthrene, and anthracene. The efficient extraction of these compounds may be explained due to the PDMS moiety in the BHEA polymer. Moreover, the significant extraction took the LODs to lower-level ranging between 0.10 ng mL^{-1} - 0.40 ng mL^{-1} (S/N = 3) with extraordinary %RSD less than 4.5 (n=3) as shown in Table 2.2.

In addition to the LODs and reproducibility of various analytes, Table 2.2. also presents the enrichment factors for all the analytes ranging from 21.0 folds to 240 folds. Higher enrichment factors lead to lower level of detection limits, hence proving the extraction ability of BHEA-BPU coated capillary.

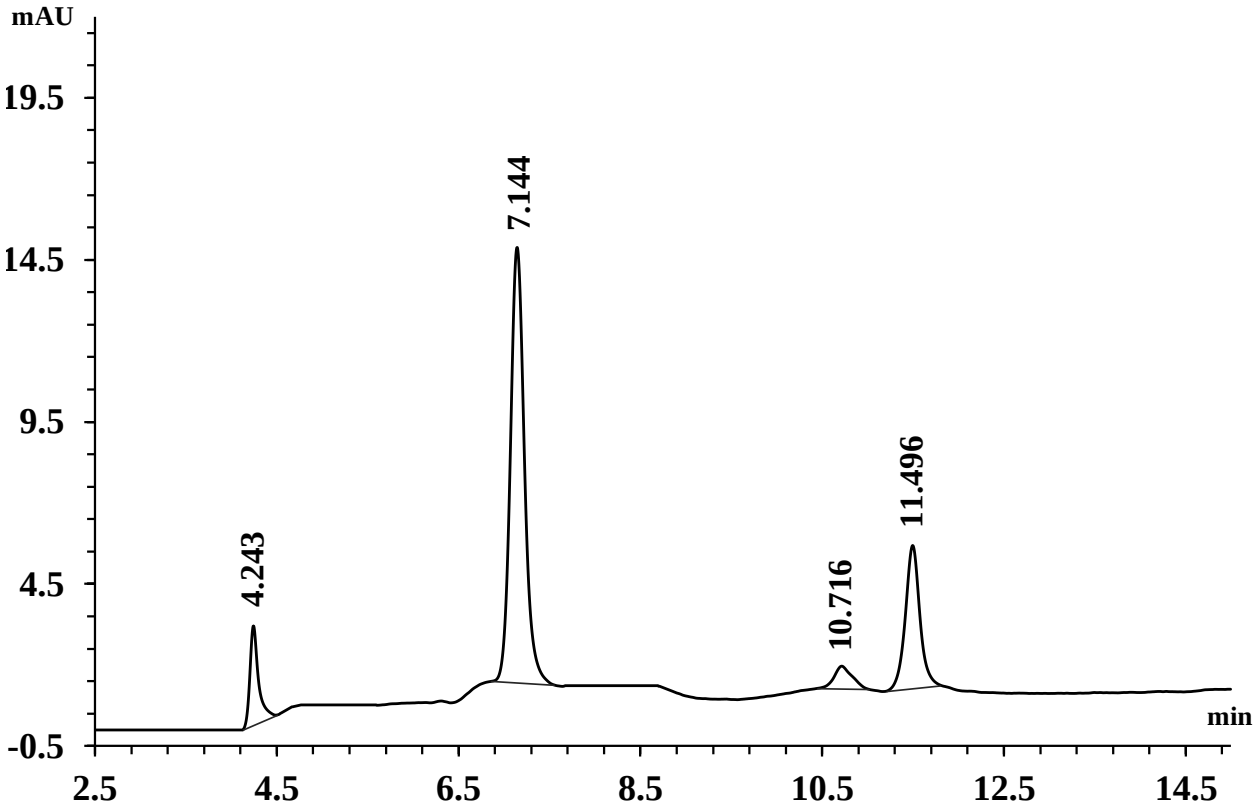


Fig 2. 12. Capillary microextraction-HPLC analysis of Aldehydes. Extraction conditions: 40 cm × 0.32 mm i.d. sol-gel BPU-BHEA- coated capillary; extraction time: 30 min: HPLC conditions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 µm dp). Gradient elution from 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH to 70% ACN till 20 min and 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH till 30 min; runtime 30 min; 0.8 mL min^{-1} flow rate, UV detection at 230 nm, ambient temperature. Peaks: (t_R = 4.243) 4-Hydroxy-3-methoxybenzaldehyde (50 ng mL^{-1}), (t_R = 7.144) 5-nitrososalisaldehyde (50 ng mL^{-1}), (t_R = 10.716) 4-chlorobenzaldehyde (50 ng mL^{-1}), (t_R = 11.496) 5-bromobenzaldehyde (50 ng mL^{-1}).

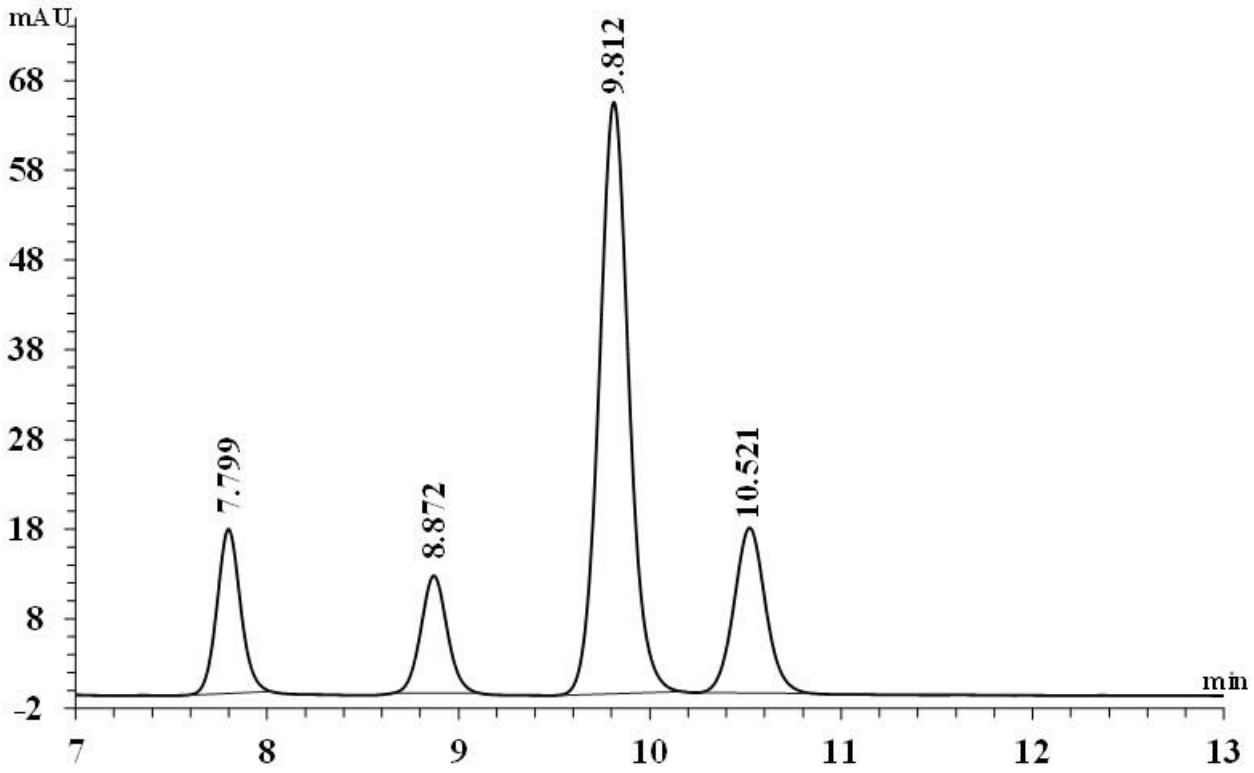


Fig 2. 13. Capillary microextraction-HPLC analysis of polyaromatic hydrocarbons. Extraction conditions: 40 cm × 0.32 mm i.d. sol-gel BPU-BHEA- coated capillary; extraction time: 30 min: HPLC conditions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 µm dp). Isocratic elution with 80:20 (v/v) ACN:Water for 15min; 0.8 mL min^{-1} flow rate; UV detection at 254 nm, ambient temperature. Peaks: (t_R = 7.799) biphenyl (01 ng mL^{-1}), (t_R = 8.872) fluorene (01 ng mL^{-1}), (t_R = 9.812) phenanthrene (01 ng mL^{-1}), (t_R = 10.521) acenaphthene (01 ng mL^{-1}).

2.3.4. CME extraction profile for BHEA-BPU coated capillary

Figure 2.14. signifies the extraction kinetic profile of the selected classes for online CME-HPLC analysis using the BHEA-BPU coated capillary. One member of each class of compound was selected, including 4-bromoacetanilide (amide, polar) 4-tert-octylphenol (phenol, polar), 2-naphthol (alcohol, polar) 4-hydroxybenzophenone (ketone, moderately polar), 5-nitrosalisaldehyde (aldehyde, moderately polar), and biphenyl (polyaromatic hydrocarbon, non-polar). A series of experiments were conducted for the extraction of these analytes from the water samples. The selected concentration for the extraction kinetic profile was based on the detection limits of the respective compound. The time for the extraction process was varied from 2 minutes to 50 minutes (2, 5, 10, 20, 30, 40, and 50) to evaluate the extraction kinetics. The average peak was plotted against the extraction time. All four analytes, a representative of each class of compounds, showed maximum peak area at 30 minutes, indicating that the sol-gel coated BHEA-BPU coated capillary achieve equilibrium after 30 minutes. However, the profile suggests the good extraction of non-polar analytes PAHs and significant extraction for moderately polar (ketones and aldehydes) and highly polar analytes (phenols).

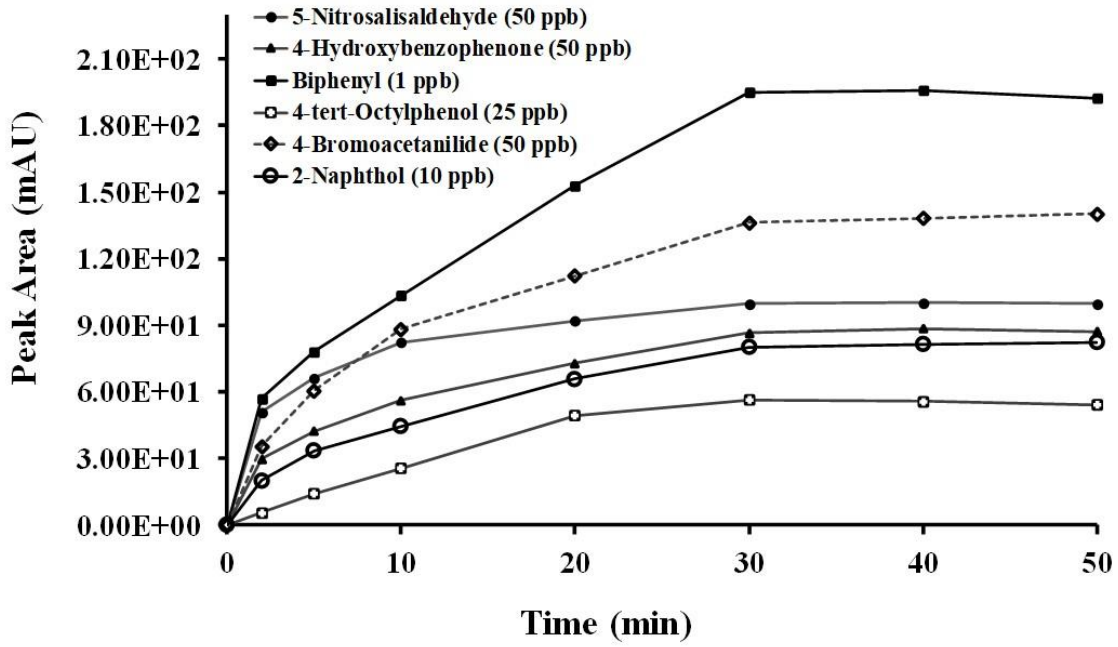


Fig 2. 14. Extraction kinetics of 4-bromoacetanilide (50 ng mL-1), 4-tert-octylphenol (25 ng mL-1), 2-naphthol (10 ng mL-1) 4-hydroxybenzophenone (50 ng mL-1), 5-nitrosalisaldehyde (50 ng mL-1), and Biphenyl (01 ng mL-1) as a representative of each class of compounds amides, phenols, alcohols, ketones, aldehydes, and polyaromatic hydrocarbon respectively.

2.3.5. Preparation method reproducibility for the BHEA-BPU coating

Moreover, to evaluate the capillary to capillary reproducibility, a special run was designed where compounds from all the classes from varied polarities were managed to be in the same chromatographic run as shown in Fig 2.15. For this purpose, the photodiode array detector was used, and all three desired wavelengths were simultaneously kept switch ON. For amides, alcohols, and aldehyde 230 nm was switched ON. Ketones and PAHs were analyzed at 254 nm. Phenols show maximum absorption at 280 nm. For an experimental procedure, three BHEA-BPU coated capillaries were cut of same size (40 cm) and used for extraction. The extraction time was kept constant (30 minutes), and a mixture of 6 compounds containing all 6 class of compounds were analyzed. In this analysis amides, alcohols, aldehydes, ketones, PAHs, and phenols showed 2.7, 5.7, 9.9, 6.3, 4.9 and 1.7 % RSD (n = 3) as shown in Table 2.3.

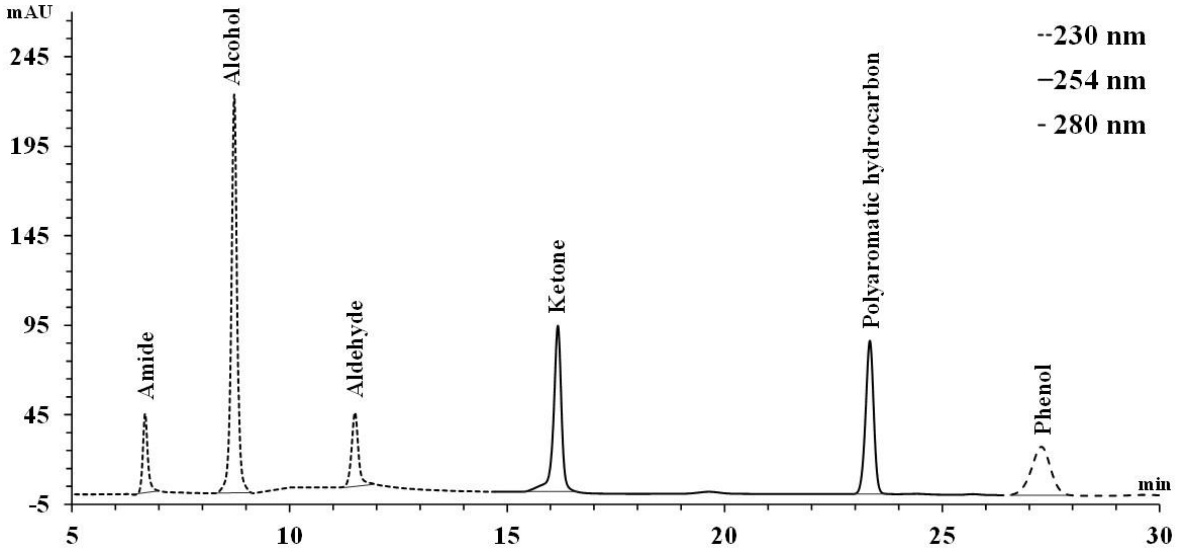


Fig 2. 15. Chromatogram showing a single run designed for determining the capillary to capillary %RSD for all different classes of compounds varied in polarities. Extraction conditions: 40 cm × 0.32 mm i.d. sol-gel BPU-BHEA- coated capillary; extraction time: 30 min: HPLC conditions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 µm d_p). Gradient elution from 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH to 70% ACN till 20 minutes and 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH till 30 minutes; runtime 30 minutes; 0.8 mL min^{-1} flow rate, UV detection using Photodiode array detector at 230 nm (amides, alcohols, and aldehydes), 254 nm (ketones, polyaromatic hydrocarbons), and 280 nm (phenols).

Table 2. 3. Reproducibility for capillary to capillary extraction, one member from each class was selected based on well resolved peaks and retention time.

Chemical class	Name	R.T.	Peak area reproducibility			Capillary to Capillary %RSD
			Mean peak area (n=3) Capillary 1	Mean peak area (n=3) Capillary 2	Mean peak area (n=3) Capillary 3	
Amides	4-bromoacetanilide	6.718	433.0	453.0	431.5	2.7
Alcohols	2-naphthol	8.821	2875.0	2688.0	2566.2	5.7
Aldehyde	5-bromobenzaldehyde	11.616	401.0	461.8	488.2	9.9
Ketone	Benzophenone	16.075	1288.0	1457.7	1344.0	6.3
PAHs	Biphenyl	23.656	1110.0	1014.8	1027.0	4.9
Phenols	4-tertoctylphenol	27.752	858.0	857.7	883.0	1.7

2.3.6. Recovery and precision in real samples

The online CME-HPLC analysis of selected analytes has shown the applicability of the BHEA-BPU coated capillary in wastewater, sea-water and pool-water. Wastewater has presented acceptable recovery between 87.5 % to 112.8 % with RSD less than 8.7 % (n = 3). Similarly, the data for sea-water and pool-water have offered adequate recovery and RSD as shown in Table 2.4. The chromatogram can be seen in supplementary information as Fig 2.16.-2.18. respectively for wastewater, sea-water, pool-water. Overall recovery range for any type of selected water and at any concentration level was ranging between 87.5 % - 114.8 with RSD less than 11.0 %.

Further utility of sol-gel BHEA-BPU coated capillary may be checked for hyphenation of capillary microextraction with gas chromatography to achieve lower detection limits due to sensitive detectors in gas chromatography as compared to UV detector in CME-HPLC analysis.

Table 2. 4 Analytical results of wastewater, sea-water and pool-water samples

Class: Analyte	**Spiked Concentration (ng mL^{-1})**	**Wastewater**		**Sea-water**		**Pool-water**	
		Recovery (%)	**RSD (%)**	**Recovery (%)**	**RSD (%)**	**Recovery (%)**	**RSD (%)**
Amides: 4-bromoacetanilide	100	105.3	8.4	113.8	9.5	107.6	10.8
	300	112.8	6.3	108.5	6.2	108.6	6.4
	500	103.1	6.2	114.8	7.4	112.9	7.5
Alcohols: 2-naphthol	100	104.8	8.4	105.2	8.8	108.6	10.9
	300	108.7	6.0	88.8	5.1	114.8	6.8
	500	97.6	5.9	114.2	7.4	113.5	7.6
Aldehydes: 5-bromobenzaldehyde	100	91.0	7.3	89.1	7.4	87.8	8.8
	300	87.8	4.9	88.2	5.0	90.7	5.3
	500	87.5	5.3	102.4	6.6	95.9	6.4
Ketones: Benzophenone	100	108.3	8.7	110.3	9.2	104.8	10.5
	300	111.2	6.2	111.0	6.3	109.4	6.4
	500	92.0	5.6	110.7	7.1	113.0	7.5
PAHs: Biphenyl	100	88.8	7.1	102.1	8.5	98.5	9.8
	300	88.1	4.9	92.0	5.3	95.1	5.6
	500	93.3	5.7	88.0	5.7	95.2	6.3
Phenols: 4-tertoctylphenol	100	108.0	8.6	113.4	9.5	108.2	9.7
	300	106.8	5.9	110.2	6.3	109.4	6.4
	500	99.7	6.0	110.3	7.1	104.7	7.0

*RSD = Relative standard deviation (n = 3)

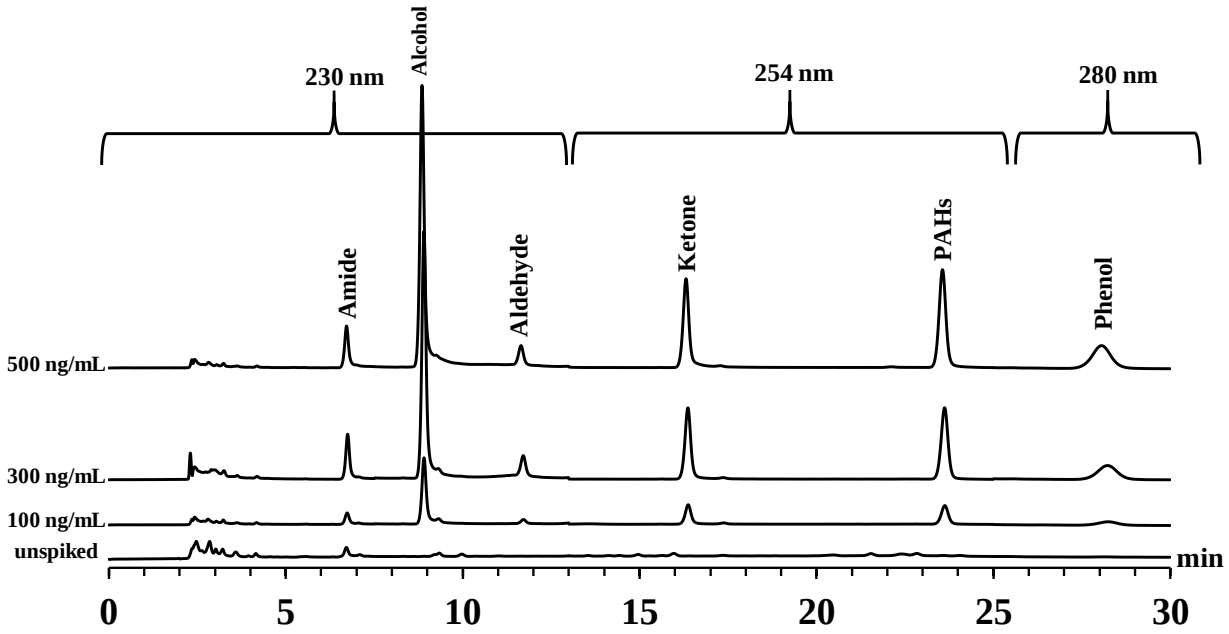


Fig 2. 16. Wastewater analysis using BHEA-BPU coated capillary: unspiked, 100 ng mL-1 spiked, 300 ng mL-1 spiked and 500 ng mL-1 spiked wastewater. Extraction conditions: 40 cm × 0.32 mm i.d. sol-gel BPU-BHEA- coated capillary; extraction time: 30 min: HPLC conditions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 µm dp). Gradient elution from 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH to 70% ACN till 20 minutes and 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH till 30 minutes; runtime 30 minutes; 0.8 mL min^{-1} flow rate, UV detection using Photodiode array detector at 230 nm (amides, alcohols, and aldehydes), 254 nm (ketones, polyaromatic hydrocarbons), and 280 nm (phenols).

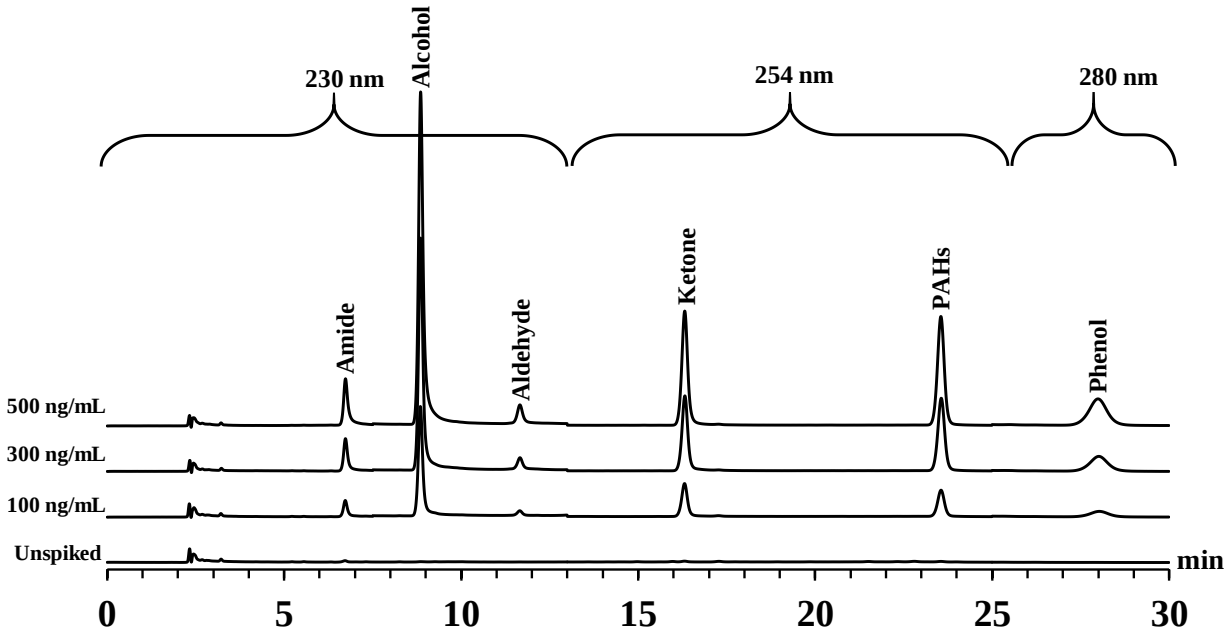


Fig 2. 17. Sea-water analysis using BHEA-BPU coated capillary: unspiked, 100 ng mL-1 spiked, 300 ng mL-1 spiked and 500 ng mL-1 spiked sea-water. Extraction conditions: 40 cm × 0.32 mm i.d. sol-gel BPU-BHEA-coated capillary; extraction time: 30 min: HPLC conditions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 μm dp). Gradient elution from 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH to 70% ACN till 20 minutes and 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH till 30 minutes; runtime 30 minutes; 0.8 mL min^{-1} flow rate, UV detection using Photodiode array detector at 230 nm (amides, alcohols, and aldehydes), 254 nm (ketones, polyaromatic hydrocarbons), and 280 nm (phenols).

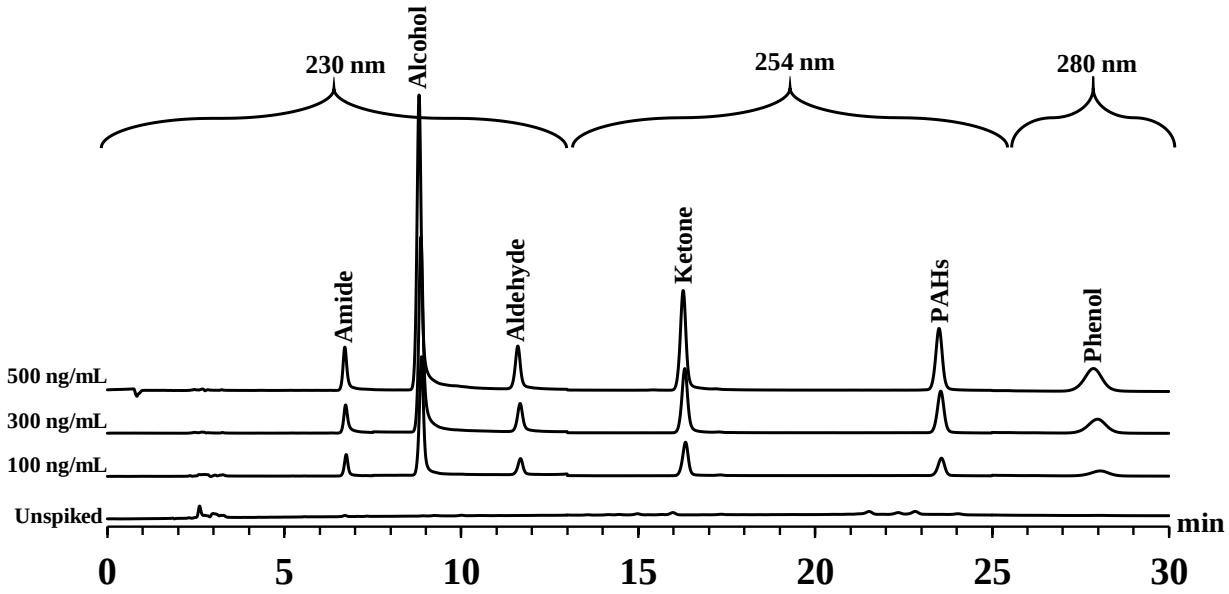


Fig 2. 18. Pool-water analysis using BHEA-BPU coated capillary: unspiked, 100 ng mL-1 spiked, 300 ng mL-1 spiked and 500 ng mL-1 spiked pool-water. Extraction conditions: 40 cm × 0.32 mm i.d. sol-gel BPU-BHEA-coated capillary; extraction time: 30 min: HPLC condit ions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 μm dp). Gradient elution from 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH to 70% ACN till 20 minutes and 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH till 30 minutes; runtime 30 minutes; 0.8 mL min^{-1} flow rate, UV detection using Photodiode array detector at 230 nm (amides, alcohols, and aldehydes), 254 nm (ketones, polyaromatic hydrocarbons), and 280 nm (phenols).

2.4. Summary of the work

This sol-gel BHEA-BPU coating is the first-time report on the creation of such surface and utilization for online CME-HPLC analysis. This coating has the direct chemical attachment of the sol material to the inner surface of the capillary which makes this coating resistant to solvent flow and best for preconcentration of analytes from the water samples. This urea functionalized sol-gel surface bonded BHEA-BPU coating has shown extraordinary extraction abilities towards a complete range of polarities of the analytes. The online CME-HPLC analysis using a UV detector has shown low and sub ppb level detection limits (0.10 ng mL^{-1} – 14.29 ng mL^{-1}) for amides, phenols, alcohols, ketones, aldehydes, and PAHs. However, the detection limits may improve by using sol-gel BHEA-BPU coated capillaries used in the CME-GC analysis due to higher detector sensitivities.

CHAPTER 3

Yttria-based sol-gel coating for capillary microextraction on-line coupled to high-performance liquid chromatography.

3.1. Introduction

The solid phase microextraction is a technique which was developed by coating the fused silica capillary on the outer surface with a polymer [9,57,58]. This technique implies a solvent-free approach for sample preparation and is known as fiber SPME. The analytes were preconcentrated on the small coated surface by various methods (headspace, or direct insertion) and then, injected into a gas chromatographic (GC) system. Fiber SPME has many shortcomings, including difficulties with thick coatings, low sample loading capacity, degradation of coating material in thermal and solvent variations [76], practical problems for hyphenation with liquid chromatographic systems [77,78] and susceptibility to mechanical damage of the fiber coating [79,80].

Introduction of the capillary microextraction (CME) [64] or in-tube solid phase microextraction [9,81] has made it easier to hyphenate the microextraction technique to liquid chromatographic techniques. CME is hugely attractive because of its suitability for the microextraction analysis of thermally liable compounds [82] that cannot be evaluated using gas chromatographic techniques. CME has removed all the major disadvantages of fiber SPME including the mechanical damage during the analysis because CME is a wall coated capillary column. The analytes of interest were extracted from aqueous samples and preconcentrated inside the wall coated capillary, later desorbed by mobile phase and analyzed. Previously, the wall coated capillary was a thin layer of adsorbent inside the capillary and it was not chemically bonded. Therefore, the wall coated capillary had some

disadvantages such as poor stability of the wall coating for thermal, solvent, varied pH environment and limited sorption for the analytes. Because of all these shortcomings, limited applications of capillary microextraction were seen as the wall coating cannot survive the HPLC mobile phases with various pH conditions [83].

The introduction of the of the sol-gel chemistry as a coating method [76] for solid phase microextraction [64] was able to diminish the effect of solvent and thermal stabilities of the coating. This is because the sol-gel chemistry involves the chemical bonding of the polymer coating inside the fused silica capillary. Utilization of sol-gel chemistry has helped to introduce different types of functional groups into the growing sol-gel network that resultantly form a polymeric coating inside the fused silica capillary and enhances the extraction and sensitivity. Right after the introduction of sol-gel chemistry for capillary microextraction, many silica-based [84–86] and non-silica based [72,87,88] polymeric coatings were introduced for better features and enhanced extraction. On the other hand, new solid phase microextraction techniques were developed but the capillary microextraction has the advantage of online hyphenation with [89] HPLC. Capillary microextraction is a simplified technique with few parameters to optimize. Resultantly, it becomes more precise than other techniques. Other solid-phase microextraction techniques have several parameters to optimize for a single analysis. Taking all these factors into consideration, there is still a need to create more new surfaces and materials [71,90–93] to make this technique more versatile.

To the best of our knowledge, we presented the first synthesis of sol-gel [bis(hydroxyethyl)amine] terminated polydimethylsiloxane - Yttrium methoxyethoxide (BHEA-Y) surface immobilized coating for capillary microextraction. This material

showed remarkable extraction sensitivity for compounds having various polarities from non-polar to highly polar. The selected analytes for online CME-HPLC analysis are well established environmental pollutants. All are reported for toxicity and persistence in our environment including PAHs, alcohols [94], aldehydes, ketones, amides [95,96] and phenols [97]. Moreover, this work also presents method validation parameters for the important contaminants phenols with real sample analysis. Phenols are used for the synthesis of pesticides, dyes, explosives and drugs in various industries [102]. The hydroxyl group of phenols react with disinfection by-products and form chlorinated phenols that have higher toxicity [103]. Similarly the interaction of nitrite with phenol in the environmental water results in the formation of nitrophenols that are more persistent and toxic in nature [104]. Keeping in mind the aforementioned hazards of the phenols, its preconcentration and real sample analysis were also added to this work as an application of sol-gel BHEA-Y coated capillary for online CME analysis.

3.2. Experimental

3.2.1. Equipment

For the analysis part, a HPLC system (Agilent Technologies, USA) equipped with a quaternary pump (G1311B/C), a DAD (G4212B), with manual injection port, an analytical column Agilent ZORBAX Eclipse XDB C-18 (5µm, 4.6mm id x 250 mm) and Chemstation software were used. An Agilent 1260 infinity isocratic pump (G13103B) was also utilized for sample flow through the coated capillary. For the preparation and homogenized mixing of BHEA-Y sol, Thermofisher Scientific MaxiMix Vortex mixer was used (model M16715). Using Sorvall^{TM} Legend^{TM} micro17 Microcentrifuge, the precipitates in the sol-solution were settled down. X-ray photoelectron spectroscopy (XPS) analysis of the BHEA-Y coating was conducted on *Thermo Scientific ESCALAB 250Xi* (PHI 5000 Versa Probe II, ULVAC-PHI Inc., UK) to determine the bonding state and surface chemical composition. Before analysis, a chunk of the polymer sample was mounted on carbon tape and subjected to high vacuum to remove impurities or moisture adsorbed on the sample. Thermal stability and decomposition of BHEA-Y coating were observed by thermogravimetry analysis (SDT Q600, V20.9 Build 20, thermal analyzer, USA) under nitrogen (N_2) environment from 30 to 600 ^oC with the constant heating rate of 10 $^oC\ min^{-1}$. The morphological information of the BHEA-Y coated in capillary fused silica was examined by field emission scanning electron microscope (FE-SEM) from TESCAN, LYRA 3 Czech Republic, using secondary electron (SE) and backscattered electron (BSE) mode at an accelerating voltage of 30 kV and equipped with energy dispersive X-ray spectrometer (EDS, Oxford Inc.) detector for elemental analysis. However, the polarity of the surface material was determined from contact angle calculations with water using

Attention theta optical tensiometer, C204A, (Biolin scientific, Finland), equipped with one-attention software (version 3.2, r5971).

3.2.2. Chemicals and materials

Fused silica capillary (320 µm I.D.) was purchased from Polymicro Technologies USA. Yttrium methoxyethoxide (YMEO) and [Bis(hydroxyethyl)Amine] Terminated Polydimethylsiloxanes (BHEA) were purchased from Gelest, USA. 4-bromoacetanilide , N-methyl-1-naphthylacetamide, benzanilide, 4-fluorophenol, 2,3-dichlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 2-benzyl-4-chlorophenol, pentachlorophenol, 4-tert-octylphenol, 2-naphthol, 1-naphthol, diphenylcarbinol, 5,5-dimethyl-1,3-cyclohexadione, 1,2-naphthoquinone, 1-indanone, 4-methoxyacetophenone, 4-hydroxybenzophenone, 2-hydroxy-2-phenylacetophenone, propiophenone, benzophenone, benzil, 4-chlorobenzophenone, 4-hydroxy-3-methoxybenzaldehyde, 5-nitrosalisaldehyde, 4-chlorobenzaldehyde, 5-bromobenzaldehyde, naphthalene, biphenyl, fluorene, phenanthrene, and anthracene were purchased from Sigma-Aldrich USA.

3.2.3. Preparation of sol-solution

The sol-solution was prepared by vortexing a sol-gel active polymer BHEA 200 µL with 200 µL ethanol in a microcentrifuge tube for 30 s. The sol-gel active precursor YMEO (100 µL) and 8.0 µL water were added to the reaction mixture and vortex for 90 s. The sol-solution was then ready for the coating inside the fused silica microextraction capillary.

3.2.4. Preparation of sol-gel BHEA-Y coated microextraction capillary

A 3.0 m long fused silica capillary (320 µm i.d.) was rinsed with methanol and dichloromethane and pretreated with 1.0 M NaOH solution, where NaOH solution was kept inside the capillary for 2 hours by closing both ends of the capillary and flushed later.

The capillary was rinsed with 0.1 M HCl to neutralize any NaOH present and later rinsed with water to confirm the cleaning. All the rinsing and etching procedures were done under helium pressure using an in-house built gas pressure-operated capillary filling device. The capillary was then kept inside the GC oven for drying at 250 ^{O}C for overnight under helium flow and later took out from GC and installed to an in-house built gas pressure-operated capillary filling device for rinsing with methanol and dichloromethane before coating.

A 1.0 m long piece of the pretreated capillary was used for sol-gel coating using gas pressure operated purging device. The sol solution was purged to the capillary and kept inside the capillary for 10 min to enhance the on-surface reaction of the sol coating. The unreacted sol solution was expelled out of the capillary using helium gas pressure and the helium flow was continued for 10 additional min. The coated capillary was then subjected to post-treatment [72] using the GC oven to make the sol-gel material more porous and cleaned.

3.2.5. Online capillary microextraction (CME) and HPLC analysis

The online CME-HPLC analysis is presented in Fig 3.1, where there is a sample flow system, manual injection port, and an HPLC system. The analytical column was pre-equilibrated with the mobile phase and kept ready for manual injection. A 40 cm long sol-gel BHEA-Y coated capillary was fixed in place of the sample loop in the manual injection port. The injection port was switched to “load” mode and an aqueous sample having the analytes of interest was pre-concentrated in the sol-gel BHEA-Y coated capillary with a constant flow of 1.0 mL/min using the isocratic pump. The deionized water was later flushed through the sol-gel BHEA-Y coated capillary to remove the sample present inside the capillary loop. The Injection port was then switched to “inject” mode for the desorption

of the extracted analytes from sol-gel BHEA-Y capillary to the analytical column. The analytical column separated the analytes based on the interaction between mobile phase and stationary phase and detected with a UV detector.

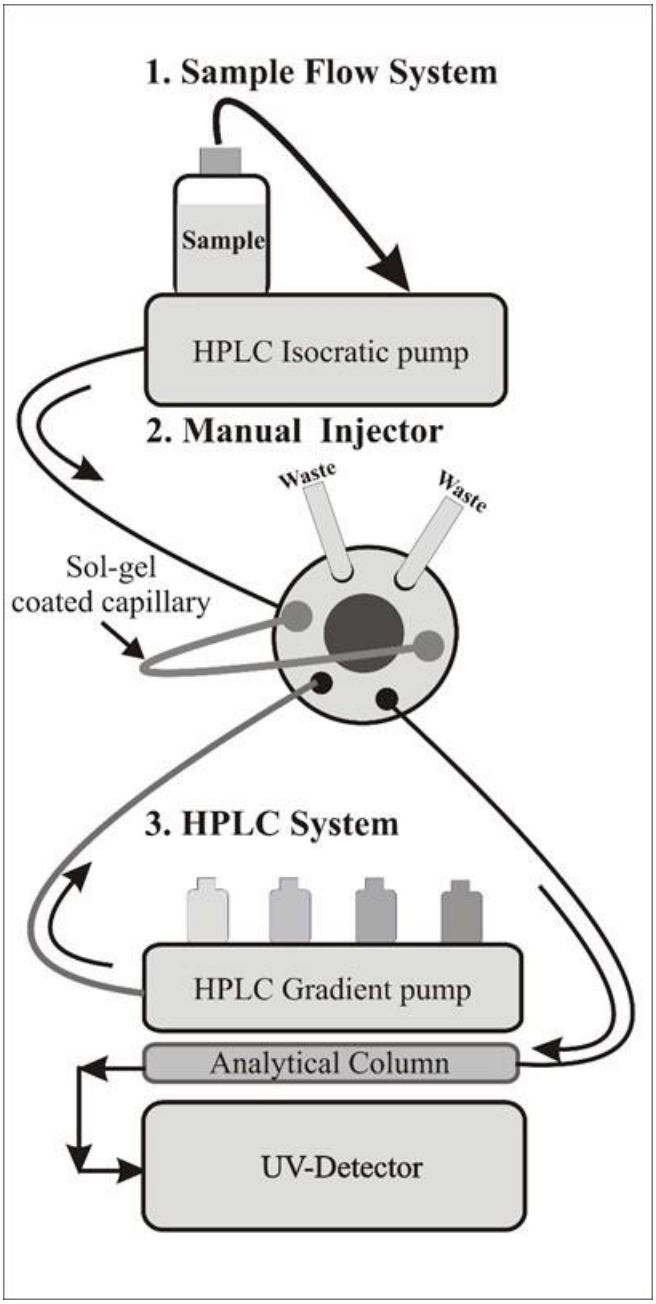


Fig 3. 1. Representation of the setup; CME-HPLC online analysis

3.2.6. Extraction comparison of Yttria based coating and BHEA-Y based coating

The extraction performance of the capillary coated with Yttria and capillary coated with BHEA-Y was compared. For the preparation of yttria-based coating, the sol-solution was prepared exactly as it was prepared for BHEA-Y except the BHEA is not added. Moreover, to support the extraction results, contact angles were determined to present the hydrophobicity or hydrophilicity. The sol-gel Yttria based coating was also characterized by FESEM and EDS. A specific run was designed containing one member from all of the different classes of compounds and the diode array detector was used by varying absorption wavelengths for each class of compounds. The variables were kept constant and extraction was tested in triplicates.

3.2.7. Analysis of the analytes with various polarities

Using this complete extraction and analysis procedure with the selected BHEA-Y coated capillary, compounds ranging in various polarities were tested for extraction ability, enrichment factors [98] and detection limits. Various classes of compounds include amides, phenols, alcohols, ketone, aldehyde, and polyaromatic hydrocarbons were tested. Experimentally, the enrichment factor was calculated by dividing the peak area of the extracted analyte to the peak of the standard analyte. The peak area for the analyte from the standard solution was obtained by injecting 20 µL of the standard solution to the HPLC manual port without any extraction involved. The limit of detection of all the analytes was calculated by signal to noise ratio method (S/N = 3).

Apart from proving the extraction ability of the coated capillary, the solvent and chemical stability of the coating material was also tested. For this purpose, extreme pH

environments were applied to the coated capillary for 24 hours. The coated capillaries were continuously rinsed using 1.0 M HCl (pH = 0) and NaOH (pH = 14) for 24 hours and then tested for the extraction of the analytes (each member from the various class of compounds). The BHEA-Y coated capillary was also tested for preparation method reproducibility. Three capillaries were prepared keeping all the synthesis and coating factors same and installed to online CME-HPLC system for the analysis to compare the extraction efficiency of the analytes.

3.2.8. Method validation and real sample analysis of phenols

The main objective of this work was to present a new sorbent surface material for the extraction of analytes having different polarities. Any well-developed and validated chromatographic method may be applied for the analytes in aqueous samples. Phenols, a polar class of compounds, are a well-known environmental pollutant, were selected to present the analytical method validation. Some of the analytical tools considered that include calibration curve, linear regression coefficient, limit of detection, limit of quantification, inter-day and intra-day precision, and capillary to capillary precision, etc. The calibration curves were plotted using 5, 10, 25, 50, 100, 200, and 400 ng mL^{-1} concentration versus peak area of the phenols. The linear trendline model was applied and linear regression equation and constant were calculated. Limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the signal to noise ratio method. Reproducibility of the results or the precision was calculated statistically from the ratio of standard error of regression to mean of the data and converted to percentage. Inter-day, intra-day, and capillary to capillary precision were evaluated by repeating the analysis thrice (n = 3).

To evaluate the applicability of the method in the real sample, wastewater and swimming pool-water samples were collected and analyzed using BHEA-Y coated capillary for online CME-HPLC analysis of phenols. The samples were filtered using 4.5-micron membrane filter paper. The filtered sample was then passed through the capillary for extraction and HPLC analysis to see the presence of phenols in the sample. The real samples were also assessed for recovery and precision by spiking with three concentrations (5, 50, and 200 ng mL^{-1}) from the linear range.

3.3. Results and discussion

The aim of this work was to develop a Yttria based surface bonded sol-gel coating for capillary microextraction in hyphenation with high-performance liquid chromatography. The distinct characteristics of sol-gel chemistry help to make a surface bonded BHEA-Y coating on the inner walls of the fused capillaries.

3.3.1. Chemical anchoring of sol-gel based BHEA-Y coating

The sol-gel precursor yttrium methoxyethoxide (YMEO) and sol-gel active polymer [Bis(hydroxyethyl)amine] terminated polydimethylsiloxanes (BHEA) continue the hydrolysis and polycondensation to form the colloidal system called sol. Ethanol acts as a solvent to dissolve the contents of the sol solution. The hydrolyzed reactive species further undergo polycondensation reactions to produce the yttria-based three-dimensional network.

The sol-gel active polymer BHEA may undergo condensation reactions with the silanol groups on the inner side of the fused silica capillary and produce a surface bonded polymer with sol-gel active precursor, forming yttria network over the surface. Later, the heat treatment of this coated capillary causes crosslinking of the polymer resultantly enhancing the porosity. Overall, the sol-gel process for the creation of yttria-based coating involves (i) hydrolysis of the yttrium methoxyethoxide precursor, (iii) polycondensation of the sol-gel active polymer BHEA with yttria sol-gel network, and (iv) chemical immobilization of the sol-gel material to the silanol groups on the inner surface of fused silica capillaries. Resultantly, this create a yttria-based sol-gel surface bonded coating for microextraction. Fig 3.2. shows the hydrolysis, polycondensation of the YMEO precursor and anchoring of the final coating material network inside the capillary wall.

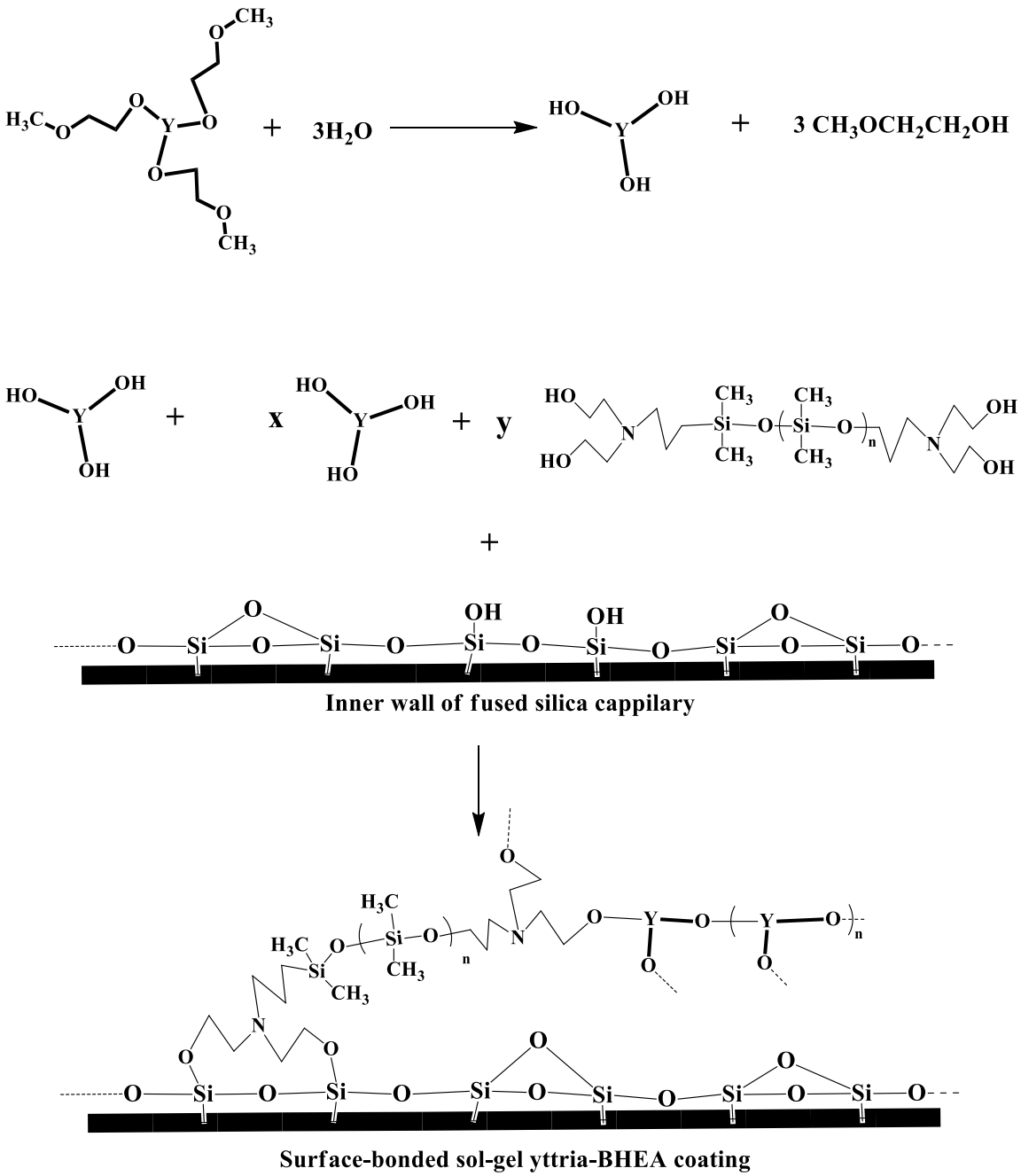


Fig 3. 2. Sol-gel BHEA-Y based coating representing hydrolysis and one step polycondensation reactions inside the capillary.

3.3.2. Yttria based coating versus BHEA-Y based coating

Two capillaries were prepared, one with BHEA polymer backbone and one without BHEA polymer and the extraction was compared (Fig 3.3.). The prepared coating without BHEA polymer was characterized using FESEM (Fig 3.4.) and EDS (Fig 3.5.). The characterization results successfully supported the chemical immobilization of yttrium oxide inside the capillary. The extraction results have shown that BHEA-Y based coating extracted all classes of the analytes 10-15 times better than yttria. This was because of the BHEA polymer provided more sorbent surface and a non-polar moiety. The sol-solution of the BHEA-Y coating and yttria coating was employed on the glass slides and a thin layer was formed. These glass slides were used to determine the contact angles with water. The yttria-based coating showed higher hydrophilicity, as the contact angle was 67.309^{o}, and the BHEA-Y coating showed the decrease in the hydrophilicity, as obvious from the increase in the contact angle to 85.478^{o}. Therefore, the BHEA-Y based coating was selected, as it provided an overall hydrophilic surface, better extraction and an ability to extract analytes of various polarities.

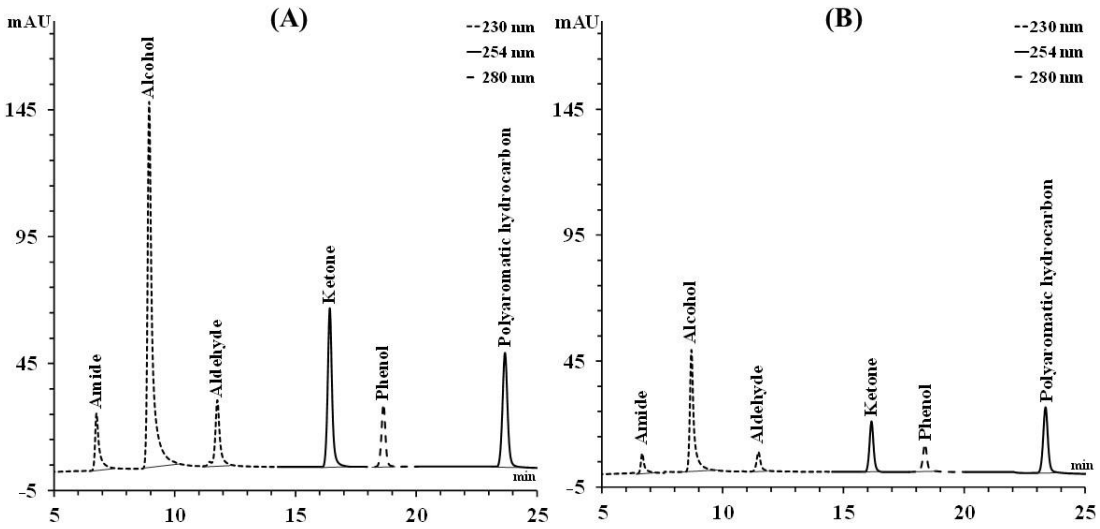


Fig 3. 3. Extraction of each representative compound of class (A) capillary coated with BHEA-Y based coating (B) capillary coated with Yttria based coating. Extraction conditions: 40 cm × 0.32 mm i.d. extraction time: 20 min: HPLC conditions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 µm d_p). Gradient elution from 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH to 70% ACN till 20 minutes and 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH till 30 minutes; runtime 30 minutes; 0.8 mL min^{-1} flow rate, UV detection using Photodiode array detector at 230 nm (amides, alcohols, and aldehydes), 254 nm (ketones, polyaromatic hydrocarbons), and 280 nm (phenols).

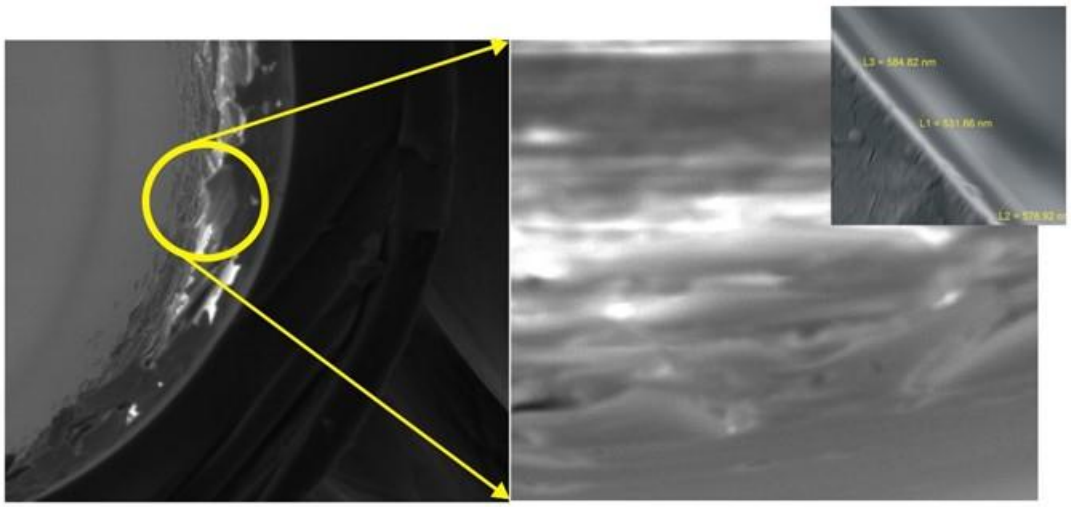


Fig 3. 4. SEM analysis of Yttrium oxide coating inside the fused silica capillary at low (A) and high (B) magnifications. The inset showing in (B) the thickness of polymer coated in capillary fused-silica.

Fig 3. 5. EDS analysis of yttrium oxide polymer coated in capillary fused-silica with inset representing atomic weight (%)

3.3.3. Characterization of sol-gel BHEA-Y coating for CME

Compositional analysis of sol-gel derived polymeric yttria material/sample/adsorbent (BHEA-Y) was analyzed by X-ray photoelectron spectroscopy (XPS). The XPS analysis survey reveals the presence of carbon, nitrogen, oxygen, silicon and yttrium. The peak deconvolution of each constituent gives quantitative information about the surface percentage with respect to their binding energies and oxidation states. Fig 3.6. presents the XPS spectrum of BHEA-Y, with carbon (C 1s) representing the major component (approximately 57 %) found at binding energy (BE) of 282.58 eV. The C 1s at this BE corresponds to carbon bonded with silicon and nitrogen atoms. Oxygen (O1s) was observed at BE 530.04 eV, corresponding to oxygen attached to metal and silicon with 21.73 % surface atomic percent [99]. The evidence of silicon (17.86 %) was found at BE of 99.94 eV, corresponding to Si 2p attached to oxygen and carbon atoms. The observed acquired spectrum revealed two forms of nitrogen at 397.51 and 400.36 eV, corresponding to N 1s in nitride-form in two different environments, and the total surface concentration of N is < 1%. Similarly, yttrium was observed in low concentration as compared to C, Si, and O. The presence of yttria bonding state at $3d_{5/2}$ $3d_{3/2}$ spin orbitals was observed in form of yttria at BE energy of 156.95 and 159.02 eV, respectively. The atomic surface percent with respect to other constituents of BHEA-Y is 2.67 %, using BE at 156.95 eV for $3d_{5/2}$ spin orbital as in Table 3.1. The observed elemental analysis with their corresponding bonding energies of BHEA-Y confirms the successful attachment of yttria on BHEA through sol-gel synthesis method for efficient and maximum extraction application.

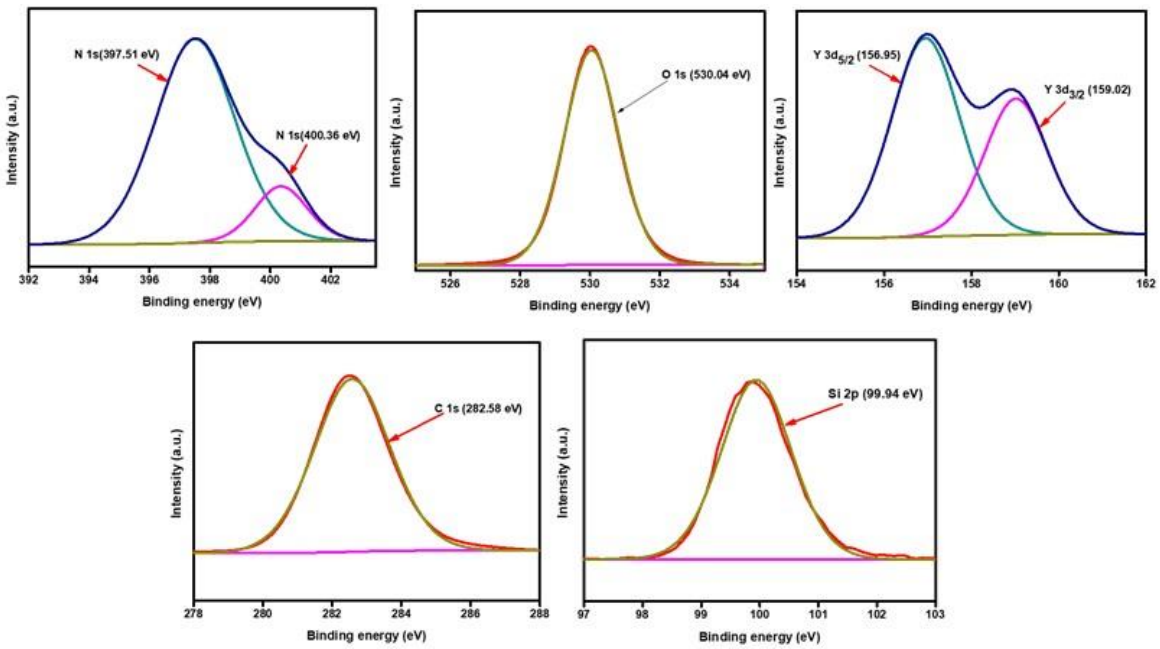


Fig 3. 6. XPS analysis of as-synthesized BHEA-Y polymer before capillary coating in fused-silica showing different bonding states of Y, C, N, O and Si.

Table 3. 1. The bonding states and atomic weight (%) of polymer composition by XPS

Name	**Peak BE**	**Atomic %**
O1s	530.04	21.73
Si2p	99.94	17.86
Y3d$_{5/2}$	156.95	2.63
N1s	397.51	0.68
N1s	400.36	0.12
C1s	282.58	56.99

The BHEA-Y polymer was kept in the inert nitrogen environment and the temperature was raised to 600 ^{o}C to evaluate the thermal and structural stability. A gradual loss of weight (approx. 10%) was seen from room temperature 30 ^{o}C to 300 ^{o}C in the first phase transition. This observation could be attributed to the loss of adsorbed water and other impurities associated with the sol-gel prepared polymer. Furthermore, gradual decomposition of the organic material network (backbone) in the polymer caused nearly 50% loss in weight between 300 to 400 ^{o}C. Between 400-600 ^{o}C, phase transition indicates the complete decomposition of polymer and formation of carbon/soot [100,101]. Therefore, the thermal stability and practical working temperature of BHEA-Y polymer will be within the range of 0 – 300 ^{o}C without significant decomposition as shown in Fig 3.7.

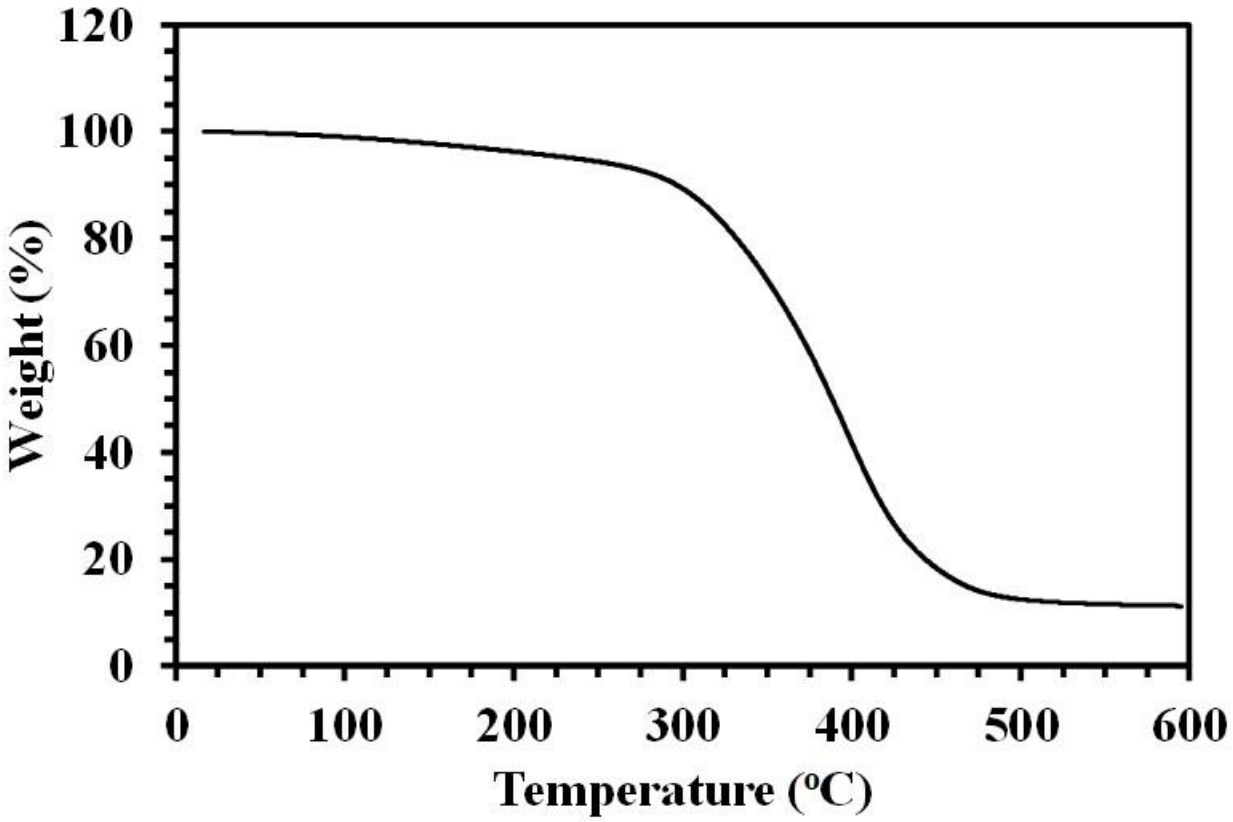


Fig 3. 7. Thermogravimetric analysis of as-synthesized BHEA-Y polymer before capillary coating in fused-silica capillary.

The successful sol-gel BHEA-Y polymer coating inside the capillary was observed by scanning electron microscope (SEM) at high resolution. As shown in Fig 3.8., the BHEA-Y polymer was uniformly coated inside the fused silica of 320 µm i.d. with estimated 8.0 µm thickness. The morphology of the inner surface reveals no cracks or discontinuity of BHEA-Y coating. This may offer better accessibility and high sorption capacity of extractant during micro-extraction operation. In addition, the energy dispersive spectroscopy (EDS) was used to confirm the elements and to complement the compositions of the polymer as observed by XPS. There exists a good correlation between the obtained elemental weight (%) by EDS and the theoretical calculation from the monomers, as shown in Fig 3.9.(inset).

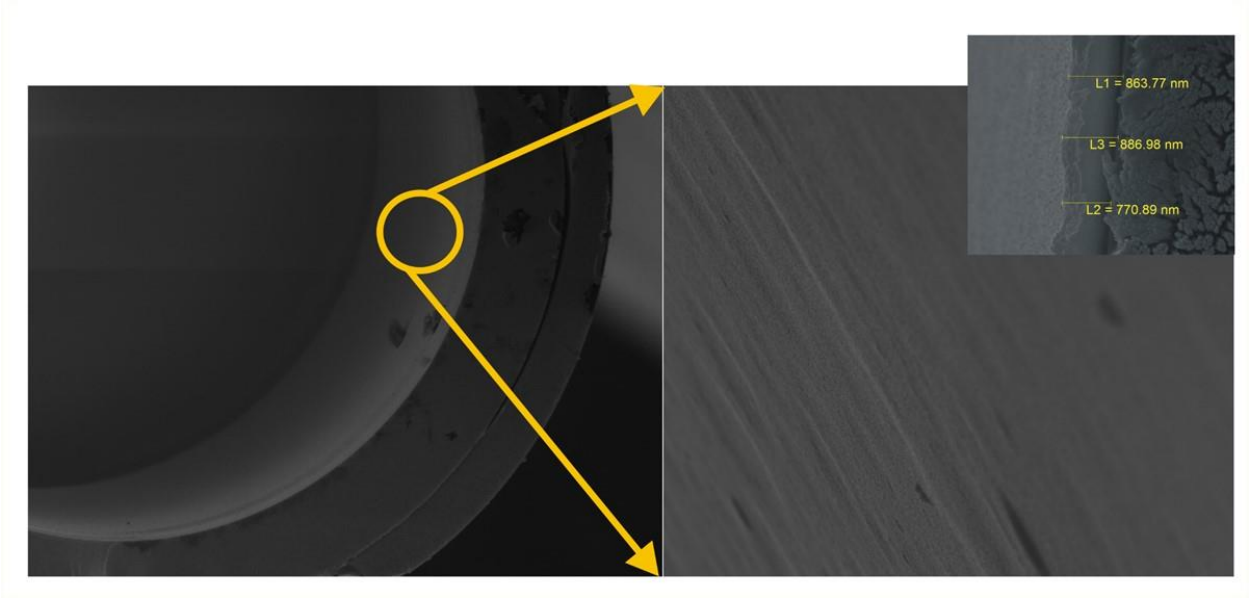


Fig 3. 8. SEM analysis of BHEA-Y coating inside the fused silica capillary at low (A) and high (B) magnifications. The inset showing in (B) the thickness of polymer coated in capillary fused-silica.

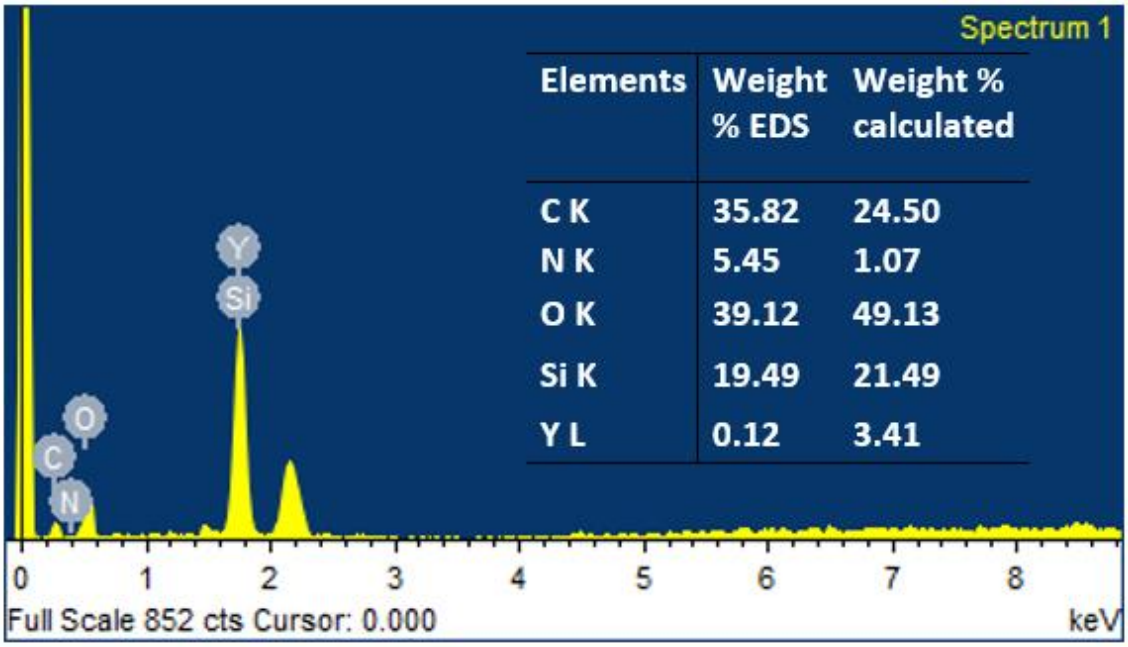


Fig 3. 9. EDS analysis of BHEA-Y polymer coated in capillary fused-silica with inset representing atomic weight (%).

3.3.4. Online CME-HPLC analysis using sol-gel coated BHEA-Y capillary

This work has presented the excellent extraction efficiencies for various classes of compounds, ranging from non-polar to highly polar compounds using BHEA-Y based coating inside the capillary. The selected classes include the polyaromatic hydrocarbons, aldehydes, ketones, alcohols, phenols, and amides. The BHEA-Y coated capillary presented exceptional ability to be equally suitable for the polar analyte and the non-polar analyte. Extraction of non-polar analytes was due to polydimethylsiloxanes (PDMS) moiety in the BHEA sol-gel active polymer and polar analytes were extracted efficiently due to hydrophilic yttrium oxide moiety over the polymeric surface. Amides are considered as polar analytes; therefore, these are selected to prove the extraction ability of the BHEA-Y coated capillary for highly polar analytes. Online CME-HPLC analysis of amides was successfully conducted using BHEA-Y based coated capillary as shown in Fig 3.10. The analysis presented appropriate enrichment factors (78.9-153.6), low detection limits ranging between 2.60-5.95 ng mL^{-1} (S/N=3) and reliable %RSD (less than 6.1%) where n=3 as shown in Table 3.2.

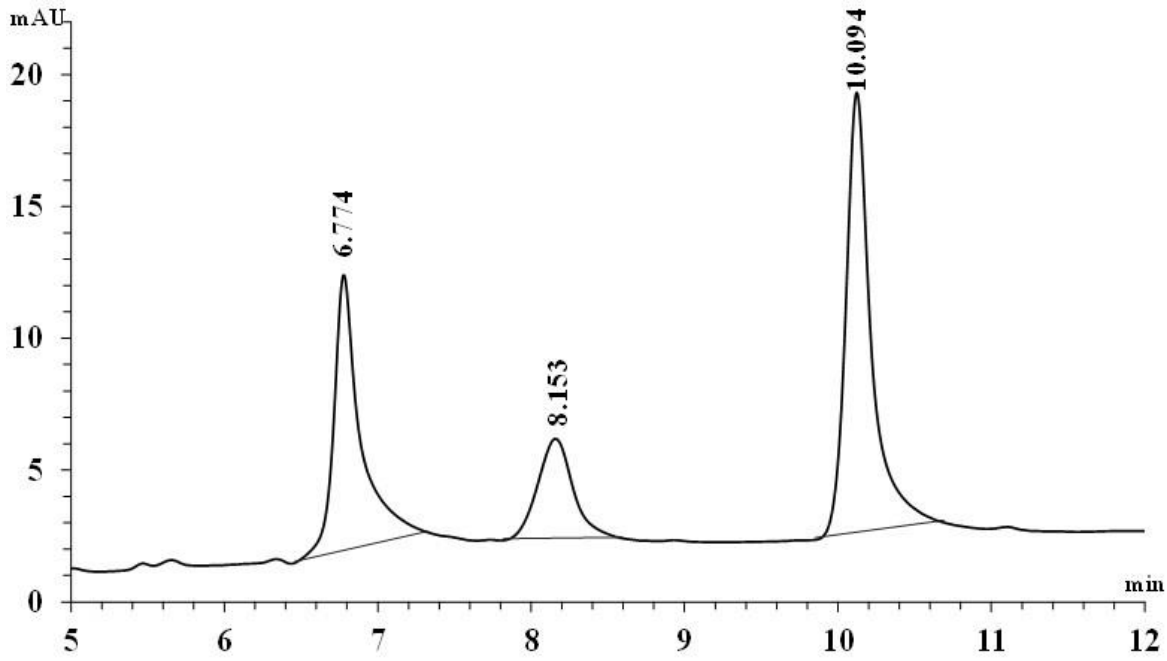


Fig 3. 10. Capillary microextraction-HPLC analysis of Amides. Extraction conditions: 40 cm × 0.32 mm i.d. sol-gel BHEA-Y- coated capillary; extraction time: 20 min: HPLC conditions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 µm dp). Gradient elution from 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH to 70 % ACN till 20 min and 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH till 30 min; runtime 30 min; 0.8 mL min^{-1} flow rate, UV detection at 230 nm, ambient temperature. Peaks: (t_R=6.774) 4-bromoacetanilide (25 ng mL^{-1}), (t_R=8.153) n-methyl-1-naphthylacetamide (25 ng mL^{-1}), (t_R= 10.094) benzanilide (25 ng mL^{-1}).

Table 3. 2. Peak area reproducibility and detection limits for Amides, phenols, alcohols, ketones, aldehydes, and PAHs in CME-HPLC using a sol-gel BHEA-Y coated microextraction capillary a

Analyte class and name	Peak area reproducibility (n=3)		Detection limit ($ng\ mL^{-1}$) (S/N=3)	Enrichment factors
	Mean peak area (milli absorbance unit)	RSD (%)		
Amides				
4-bromoacetanilide	142.2	6.1	3.62	110.4
N-methyl-1-naphthylacetamide	61.9	5.3	5.95	78.9
Benzanilide	204.0	2.9	2.60	153.6
Phenols				
4-flourophenol	13.1	4.2	1.35	95.5
2,3-dichlorophenol	22.8	4.7	1.19	135.8
2,4-dichlorophenol	30.5	3.3	0.94	160.0
2,4,6-trichloropehnol	29.5	6.3	0.91	175.5
2-benzyl-4-chlorophenol	30.1	2.7	0.96	155.4
Pentachlorophenol	11.0	6.2	1.28	116.5
4-tertoctylphenol	7.2	2.8	1.39	93.0
Alcohols				
2-naphthol	462.6	1.9	0.83	300.0
1-naphthol	198.0	2.9	1.04	240.0
Diphenylcarbinol	158.3	1.7	1.25	200.0
Ketones				
5,5-dimethyl-1,3-cyclohexadione	25.6	5.6	7.35	54.4
1,2-naphthoquinone	37.8	3.2	6.85	58.4
1-indanone	78.7	1.7	5.68	70.4
4-methoxyacetophenone	135.3	3.3	3.85	104.0
4-hydroxybenzophenone	145.0	4.9	3.65	109.6
2-Hydroxy-2-phenylacetophenone	65.3	5.3	5.95	67.2
Propiophenone	159.4	5.2	3.57	112.0
Benzophenone	375.6	3.7	1.67	240.0
Benzil	361.3	5.3	1.79	224.0
4-chlorobenzophenone	392.3	3.4	1.56	256.0
Aldehydes				
4-Hydroxy-3-methoxybenzaldehyde	147.0	0.6	3.68	108.8
5-Nitrosalisaldehyde	210.2	5.0	2.59	154.4
4-chlorobenzaldehyde	28.1	5.7	7.35	60.4
5-bromobenzaldehyde	201.4	6.1	2.78	144.0
Polyaromatic hydrocarbons				
Naphthalene	61.7	6.8	0.24	1064.4
Biphenyl	212.2	5.3	0.18	1378.1
Fluorene	77.5	1.5	0.23	1101.3
Phenanthrene	85.8	3.5	0.22	1102.4
Anthracene	59.6	5.8	0.29	856.3

For amides (25 $ng\ mL^{-1}$), phenols (5 $ng\ mL^{-1}$), alcohols (10 $ng\ mL^{-1}$), ketones (25 $ng\ mL^{-1}$), and aldehydes (25 $ng\ mL^{-1}$): gradient elution from 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH to 70% ACN for 20 min and 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH till 30 min; runtime 30 min; 0.8 mL min^{-1} flow rate, UV detection at 230 nm (amides, alcohols and aldehydes), 280 nm (phenols), 254 nm (ketone)

For PAHs (01 $ng\ mL^{-1}$): gradient elution from 80:20 (v/v) ACN:Water to 100 % ACN for 20 min; 0.8 mL min^{-1} flow rate; UV detection at 254 nm.

[a] Extraction conditions: 40 cm × 0.32 mm i.d. sol-gel BHEA-Y- coated capillary; extraction time: 20 min: HPLC conditions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 µm d_p).

The CME-HPLC analysis of the polar phenols using BHEA-Y coated capillary is presented in Fig 3.11. The selected phenols have higher polarity because they are halogenated. The analysis of the seven selected phenols has shown in Fig 3.11. with the concentration of 5 ng mL^{-1}. The sol-gel BHEA-Y coated capillary showed extraordinary enrichment factors ranging from 93.0-175.5, and reproducibility less than 6.5 (n = 3) with detection limits (0.91 ng mL^{-1}- 1.39 ng mL^{-1}) as shown in Table 1. These low detection limits and efficient extraction of polar moieties may be explained by the polar yttrium oxide moiety in the sol-gel BHEA-Y coated capillary.

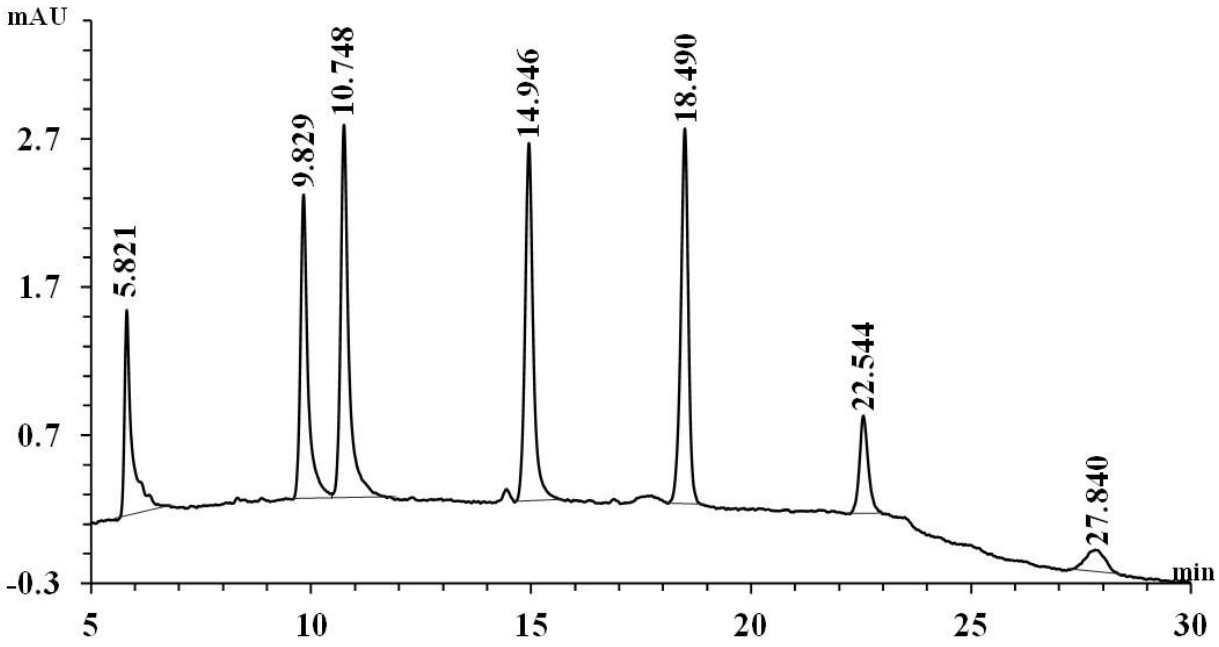


Fig 3. 11. Capillary microextraction-HPLC analysis of Phenols. Extraction conditions: 40cm × 0.32mm i.d. sol-gel BHEA-Y- coated capillary; extraction time: 20 min: HPLC conditions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 µm dp). Gradient elution from 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH to 70% ACN till 20 minutes and 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH till 30 minutes; runtime 30 minutes; 0.8 mL min^{-1} flow rate, UV detection at 280 nm, ambient temperature. Peaks: (t_R=5.821) 4-fluorophenol (5 ng mL^{-1}) (t_R= 9.829) 2,3-dichlorophenol (5 ng mL^{-1}), (t_R=10.748) 2,4-dichlorophenol (5 ng mL^{-1}), (t_R=14.946) 2,4,6-trichlorophenol (5 ng mL^{-1}), (t_R=18.490) 2-benzyl-4-chlorophenol (5 ng mL^{-1}), (t_R=22.544) pentachlorophenol (5 ng mL^{-1}), (t_R=27.840) 4-tert-octylphenol (5 ng mL^{-1}).

The CME-HPLC analysis of the alcohols is shown in Fig 3.12. with 10 ng mL^{-1} concentration. Alcohols are less polar than phenols but still on the higher side in polarity. Since the selected alcohols have multiple benzene rings that make them suitable for increased interactions with the capillary coating, the online CME-HPLC analysis showed better performance. The benzene rings were attracted towards non-polar groups like polydimethylsiloxane in BHEA polymer and the alcohol functionalized part interacted with yttrium oxide moiety in the coating. These interactions resulted in higher enrichment factors (200-300), lower detection limits between 0.83-1.25 ng mL^{-1} (S/N=3) and %RSD as less than 3.0 (n=3).

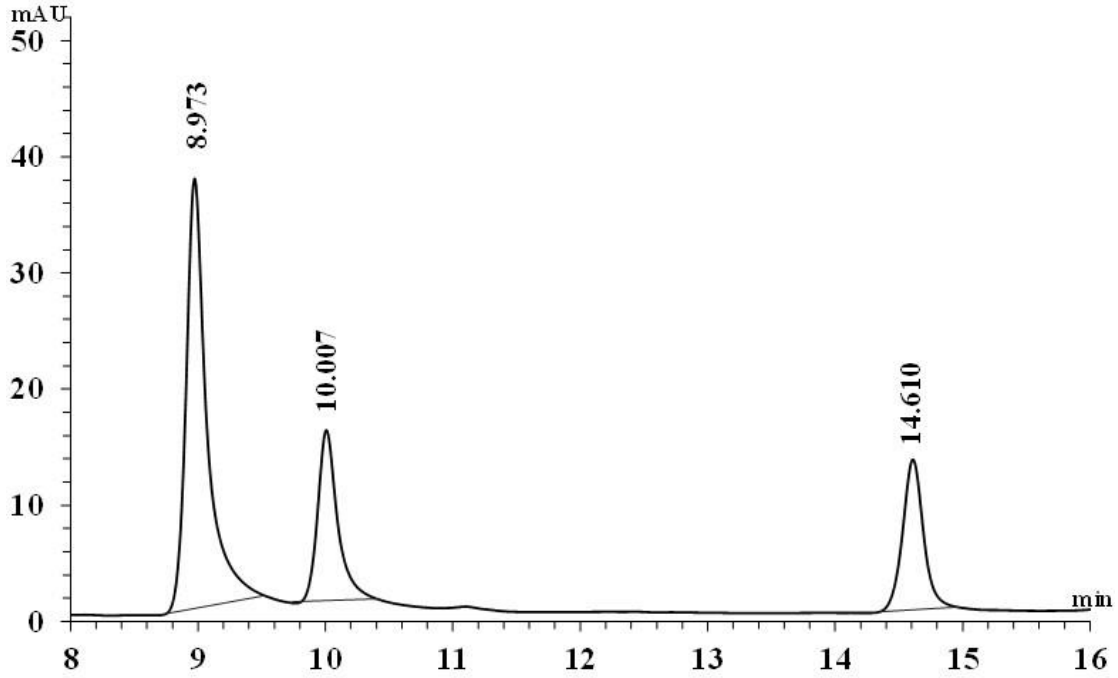


Fig 3. 12. Capillary microextraction-HPLC analysis of Alcohols. Extraction conditions: 40 cm × 0.32 mm i.d. sol-gel BHEA-Y-coated capillary; extraction time: 20 min: HPLC conditions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 μm dp). Gradient elution from 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH to 70% ACN till 20 min and 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH till 30 min; runtime 30 min; 0.8 mL min^{-1} flow rate, UV detection at 230 nm, ambient temperature. Peaks: (t_R=8.973) 2-naphthol (10 ng mL^{-1}), (t_R=10.007) 1-naphthol (10 ng mL^{-1}), (t_R=14.610) diphenylcarbinol (10 ng mL^{-1}).

Fig 3.13. illustrates the online CME-HPLC analysis of moderately polar ketones. For this purpose, ten members of this class were selected including 5,5-dimethyl-1,3-cyclohexadione, 1,2-naphthoquinone, 1-indanone, 4-methoxyacetophenone, 4-hydroxybenzophenone, 2-hydroxy-2-phenylacetophenone, propiophenone, benzophenone, and benzil. The sol-gel BHEA-Y coated capillary presented excellent enrichment factors (54.4-256.0) for moderately polar analytes with an excellent limit of detection of 1.56 ng mL^{-1} – 7.35 ng mL^{-1} and reproducibility of the extraction process within 5.6% (n = 3), clearly represented in Table 3.2. The similar extraction interactions were presented by aldehydes due to similar and comparable polarities of aldehydes and ketones. Fig 3.14 shows the CME-HPLC analysis of four selected aldehydes at 25 ng mL^{-1} concentrations. The analysis was very efficient for all the compounds, presenting reproducibility less than 6.1% (n=3), lower LOD ranging between 2.59 ng mL^{-1} to 7.35 ng mL^{-1} (S/N=3) and excellent enrichment factors (60.4-154.4).

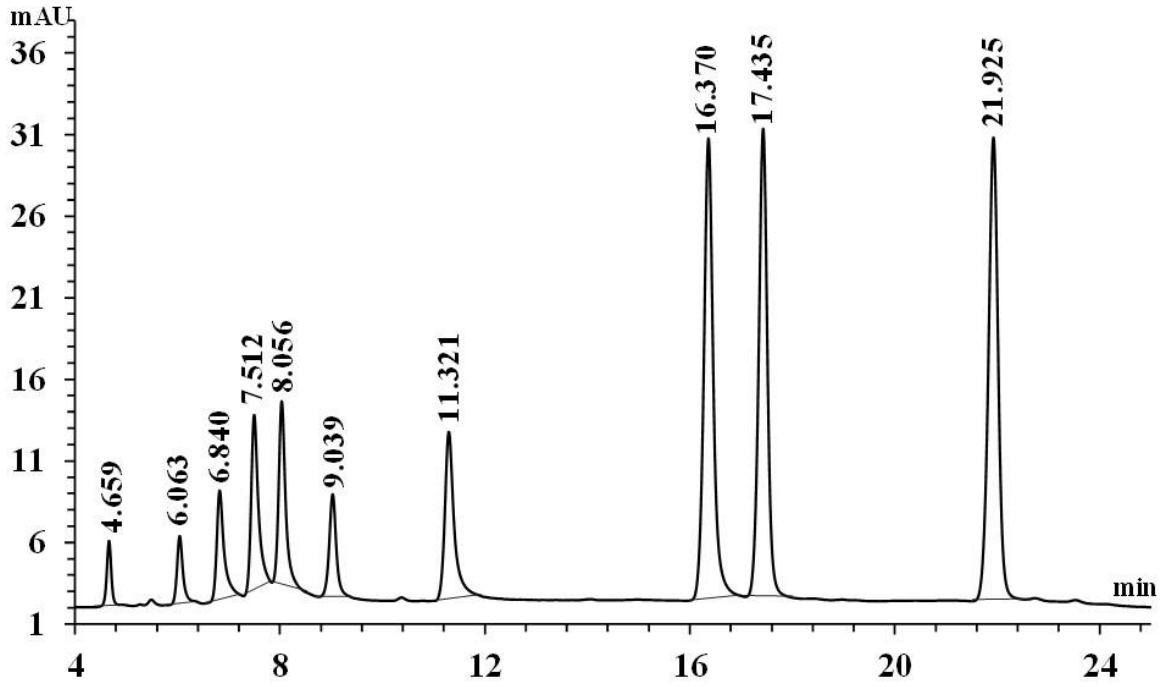


Fig 3. 13. Capillary microextraction-HPLC analysis of Ketones. Extraction conditions: 40 cm × 0.32 mm i.d. sol-gel BHEA-Y-coated capillary; extraction time: 20 min: HPLC conditions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 µm dp). Gradient elution from 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH to 70% ACN till 20 minutes and 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH till 30 minutes; runtime 30 minutes; 0.8 mL min^{-1} flow rate, UV detection at 254 nm, ambient temperature. Peaks: (t_R=4.659) 5,5-dimethyl-1,3-cyclohexadione (25 ng mL^{-1}), (t_R=6.063) 1,2-naphthaquinone (25 ng mL^{-1}), (t_R=6.840) 1-indanone (25 ng mL^{-1}), (t_R=7.512) 4-methoxyacetophenone (25 ng mL^{-1}), (t_R=8.056) 4-hydroxybenzophenone (25 ng mL^{-1}), (t_R=9.039) 2-Hydroxy-2-phenylacetophenone (25 ng mL^{-1}), (t_R=11.321) propiophenone (25 ng mL^{-1}), (t_R=16.370) benzophenone (25 ng mL^{-1}), (t_R=17.435) benzil (25 ng mL^{-1}), (t_R=21.925) 4-chlorobenzophenone (25 ng mL^{-1})

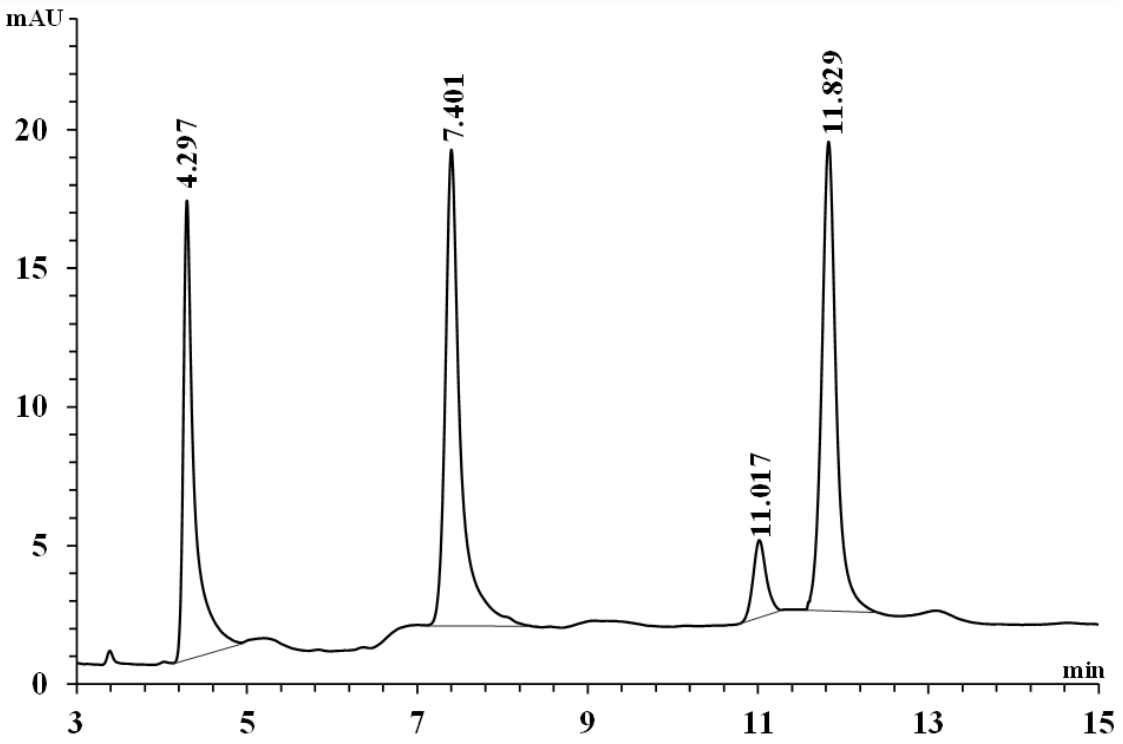


Fig 3. 14. Capillary microextraction-HPLC analysis of Aldehydes. Extraction conditions: 40 cm × 0.32 mm i.d. sol-gel BHEA-Y-coated capillary; extraction time: 20 min: HPLC conditions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 µm dp). Gradient elution from 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH to 70% ACN till 20 min and 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH till 30 min; runtime 30 min; 0.8 mL min^{-1} flow rate, UV detection at 230 nm, ambient temperature. Peaks: (t_R=4.297) 4-Hydroxy-3-methoxybenzaldehyde (25 ng mL^{-1}), (t_R=7.401) 5-nitrososalisaldehyde (25 ng mL^{-1}), (t_R=11.017) 4-chlorobenzaldehyde (25 ng mL^{-1}), (t_R=11.829) 5-bromobenzaldehyde (25 ng mL^{-1}).

The online CME-HPLC analysis of a non-polar group of analytes (i.e., polyaromatic hydrocarbons) using sol-gel BHEA-Y coated capillary is shown in Fig 3.15. For the extraction procedure, five members of this class were selected including naphthalene, biphenyl, fluorene, phenanthrene, and anthracene. The excellent extraction of these compounds may be explained due to the PDMS moiety in the BHEA polymer. Moreover, the significant enrichment factors (856.3-1378.1) took the LODs to lower-levels ranging between 0.18 ng mL^{-1} - 0.29 ng mL^{-1} (S/N = 3), with extraordinary %RSD less than 6.8 (n=3) as shown in Table 3.2.

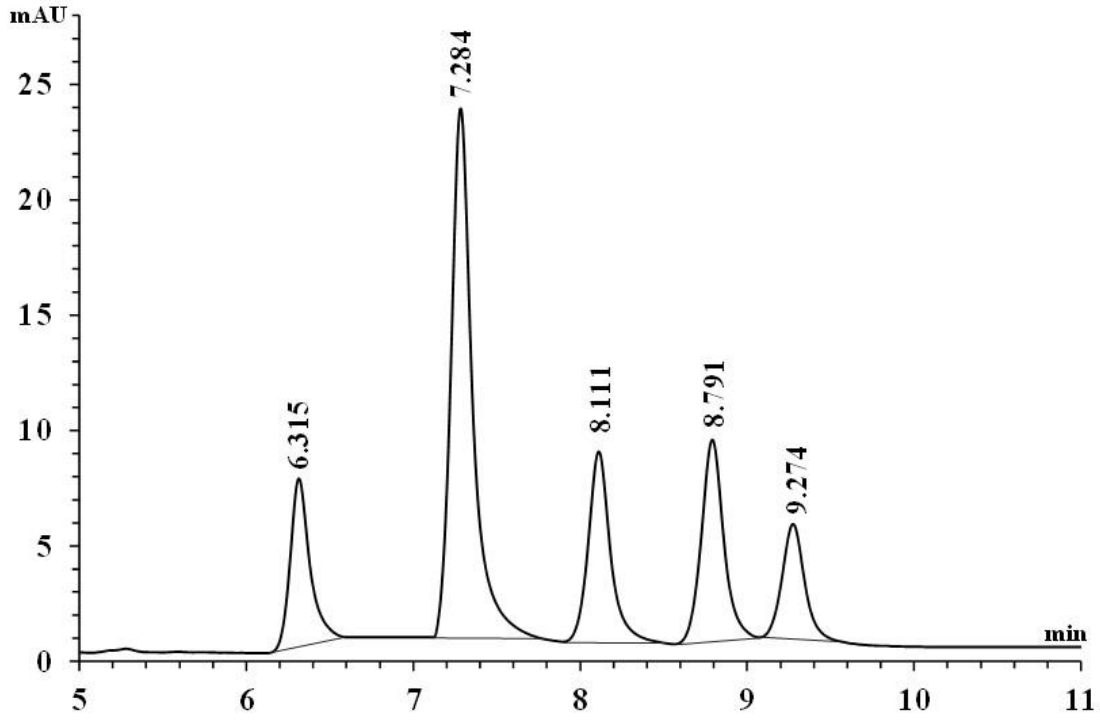


Fig 3. 15. Capillary microextraction-HPLC analysis of polyaromatic hydrocarbons. Extraction conditions: 40cm × 0.32mm i.d. sol-gel BHEA-Y-coated capillary; extraction time: 20 min: HPLC conditions: 25cm × 4.6mm i.d. Eclipse XDB C-18 column (5 µm dp). Gradient elution from 80:20 (v/v) ACN:Water to 100% ACN for 20min; 0.8mL min^{-1} flow rate; UV detection at 254 nm, ambient temperature. Peaks: (t_R=6.315) Naphthalene (01 ng mL^{-1}), (t_R=7.284) Biphenyl (01 ng mL^{-1}), (t_R=8.111) Fluorene (01 ng mL^{-1}), (t_R=8.791) Phenanthrene (01 ng mL^{-1}), (t_R=9.274) Anthracene (01 ng mL^{-1}).

3.3.5. CME extraction profile for BHEA-Y coated capillary

Fig 3.16. implies the extraction kinetic profile of the selected classes for online CME-HPLC analysis using the BHEA-Y coated capillary. One member of each class of compound was selected, including n-methyl-1-naphthylacetamide (amide, polar), 2,4-dichlorophenol (phenol, polar), 1-naphthol (alcohol, polar), propiophenone (ketone, moderately polar), 5-bromobenzaldehyde (aldehyde, moderately polar), and biphenyl (polyaromatic hydrocarbon, non-polar). Several trials were conducted for the extraction of these analytes from the aqueous standard solutions. The concentration of the analytes for the extraction kinetic profile was selected based on the quantification limit of the respective compound, where the results are reproducible. The time for the extraction process was varied from 2 min to 30 min (2, 5, 10, 15, 20, 25, and 30) to evaluate the extraction kinetics. The average peak area was plotted against the extraction time. All six analytes, a representative of each class of compounds, showed a maximum peak area at 20 min, indicating that the sol-gel coated BHEA-Y coated capillary has achieved equilibrium after 20 min. However, the profile suggests the remarkable extraction of non-polar analyte PAHs and significant extraction for moderately polar (ketones and aldehydes) and highly polar analytes (phenols).

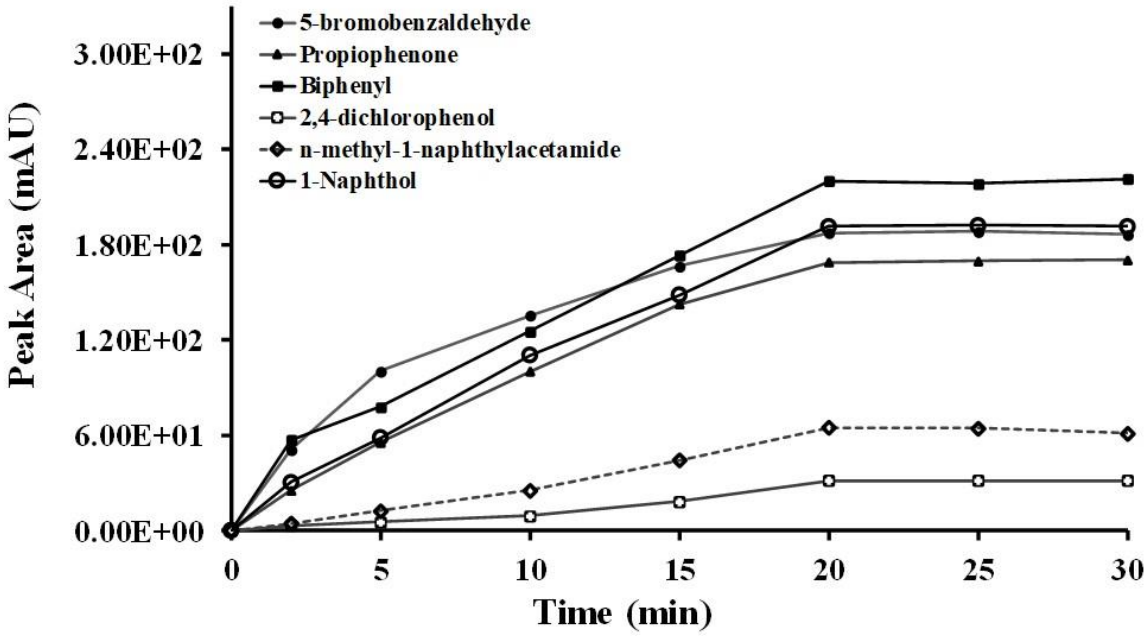


Fig 3. 16. Extraction kinetics of one representative analyte from class of compound; Amides (n-methyl-1-naphthylacetamide 25 ng mL-1), Phenols (2,4-dichlorophenol 05 ng mL-1), Alcohols (1-naphthol 10 ng mL-1), Ketones (propiophenone 25 ng mL-1), Aldehydes (5-brombenzaldehyde 25 ng mL^{-1}), and PAHs (biphenyl 01 ng mL^{-1}).

3.3.6. Preparation method reproducibility for the BHEA-Y coating

To evaluate the capillary to capillary reproducibility, a different run was designed where compounds from all the classes of varied polarities were included in the same chromatographic run. For this purpose, the photodiode array detector was used, and all three desired wavelengths were simultaneously kept switched ON. For amides, alcohols, and aldehydes 230 nm were switched ON. Ketones and PAHs were analyzed at 254 nm. Phenols show maximum absorption at 280 nm. For an experimental procedure, three BHEA-Y coated capillaries were cut of the same size (40 cm) and used for extraction. The extraction time was kept constant (20 min) and a mixture of 6 compounds containing all 6 different classes was analyzed. In this analysis amides, alcohols, aldehydes, ketones, phenols, and PAHs showed 8.3, 9.9, 9.5, 4.1, 7.6 and 7.0 % RSD (n = 3) as shown in Table 3.3.

Table 3. 3. Reproducibility for capillary to capillary extraction, one member from each class was selected based on well resolved peaks and retention time.

Chemical class	Name	t_R	Peak area reproducibility			Capillary to Capillary % RSD
			Mean peak area (n=3) Capillary 1	Mean peak area (n=3) Capillary 2	Mean peak area (n=3) Capillary 3	
Amides	4-bromoacetanilide	6.760	310.5	350.5	365.2	8.3
Alcohols	2-naphthol	8.941	1795.2	1575.2	1918.3	9.9
Aldehyde	5-bromobenzaldehyde	11.763	371.8	420.5	350.2	9.5
Ketone	Benzophenone	16.416	710.2	750.6	770.5	4.1
Phenols	2-benzyl-4-chlorophenol	18.640	291.2	250.2	271.5	7.6
PAHs	Biphenyl	23.669	483.5	453.2	420.5	7.0

3.3.7. Effect of extreme pH conditions on extraction:

The HPLC run designed for the capillary to capillary reproducibility analysis was also used for determining the stability of BHEA-Y coating. For this purpose, the coated capillaries were flushed with acidic and basic aqueous solutions for 24 hours and tested for the extraction of each analyte with different classes for organic compounds. Fig 3.17. presents good reproducibility ± 5.0 %. The extraction is a little enhanced in the case of NaOH treatment. This may be inferred because of cleaning the inner surface and increasing the porosity of the sol-gel network. However, the sol-gel based BHEA-Y based coating proved excellently stable in extreme pH environment.

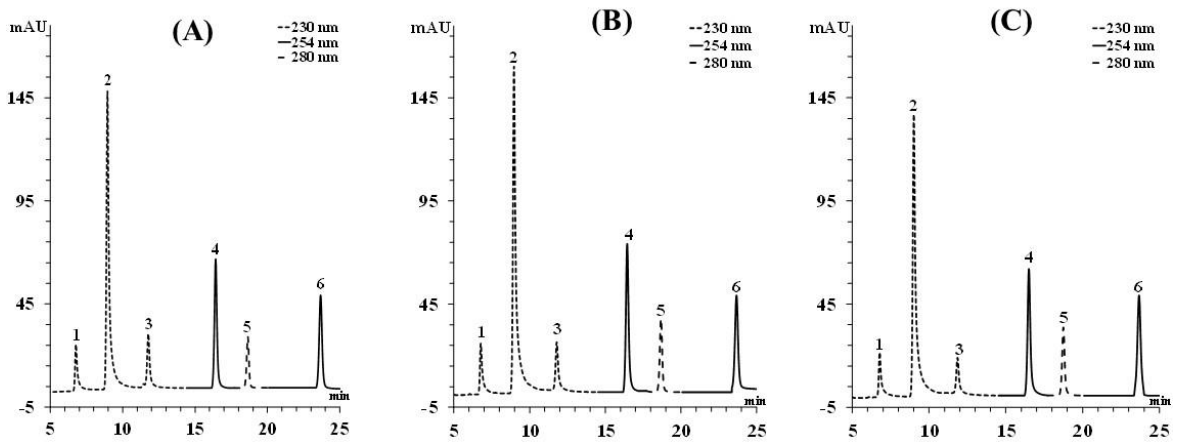


Fig 3. 17. CME-HPLC-UV extraction comparison of the sol-gel BHEA-Y coated capillary: Before exposing to acid/base conditions (A) and after exposing to 1.0 M NaOH for 24 h (B) and 1.0 M HCl for 24 h (C). Extraction conditions: 40 cm × 0.32 mm i.d. extraction time: 20 min: HPLC conditions: 25 cm × 4.6 mm i.d. Eclipse XDB C-18 column (5 µm d_p). Gradient elution from 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH to 70% ACN till 20 min and 45:55 (v/v) ACN:15 mM phosphate buffer 2.5 pH till 30 min; runtime 30 min; 0.8 mL min^{-1} flow rate, UV detection using Photodiode array detector at 230 nm (amides, alcohols, and aldehydes), 254 nm (ketones, polyaromatic hydrocarbons), and 280 nm (phenols). Peak 1 = Amides (4-bromoacetanilide), Peak 2 = Alcohols (2-naphthol), Peak 3 = Aldehydes (5-bromobenzaldehyde), Peak 4 = Ketones (benzophenone), Peak 5 = Phenols (2-benzyl-4-chlorophenol) and Peak 6 = PAHs (biphenyl).

3.3.8. Method validation parameters for online CME-HPLC analysis of phenols:

Phenols being an established environmental pollutant was considered for a detailed analysis and various parameters were conducted to validate the HPLC method. Specifically, for 7 selected phenols, a calibration curve was established, and it was found out that the described online CME-HPLC analysis provides a linear response of the phenols from 5 ng mL^{-1} to 200 ng mL^{-1}. This calibration curve was also accompanied with excellent R^2 ranging from 0.9971 to 0.9998. Moreover, the higher enrichment factors (93.0 to 175.5), lead to the lower limit of detection (0.91 ng mL^{-1} to 1.39 ng mL^{-1}) and quantification (3.0 ng mL^{-1} to 4.6 ng mL^{-1}). The intra-day, inter-day, and capillary to capillary reproducibility were also tested to be within 10% as shown in Table 3.4. The results proved the BHEA-Y coating as an excellent coating material for the extraction of phenols with reliable data.

Table 3. 4. Analytical parameters for selected phenols.

Analyte	Regression equation	R^2	Linear range (ng mL^{-1}	LOD	LOQ	Enrichment factor	RSD % (n=3)		
							One capillary		C to C*
							Intra-day	Inter-day	
4-flourophenol	y = 1.2975x + 1.2668	0.9992	5-400	1.35	4.5	95.5	4.2	4.8	5.8
2,3-dichlorophenol	y = 1.7986x + 3.9888	0.9998	5-400	1.19	3.9	135.8	4.7	5.0	6.5
2,4-dichlorophenol	y = 3.1140x + 3.7740	0.9988	5-400	0.94	3.1	160.0	3.3	4.2	7.5
2,4,6-trichloropehnol	y = 2.8494x + 1.2927	0.9990	5-400	0.91	3.0	175.5	6.3	6.0	9.2
2-benzyl-4-chlorophenol	y = 2.4693x + 4.9543	0.9979	5-400	0.96	3.2	155.4	2.7	3.5	7.6
Pentachlorophenol	y = 1.3099x + 0.3402	0.9995	5-400	1.28	4.2	116.5	6.2	6.8	8.5
4-tertoctylphenol	y = 0.9870x + 0.7340	0.9971	5-400	1.39	4.6	93.0	2.8	4.0	6.8

* c to c capillary to capillary

3.3.9. Online CME-HPLC analysis of phenols in real samples:

To evaluate the applicability of the CME-HPLC analysis to real sample, waste water and swimming pool water was collected and filtered using 4.5-micron filter paper. The filtered real sample was passed through the BHEA-Y coated capillary installed at the HPLC manual injection port for extraction and online HPLC analysis. The wastewater and pool-water samples did not show the presence of the selected phenols. However, the sample was spiked using seven phenols with 5 ng mL^{-1}, 50 ng mL^{-1}, and 200 ng mL^{-1} concentrations and evaluated for recovery and reproducibility. Fig 3.18. shows the online CME-HPLC analysis of wastewater un-spiked and spiked with different concentrations. Table 3.5. presents the overall recoveries in wastewater ranging between 84.7- 92.1 % and in swimming pool-water 86.1-94.3 %. The reproducibility of the results in the real sample was also excellent that is within 7.6 %.

A further utility of sol-gel BHEA-Y may be tested for different classes of hazardous pollutants and hyphenating the coated capillary with multiple analytical techniques, including gas chromatography etc.

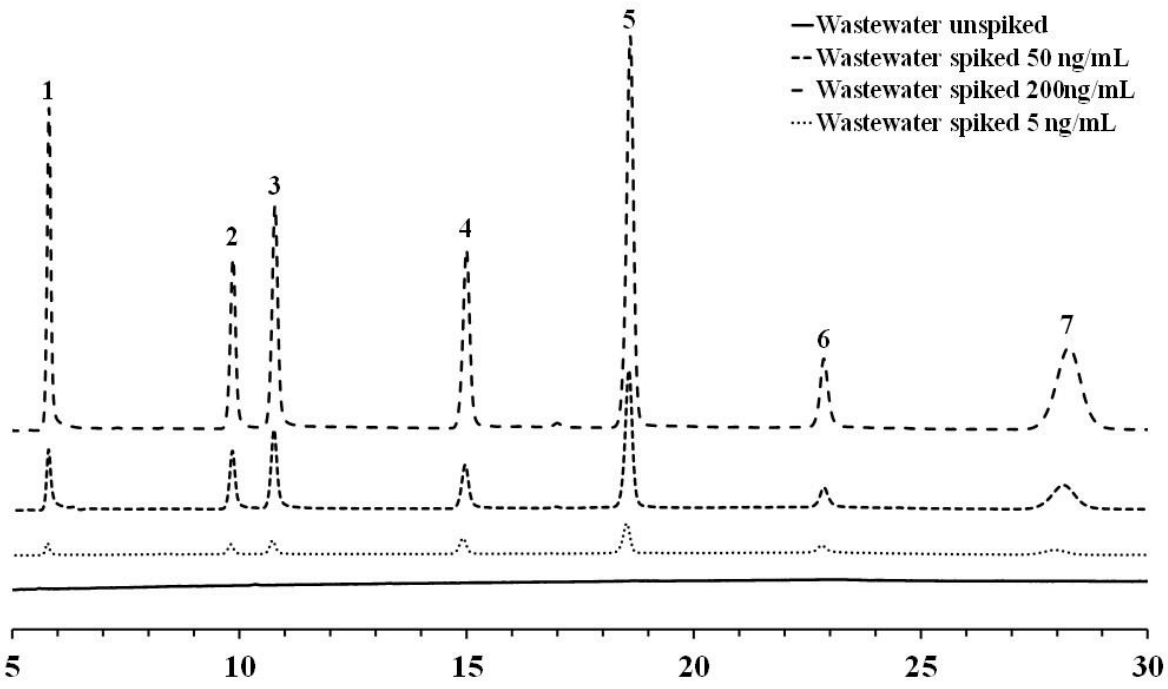


Fig 3. 18. Capillary microextraction-HPLC analysis of wastewater unspiked, and spiked with 5 ng mL-1, 50 ng mL-1, 200 ng mL-1 of mixture of phenols. Peaks: (1) 4-fluorophenol, (2) 2,3-dichlorophenol, (3) 2,4-dichlorophenol, (4) 2,4,6-trichlorophenol, (5) 2-benzyl-4-chlorophenol, (6) pentachlorophenol, (7) 4-tert-octylphenol.

Table 3. 5. Analytical results of wastewater and pool water samples

Analyte	Spiked Concentration (ng mL^{-1})	Wastewater		Pool-water	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
4-flourophenol	5	84.7	4.6	86.2	5.2
	50	86.9	6.1	89.0	5.1
	200	91.1	3.5	92.2	3.0
2,3-dichlorophenol	5	89.0	3.4	86.1	3.6
	50	87.5	4.1	88.6	3.5
	200	88.7	6.7	91.1	4.4
2,4-dichlorophenol	5	87.8	2.3	86.2	2.9
	50	91.8	6.2	90.9	5.2
	200	90.6	5.9	91.9	5.1
2,4,6-trichloropehnol	5	90.2	4.4	92.3	4.6
	50	90.1	7.1	88.1	4.5
	200	90.8	4.6	89.6	3.2
2-benzyl-4-chlorophenol	5	89.2	5.7	90.0	6.2
	50	91.7	7.6	90.7	6.9
	200	90.9	4.1	91.8	6.5
Pentachlorophenol	5	89.7	3.1	90.5	3.3
	50	88.4	5.7	87.6	4.9
	200	87.0	6.4	89.2	5.4
4-tertoctylphenol	5	90.3	5.5	94.3	4.0
	50	92.1	5.2	89.8	6.4
	200	91.8	5.6	90.6	4.8

*RSD = Relative standard deviation

3.4. Summary of the work

This sol-gel BHEA-Y coating is the first-time synthesis and immobilization of the coating inside the capillary and utilization for online CME-HPLC analysis. The sol-gel technique enables the direct chemical attachment of the coating to the inner surface of the capillary which makes this coating resistant to solvent flow and extreme pH. This BHEA-Y based coating has shown extraordinary enrichment factors towards a complete range of polarities of the analytes with lower detection limits and reproducible results. The method validation for phenols also proved the coating ability to produce linear and reliable data with acceptable recovery and reproducibility in real samples like wastewater and pool-water. However, there is a scope for the analysis of various other pollutants using this sol-gel BHEA-Y coated capillaries and to hyphenate this capillary with other analytical techniques, for example, gas chromatography.

CHAPTER 4

Analysis of 4-chloro-1-naphthol using germania-based, hybrid organic-inorganic coating for stir bar sorptive extraction with RP-HPLC-UV

4.1. Introduction

4-chloro-1-naphthol (4-CN) is toxic for the environment and is considered as a hazardous compound (OSHA 2012, 29 CFR 1910.1200). It is considered to be a category 2 level for eye and skin damage, and category 3 level toxic for the respiratory system [105]. So far, the concentration of 4-CN that poses a risk in the human body has not yet been determined. 4-CN is a member of naphthols that are well-known as intermediates for the commercial synthesis of pharmaceutical products, synthetic rubbers, and dyes. Moreover, this class has been recognized as an environmental pollutant with serious impacts on human health [106]. Naphthols are the by-product of naphthalene, which is a polyaromatic hydrocarbon (PAH) that is readily absorbed in the respiratory system [107]. These PAHs are commonly found in wastewater, tobacco smoke [108], and industrial air; however, diet is also a large contributor to human intake of PAHs [109].

There is not a single method reported for the specific determination of 4-CN, but other members of the naphthols (1 & 2- naphthols) have been identified in aqueous and

biological samples. All recent methods used for the isolation of these naphthols (1 & 2-naphthols) are based on the microextraction tools. These methods include determination of 1-naphthol using fiber-SPME for small sample quantities in glass capillaries [110], use of monolithic polymers for in-capillary microextraction of carbamate pesticides and 1-naphthol as a degradation product [111], the quantification of 1-naphthol and 2-naphthol by ionic liquid based dispersive liquid-liquid microextraction [112], analysis of both naphthols using a single drop microextraction method [113], and the sensitive analysis of the said analytes through stir-bar sorptive extraction. Similarly, it is possible to simply develop and validate a liquid chromatographic method for the determination of 4-CN in a biological sample, but the expectation of the 4-CN level determined in the real samples was very low. Due to these low levels determination/quantification of 4-CN, there is need for a sample preconcentration [114].

In recent times, the research trend for sample preparation is miniaturization which affords solvent free for environmentally benign approach. Stir bar sorptive extraction (SBSE) [21,115] is selected and preferred over solid phase microextraction (SPME) technique, because SBSE has proven to have better extraction results / performance when subjected to samples contain complex matrices. In addition, SBSE techniques have also improved the reproducibility and recoveries [116]. SBSE has a green preparation step where a stir bar containing adsorbent on its surface is added to the sample matrix and allowed to stir while extracting the analyte. This stir bar is then taken out to ultrasonically desorb the analyte of interest into a small volume of solvent (typically 100 µL) and then injected into HPLC system. The advantages of SBSE are that it has an easy preparation, cheap materials, simple working, good reproducibility [117], high sensitivity, and large sorbent surface

[118]. However, the number of commercially available stir bar surfaces is limited [56], and the SBSE applications are rarely reported. Therefore, there is need to develop more sorbent surfaces for wider / broader analytical / environmental applications.

The sol-gel approach provides a convenient method to synthesize the adsorbents of our choice with desired mechanical and chemical stabilities [119]. Many lab-made coatings have been reported as potential adsorbents for various analytes [120–124]. Similarly, germania-based organic-inorganic hybrid sol was coated to the inside of fused silica capillary and used as capillary microextraction tool for a range of analytes. The germania-based coating proved to have excellent stability for harsh pHs, temperatures, and aggressive solvents [73]. Moreover, the germania based triblock polymeric network is also reported for better temperature and solvent stabilities [125].

In this work, a germania-based organic-inorganic hybrid coating was immobilized on the outer surface of ordinary glass bars. The germania-based coating has never been used for stir-bar sorptive extraction. Also, to the best of our knowledge and literature, there is not a single research work that involves the analysis of 4-CN with a germania-based coating. Although 1-naphthol and 2-naphthols have been detected a number of times in human urine [126,127] and blood samples [108], but 4-CN has so far not been quantified in biological samples. In this work, the selectivity of the germania-based coating method is also analyzed for the most susceptible interference (i.e. 1-naphthol and 2-naphthol). The extraction is also compared with the in-lab prepared PDMS coated stir bar.

4.2. Experimental

4.2.1. Reagent and standards

Hydroxy-terminated polydimethylsiloxane (OH-PDMS), tetramethoxygermane (TMOG) and trifluoroacetic acid (TFA) 99% were purchased from Sigma-Aldrich. HPLC grade solvents like acetonitrile (ACN), methanol and dichloromethane were purchased from Fisher Scientific. The analyte of interest, 4-chloro-1-naphthol, was obtained from Sigma-Aldrich.

A 1.0 mg mL^{-1} stock solution of the 4-CN was prepared in methanol. All the working solutions or the extraction solutions were prepared freshly each time by diluting the stock solution in de-ionized water. All the optimizations of the major parameters were conducted at 200 ng mL^{-1} of 4-CN. All the standards and stock solutions were kept at 4 ^{o}C in the refrigerator.

4.2.2. Instrumentation

HPLC system (Agilent Technologies, USA) equipped with a quaternary pump (G1311B/C), a DAD (G4212B), an auto-sampler (G1329B), and Chemstation for LC software Rev.B.04.03[16] was used. The column used for separation was Agilent ZORBAX Eclipse XDB C-18 (5 µm, 4.6 mm id x 250 mm). For the preparation and homogenized mixing of PDMS-Ge sol, Thermofisher Scientific MaxiMix vortex mixer was used (model M16715). The precipitates of the sol were separated by using Sorvall™ Legend™ micro17 Microcentrifuge. The extraction from the sample was done using Fisher Scientific™ Isotemp™ basic stirring hotplate and desorption was done in Branson 3800 ultrasonic cleaner.

Thermo Scientific ESCALAB 250Xi (PHI 5000 Versa Probe II, ULVAC-PHI Inc.) X-ray photoelectron spectroscopy (XPS) was used to determine the bonding state and surface chemical composition. Prior to analysis, a representative sample from polymer sample was mounted on holder made of carbon tape, and vacuumed under high pressure to remove impurities and/or moisture adsorbed on the sample. Thermogravimetry analysis (TGA) was conducted from room temperature (RT) to 600 ^{o}C, at constant heating rate of 10 ^{o}C, under the flow of pure N_2 gas environment by using SDT Q600, V20.9 Build 20, thermal analyzer. The morphological information of the polymer was examined by field emission scanning electron microscope (FE-SEM) from TESCAN (LYRA 3 Czech Republic), using both secondary electron (SE) and back scattered electron (BSE) modes at accelerating voltage of 30 kV. The FE-SEM is equipped with energy dispersive X-ray spectrometer (EDS, Oxford Inc.) detector for elemental analysis.

4.2.3. Preparation of PDMS/Ge coated Stir bars

4.2.3.1. Preparation & Pretreatment of glass bars

The preparation and pretreatment of the stir bar were done by using previously published methods [119,123,124] where a 20 mm ordinary glass capillary having 15 mm iron wire was sealed with a flame. The sealed glass bars were cleaned with water and dichloromethane, respectively. To achieve the maximum silanol groups on the glass surface, stir bars were dipped in 1.0 M NaOH solution for 24 hours with continuous stirring. Afterward, stir bars were cleaned with 0.1 M HCl and water respectively with subsequent drying in an oven at 60 ^{o}C for 3 hours.

4.2.3.2. Preparation of sol-gel PDMS/Ge solution

The PDMS-Ge sol-gel solution was prepared according to published work [73] where 100 mg OH-PDMS was dissolved in 100 µL dichloromethane by vortexing for 1 minute in bullet-shaped microcentrifuge tubes. Then, 20 µL TMOG was added to the solution followed by 30 sec vortexing. Afterward, 30 µL of 5 % water solution in TFA was added as a catalyst. This reaction mixture was vortexed for 1 minute followed by centrifugation for 5 minutes for the removal of possible precipitates.

4.2.3.3. Coating the PDMS/Ge surface on glass bars

The pretreated sealed glass bars were immersed vertically in the PDMS-Ge sol-gel solution for 30 minutes. After taken out, they were placed in an oven at 60 ^{o}C for 24 hours for cross-linking of the polymer. Prior to use, the PDMS/Ge coated stir bars were ultrasonically washed in methanol for 5 minutes for the expected removal of organic moieties.

4.2.4. SBSE procedure and optimizations

For the SBSE procedure, extraction of 4-CN was done by stirring, and liquid desorption was carried out by ultrasonication. Typically, the coated stir bar was introduced into a 15.0 mL of a 5 % w/v NaCl aqueous solution of 4-CN at room temperature and stirred for 15 minutes at 500 rpm. Afterward, the stir bars were taken out, rinsed with de-ionized water, and dried using lint-free tissue paper. For the desorption step, the stir bar was placed in a microcentrifuge tube containing 100 µL methanol and kept in an ultrasonic bath for 10 minutes then followed by 20 µL injection into the HPLC system. After each extraction and desorption step, the stir bar was added to the 1.0 mL ACN and kept inside the ultrasonic bath for 10 minutes to ensure cleaning and removal of expected carry-over.

Furthermore, various important parameters of the extraction procedure were optimized, and these parameters include ionic strength, a solvent for the desorption, extraction time, and sample volume for extraction and desorption time. The extraction efficiency of germania-coated stir bar was also compared with PDMS-coated stir bar. All the experiments were done in triplicates.

4.2.5. Chromatographic Condition

The chromatographic analysis was performed in reverse phase mode using an isocratic mobile phase of acetonitrile and water (v/v) 80:20 respectively with a flow rate of 1.00 ml min^{-1}. An Agilent Eclipse XDB-C18 4.6 mm ID x 250 mm (5 µm) was selected. The analysis was carried at 274 nm.

4.2.6. Real sample analysis

The literature suggests that the chances of the hydroxyl group containing degradation products of naphthalene are mostly in water resources, blood, and urine. Therefore, three different types of real samples were collected and tested for 4-CN. Firstly, the wastewater sample was collected from a drain leading to the ocean. Secondly, a sample was collected from a swimming pool. Lastly, to check the presence and the recovery of 4-CN, a urine sample was also collected.

For the extraction purpose, 5.0 mL of the real sample was taken and diluted with 5.0 mL of deionized water. The germania-coated stir bar was added to the sample along with 35 % NaCl and kept in it for 10 minutes at a 600 rpm stirring speed. After the germania-coated stir bar was taken out of the real sample, it was rinsed with DI water for the removal of any sludge or impurity physically attached on the surface. Then the analyte was desorbed into

a 100 µL ACN by ultrasonication for 20 minutes. Finally, from the 100 µl desorbed solvent, an aliquot of 20 µL was injected into HPLC system.

4.3. Results and Discussion

4.3.1. Characterization of PDMS/Ge coated Stir bars

XPS analysis of polymer obtained from tetramethoxygermane and hydroxy-terminated polydimethylsiloxane (PDMS) is presented in Fig 4.1. to identify the bonding state and oxidation state of the polymer's constituent. As shown in the figure, germanium (Ge 3d), is present in (+2) oxidation state by the presence of peak at binding energy (BE) of 31.15 eV, which is mainly attributed to GeO in -Ge-O-Si- network [128,129]. The Si 2p peak in the as-synthesized polymer is symmetrical and binding energy is centered at 100.07 eV, which is a characteristic of Si bounded with mainly oxygen. The BEs of O 1s as observed are found at 530.06 eV (22%) and 530.85 eV (78%), corresponding to two different components bounded with oxygen. These two components can be referenced to oxygen bounded elements in form of Si-O (530.06) and Ge-O (530.85) [130]. C 1s is observed in a single environment, which can be associated with the carbon from PDMS employed for the synthesis polymer coated on the of stir bar. The corresponding BE of this carbon is found at 282.48 eV, and is a characteristic of carbon bonded with silicon in PDMS. Table 4.1. shows the surface composition of the polymer with carbon and silicon being the main elements with total contribution of 47% and 27%, respectively. The surface contribution of oxygen is about 25 %, while the germanium concentration found on the surface is less than 1%.

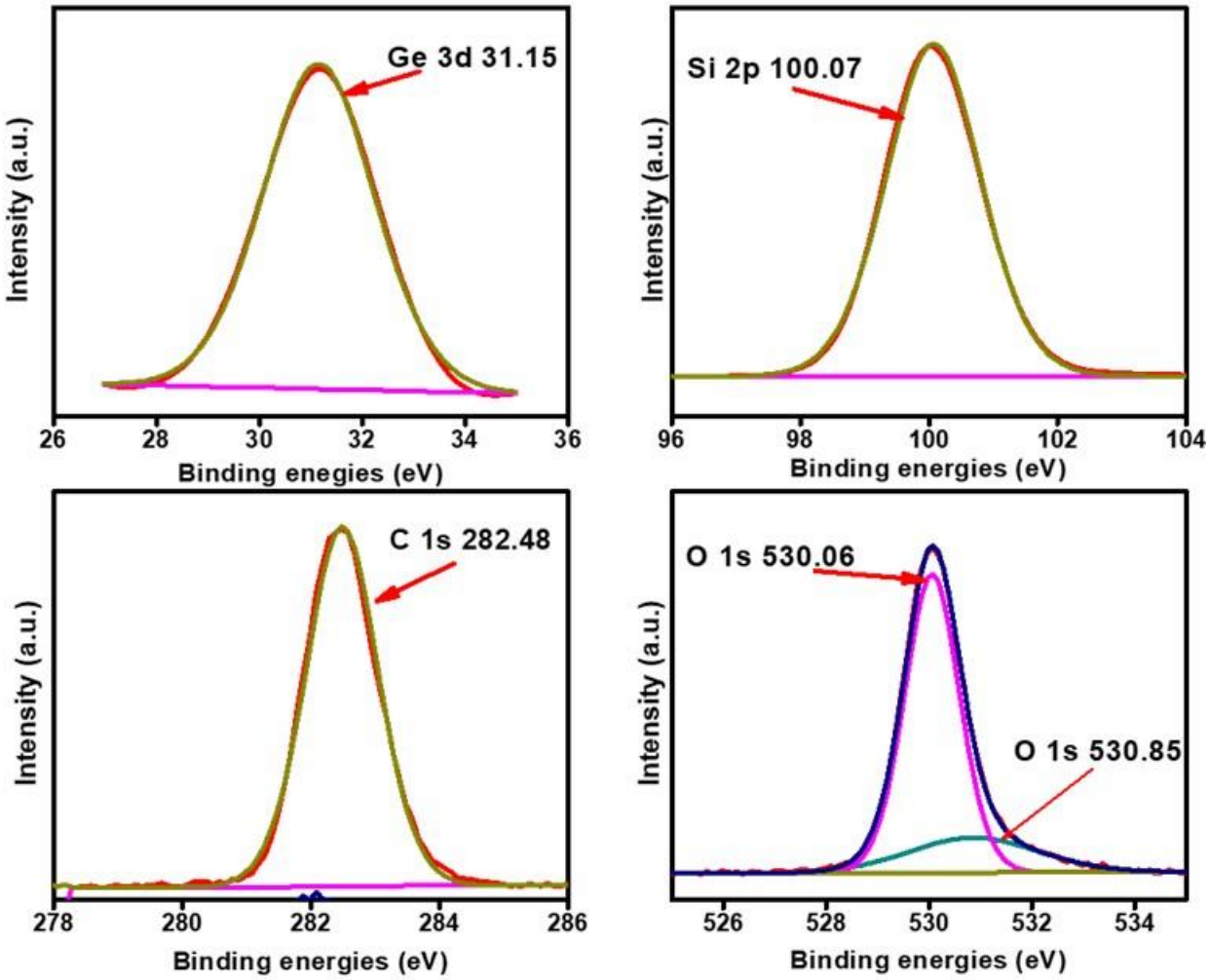


Fig 4. 1. XPS analysis of as-synthesized polymer (PDMS/Ge) showing different bonding states of the constituents (Ge, So, C and O).

Table 4. 1. The binding energies and surface atomic weight (%) of polymer constituents by XPS

Name	**Peak BE**	**Atomic %**
O1s	530.85	5.55
O1s	530.06	19.96
Si2p	100.07	26.97
C1s	282.48	46.65
Ge3d	31.15	0.87

The PDMS/Ge polymer was subjected to thermal and structural stability test under pure N_2-gas environment by thermogravimetry analysis. As shown in Fig 4.2. there is no significant mass loss (approx. 4%) and phase transition up to temperature of 300 ^oC. The total mass loss due to adsorbed water and impurities associated with the polymer is approximately 8% up, with no phase change. At a temperature relatively above 450 ^oC, there is a drastic loss of weight until 600 ^oC, which accounted for 75% of total mass loss. In addition, a phase transition or change is observed and could be attributed to decomposition of organic framework of the polymer with the formation of carbon or soot [131,132]. Therefore, the potential application of this polymer in high temperature environment will be from room temperature (RT) to 400 ^oC, with minimal loss of material due to heating effect.

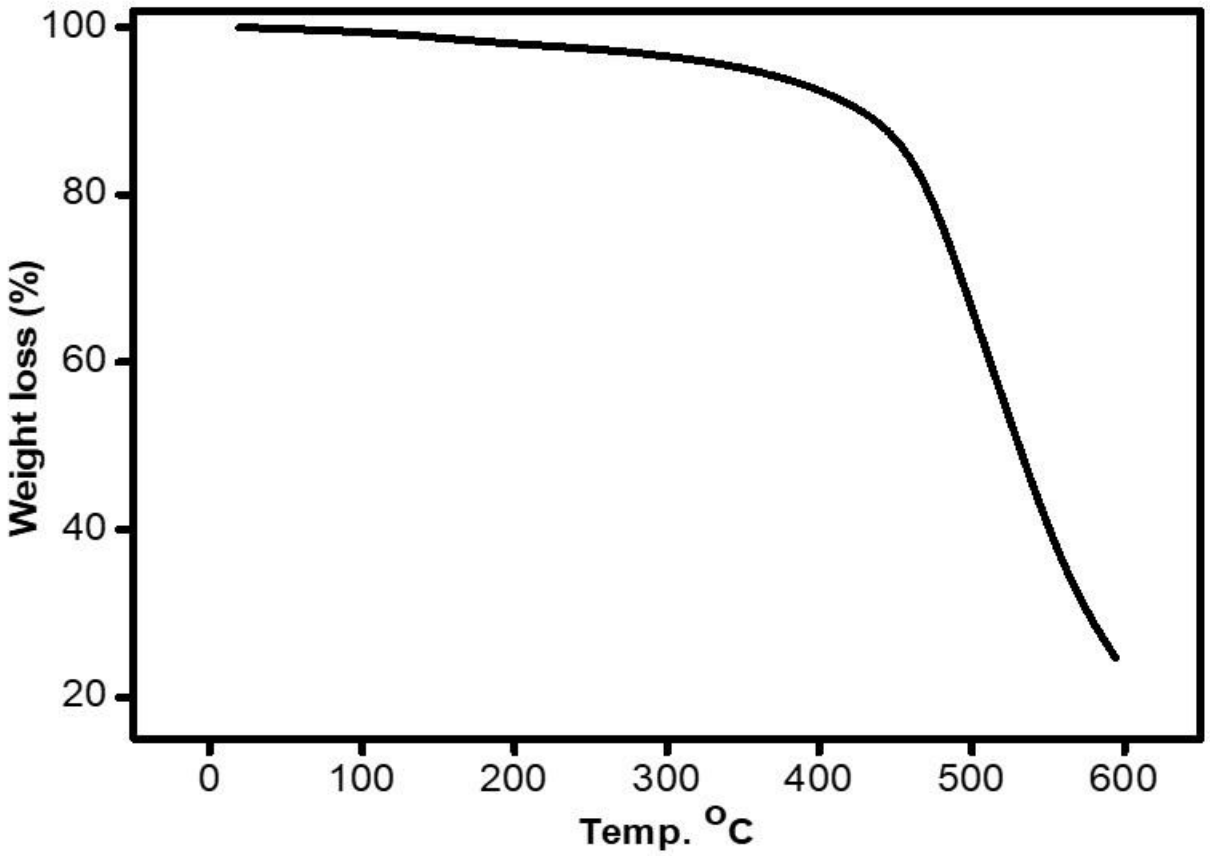


Fig 4. 2. Thermogravimetry analysis of as-synthesized polymer (PDMS/Ge) showing weight loss as a function of temperature

Since Stir bar sorptive extraction (SBSE) involves coating of polymeric materials onto the surface of stir-bar for extraction and pre-concentration of analytes, the distribution and uniformity of the coated PDMS/Ge polymer will be an important parameter for maximum and efficient extraction of analytes in the liquid phase. Fig 4.3. (a and b) presents the SEM micrographs of the PDMS/Ge coated on the stir bar at lower and higher magnifications, respectively. As shown in the figure, there is uniform distribution of polymeric materials on the stir bar without cracks, which confirms the successful coating operation for extraction of 4-CN at optimum conditions. In addition to X-ray photoelectron spectroscopy (XPS), the elemental analysis confirmed the presence of all constituents (Ge, Si, C and O) of the as-synthesized polymer coated on the bar, and there is a good agreement between the calculated (theoretical) and instrumental results, as obtained from energy dispersive X-ray (EDX) analysis shown in Fig. 4.4 (inset).

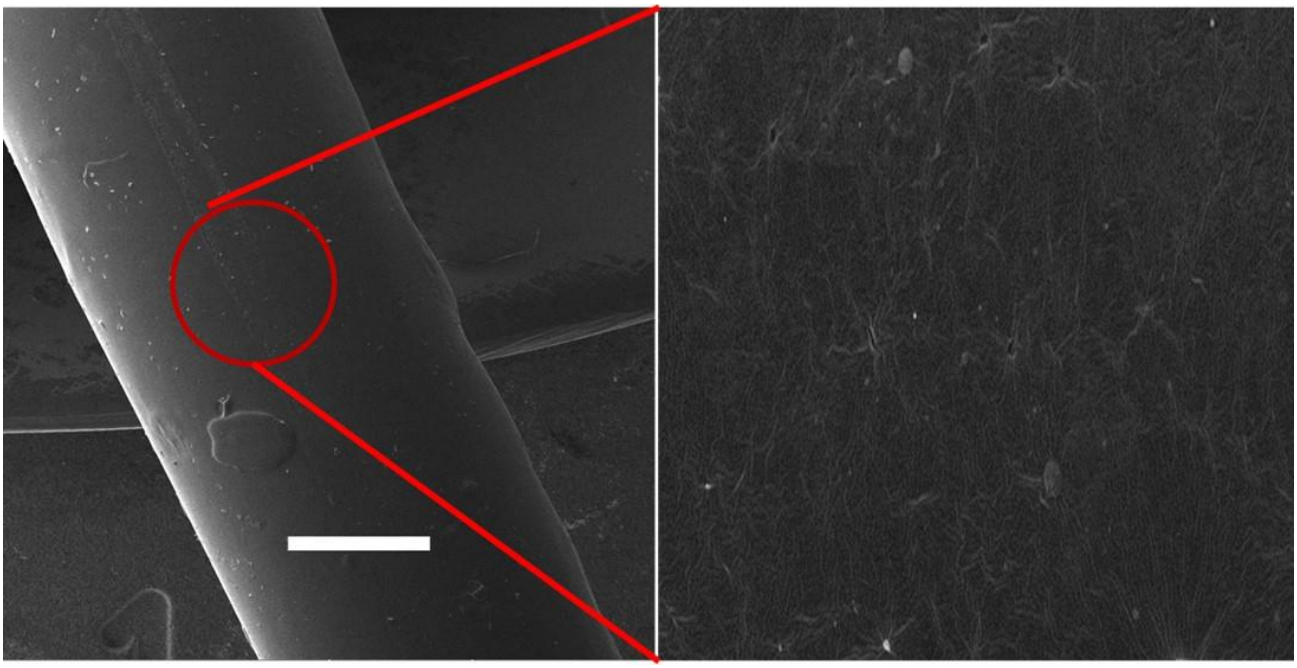
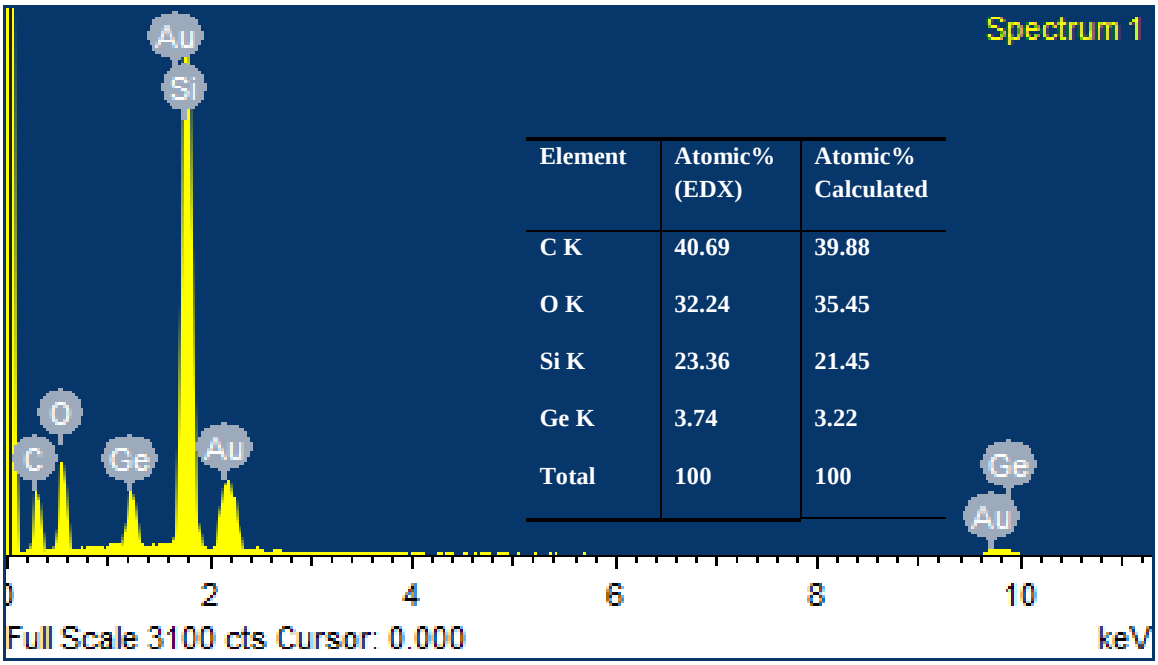


Fig 4. 3. Scanning electron microscope (SEM) analysis of as-synthesized polymer (PDMS/Ge) coated on the stir bar, at lower and higher magnifications (a and b), respectively



Element	Atomic% (EDX)	Atomic% Calculated
C K	40.69	39.88
O K	32.24	35.45
Si K	23.36	21.45
Ge K	3.74	3.22
Total	100	100

Fig 4. 4. Elemental dispersive X-ray analysis of polymer (PDMS/Ge) with the inset representing atomic weight (%)

4.3.2. Optimization of PDMS/Ge SBSE parameters

4.3.2.1. Sample volume

Sample volume is a simple but important factor for the extraction process. Considering the practical application of the analysis, it is important to determine the amount of sample that is sufficient for analysis. The sample volume was varied between 2.0-30.0 mL. The other parameters were 5 %w/v NaCl salt, 15 min extraction time with stirring rate at 500 rpm, and 10 min desorption in methanol. The increment in the volume of sample leads to an increase in the response, but the larger volumes have no significant effect on the extraction process. The data presented in Fig. 4.5. shows the optimized sample volume to be at 10.0 mL.

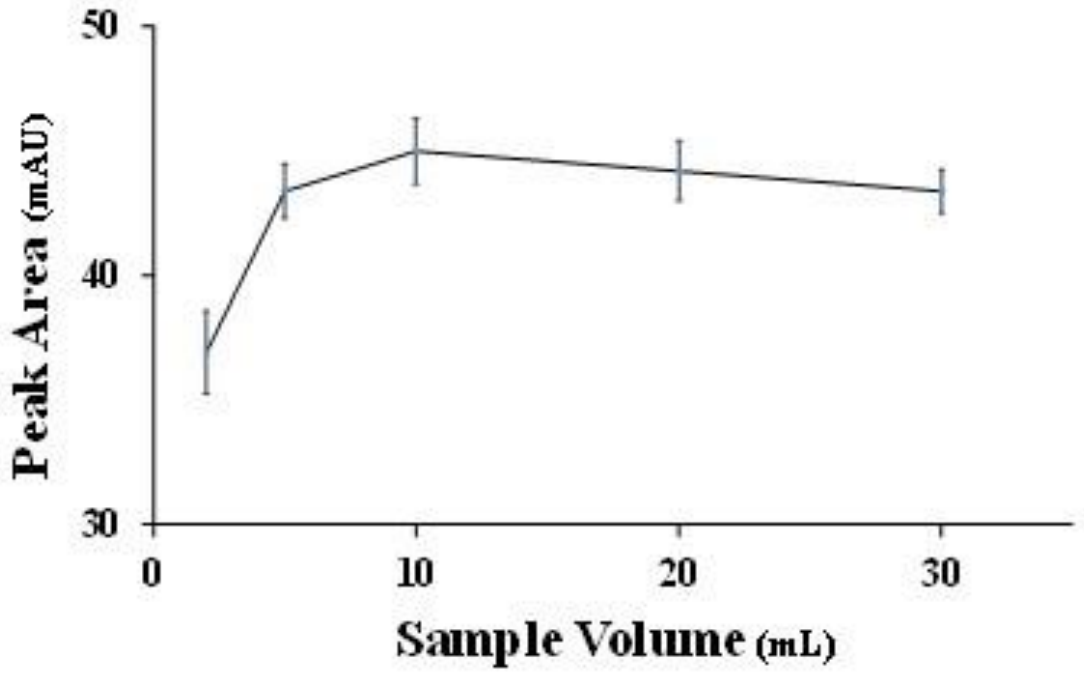


Fig 4. 5. Effect of the sample volume (2-30 mL), Conditions: concentration 200 ng/mL, salt addition 5 % w/v, extraction time 15 min, stirring rate 500 rpm, desorption time 10 min, desorption solvent Methanol.

4.3.2.2. Extraction time and stirring rate

To make the extraction process fast and effective, the extraction time was varied between 2 to 30 minutes. It can be seen in Fig 4.6. that the extraction efficiency of 4-CN increases significantly between 2-10 minutes; however, it becomes almost constant from 10-30 min. It simply shows that the equilibrium between the analyte and adsorbent surface takes 10 minutes to achieve. Therefore, 10 minutes were taken as the optimized time for the extraction process.

Similarly, the factor of the stirring rate has its own importance in the SBS extraction process. It depends on the type of adsorbent and analyte. The mechanism followed by the analyte to adsorb on the surface of the adsorbent may vary. Considering these facts, the stirring rate was evaluated at 300,600,900 and 1200 rpm. Fig 4.7. shows that the medium stirring was found to be the best for the extraction of 4-CN on PDMS/Ge coating. However, it can be inferred that at the high stirring rate, the fast movement of the sample through surface leads to desorption of analyte, so 600 rpm was found as the optimized rate for stirring.

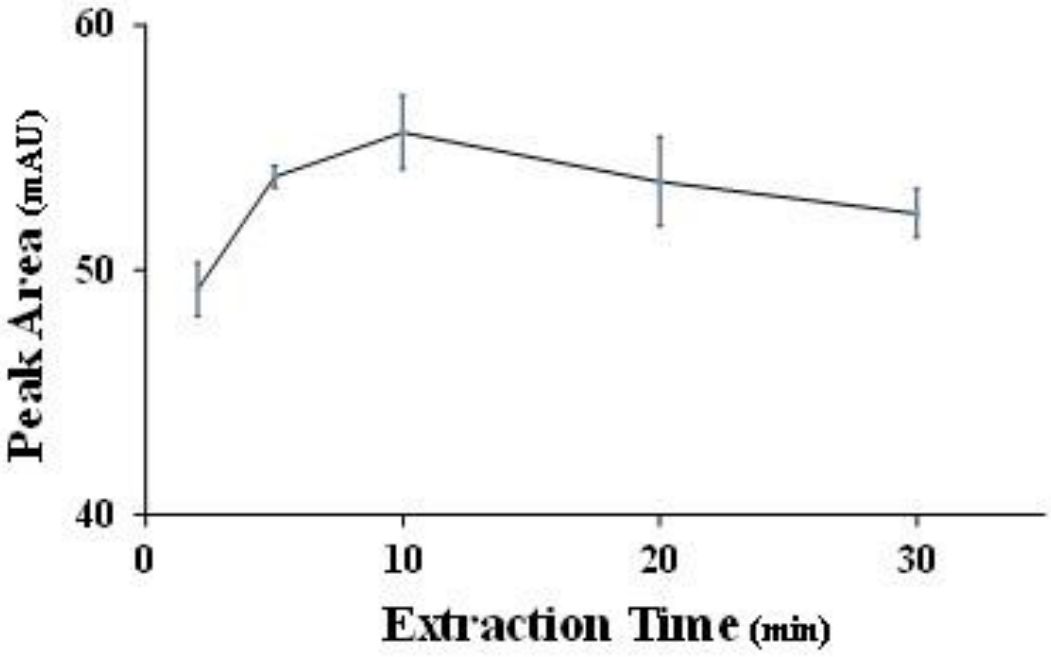


Fig 4. 6. Effect of Extraction time, (2-30 min): Conditions: Sample volume 10 mL, concentration 200 ng/mL, salt addition 5 % w/v, stirring rate 500 rpm, desorption time 10 min, desorption solvent Methanol.

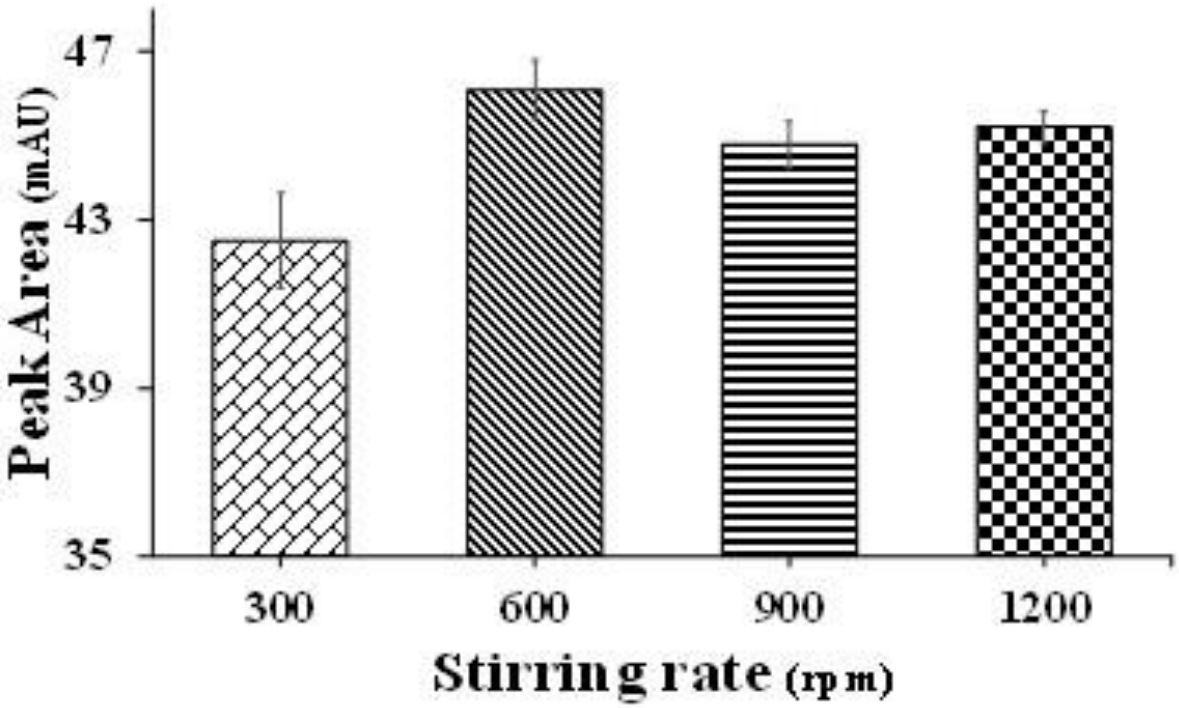


Fig 4. 7. Effect of Stirring rate, (300-1200 rpm): Conditions: Sample volume 10 mL, concentration 200 ng/mL, salt addition 5 % w/v, extraction time 10 min, desorption time 10 min, desorption solvent Methanol.

4.3.2.3. Desorption Solvent and time

Optimization of a medium is very crucial for the selection of the best-suited solvent for desorption process. Methanol, acetonitrile, and mobile phase were tested for this purpose. Contrastingly, the methanol did not show the maximum expected desorption. The reason may be resolved as 4-CN having two bulky phenyl rings which made it more suitable to be desorbed in a moderately polar solvent. Consequently, acetonitrile showed maximum extraction towards 4-CN as shown in Fig. 4.8.

The time to keep the adsorbent surface in desorption solvent under ultrasonication is a large contributing factor towards the extraction efficiency. Therefore, we used ACN as a desorbing solvent, and desorption time was varied between the range of 5-40 minutes. After each increment of time, the extraction increased, but the extraction profile showed a maximum desorption at 20 minutes as in Fig 4.9.

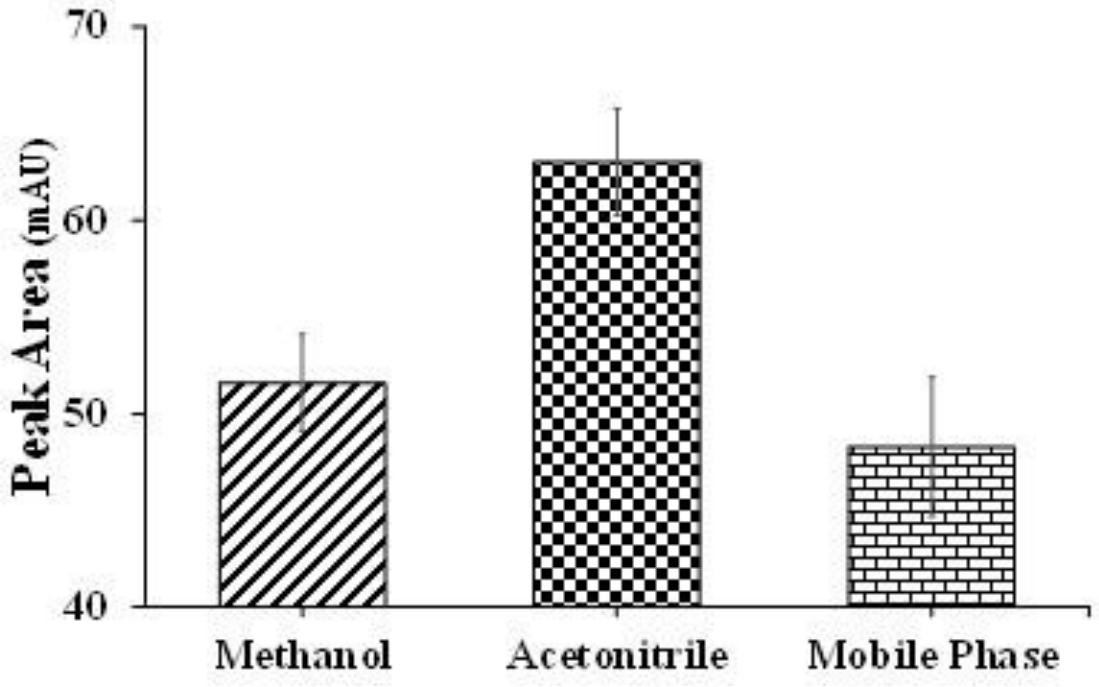


Fig 4. 8. Effect of the type of solvent for desorption: Methanol, Acetonitrile, Mobile phase (Acetonitrile:water 80:20). Conditions: Sample volume 10 mL, concentration 200 ng/mL, salt addition 5 % w/v, extraction time 10 min, stirring rate 600 rpm, desorption time 10 min

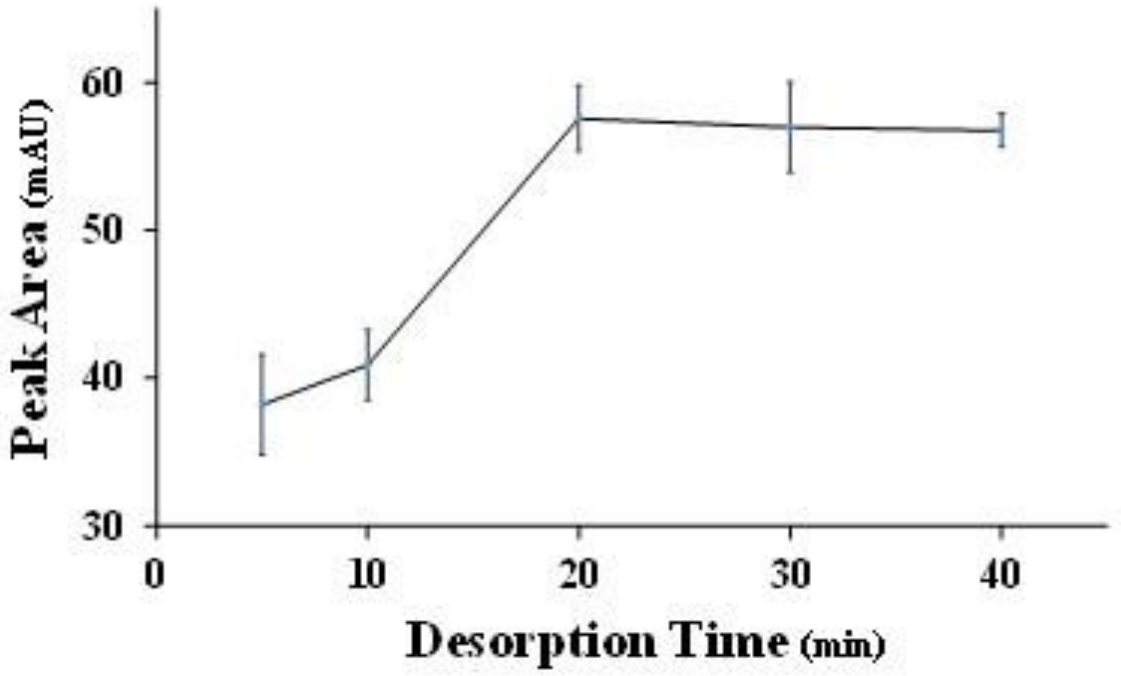


Fig 4. 9. Effect of the desorption time, (5-40 min): Conditions: Sample volume 10 mL, concentration 200 ng/mL, salt addition 5 %w/v, extraction time 10 min, stirring rate 600 rpm. Desorption solvent Acetonitrile.

4.3.2.4. Ionic strength of the sample

The concentration of NaCl in extraction medium pose high impact on the extraction efficiency of the stir bar. The concentration of salt was varied between 5-50 % w/v. However, the reported maximum solubility of NaCl in water is 35.4 % w/v. As a result of optimization of ionic strength, the extraction efficiency of 4-CN was increased 5.5 times. Fig 4.10. shows that when more salt was added, there was a significant increase in the signal. The maximum extraction efficiency was observed at 35 % w/v of NaCl to sample ratio. Hence, this ratio was selected as the optimized ionic strength of the sample.

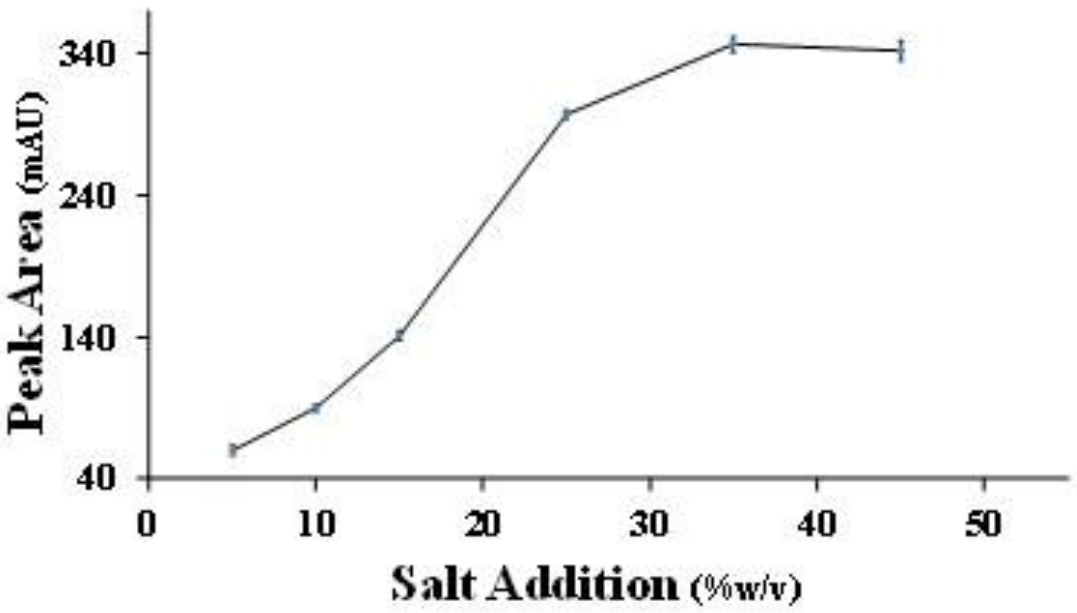


Fig 4. 10. Effect of the Salt addition, (5-50 %w/v): Conditions: Sample volume 10 mL, concentration 200 ng/mL, extraction time 10 min, stirring rate 600 rpm. Desorption solvent Acetonitrile, Desorption time 20 minutes

4.3.3. Analytical Performance

Under the optimum condition for extraction and desorption for the proposed PDMS-Ge SBSE HPLC-UV method, a calibration curve was obtained as shown in Fig 4.11. (Overlaid Chromatograms in Fig 4.12.). The analyte (4-CN) showed linearity between the range of 0.4-800 ng mL^{-1}. The chromatograms of the lowest and highest concentrations within the linear ranges are shown in Fig 4.13. & 4.14., respectively. It also displayed very good linear equation $y = 1.3419x + 47.458$ and $R^2 = 0.9992$. The analysis proved remarkably good in terms of the limit of detection (LOD) and limit of quantification (LOQ), 0.034 (S/N=3) and 0.114 ng mL^{-1}, respectively. The carryover of the stir bar after the complete SBSE process was also determined by desorbing the stir bar in the desorption solvent and later injecting into HPLC system, where no peak at the desired retention time was seen. Therefore, the PDMS/Ge coated stir bars proved to produce reliable results.

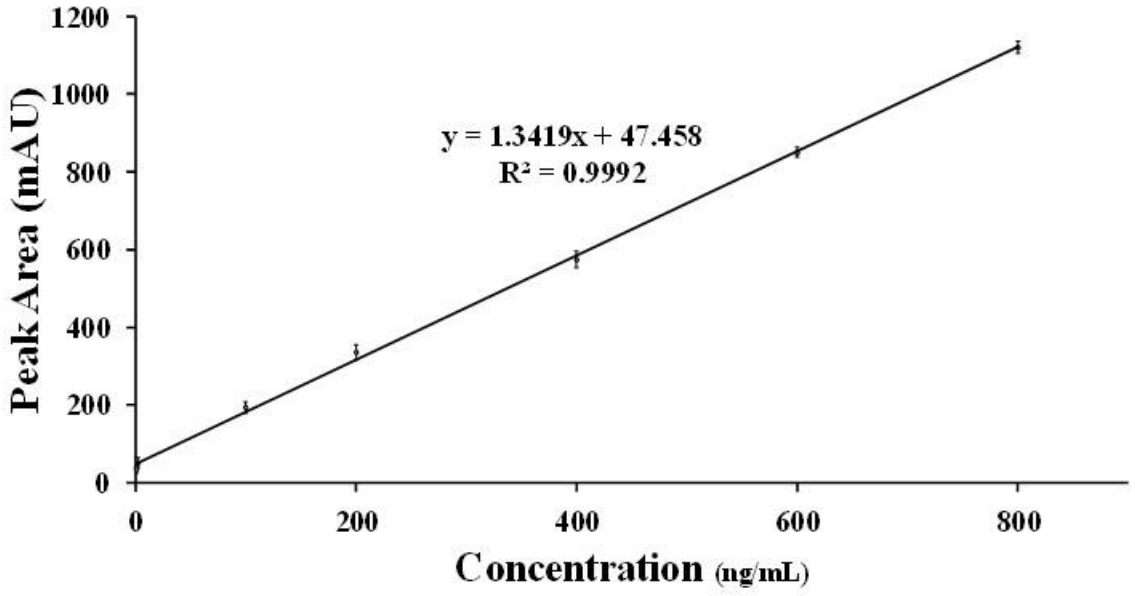


Fig 4. 11. Calibration curve (0.4-800ng/mL): Optimized extraction parameters, Sample volume 10 mL, extraction time 10 min, stirring rate 600 rpm. Desorption solvent Acetonitrile, Desorption time 20 minutes, salt addition 35 %w/v.

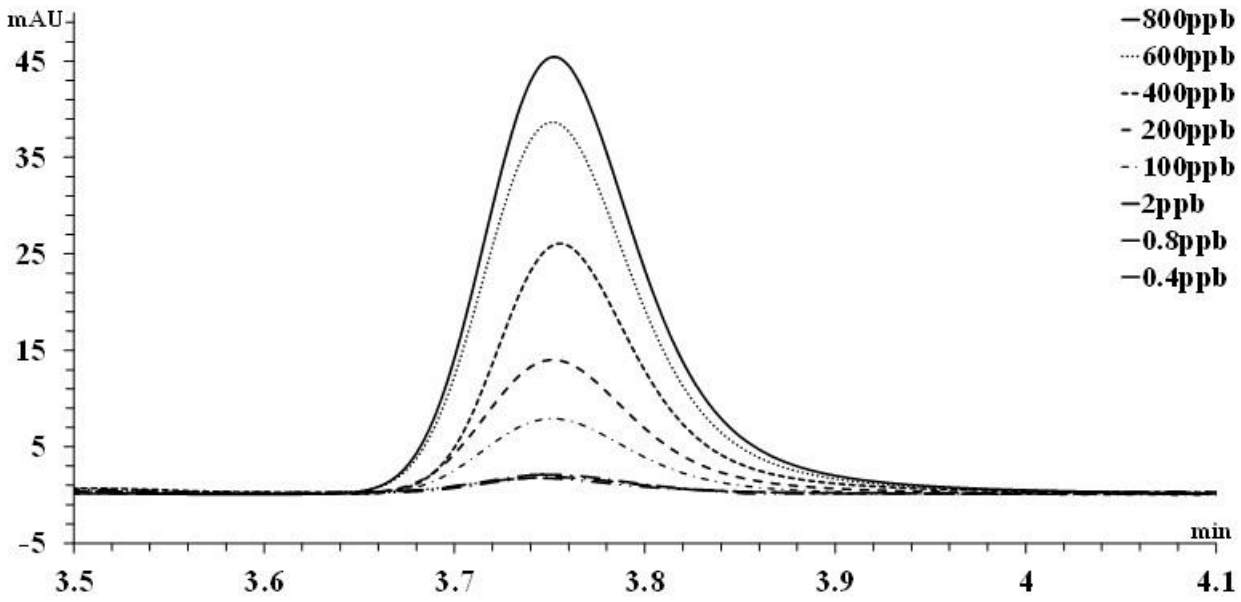


Fig 4. 12. Overlaid chromatograms showing complete linear dynamic range for 4-CN.

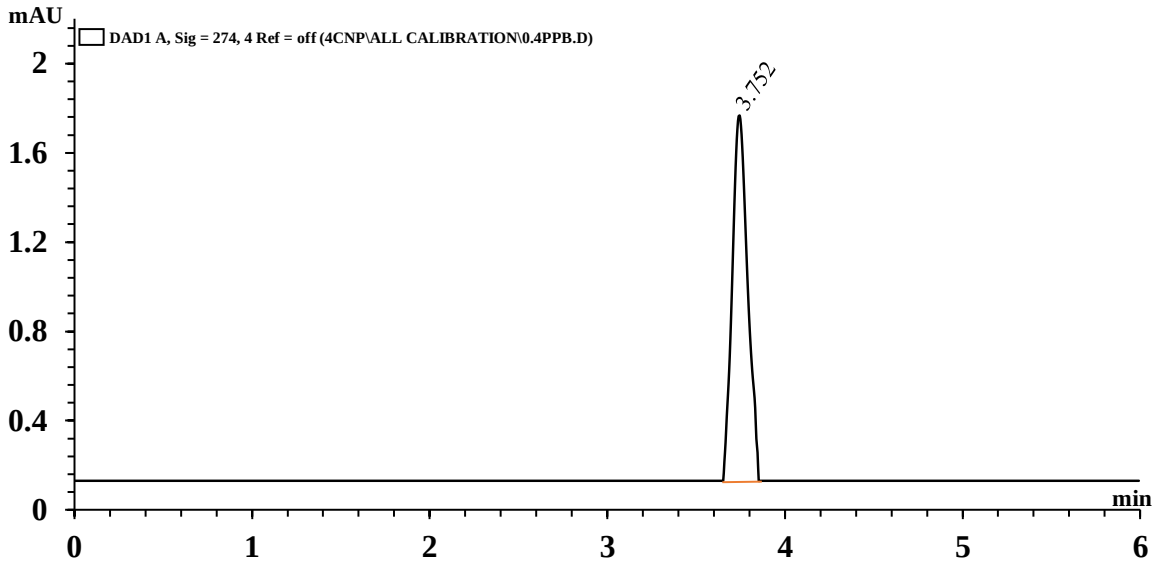


Fig 4. 13. Chromatogram showing Lowest concentration from the linear dynamic range of 4-CN 0.4 ppb

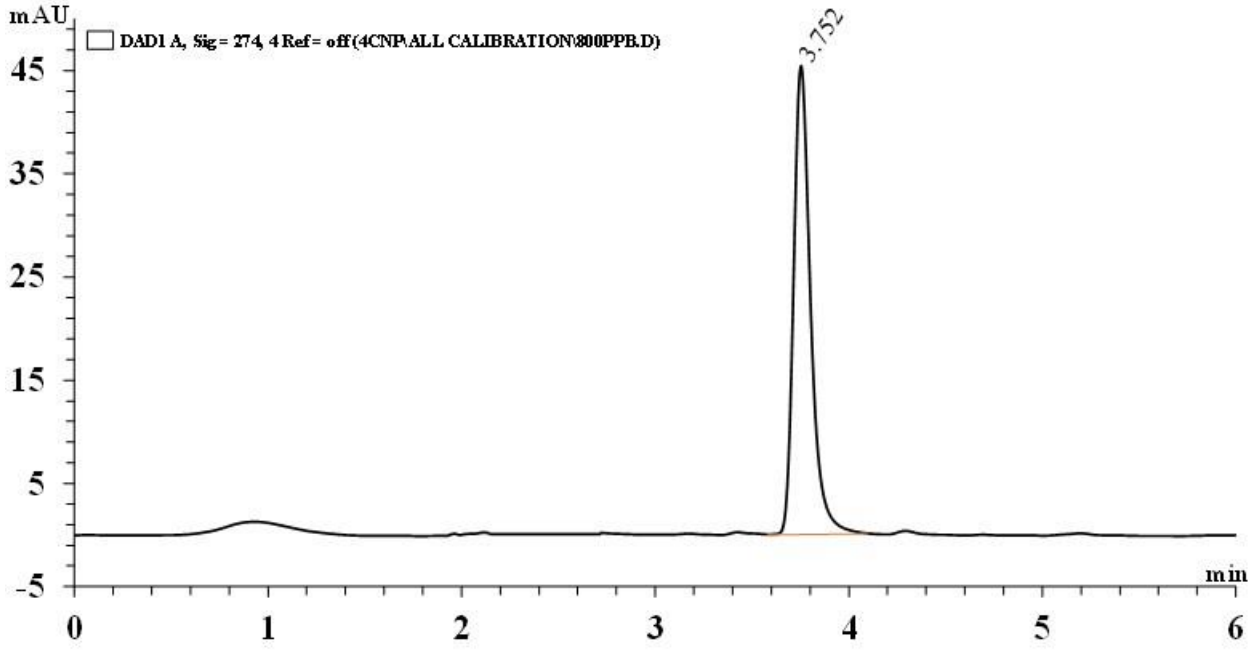


Fig 4. 14. Chromatogram showing highest concentration from the linear dynamic range of 4CN 800 ppb

4.3.4. Preparation reproducibility of PDMS/Ge coated stir bars

In this work, preparation reproducibility of the PDMS/Ge stir bars were also analyzed. All the experimental conditions were kept similar, and two different sol-gel solutions were prepared. Two pretreated glass bars of identical size were kept inside the sol for 30 minutes separately as described in section 2.3. The extraction efficiency of these two different bars was tested, and both produced similar results with 1.7 % RSD for the same batch and 3.5 % RSD for different batches from each other as shown in Fig 4.15.

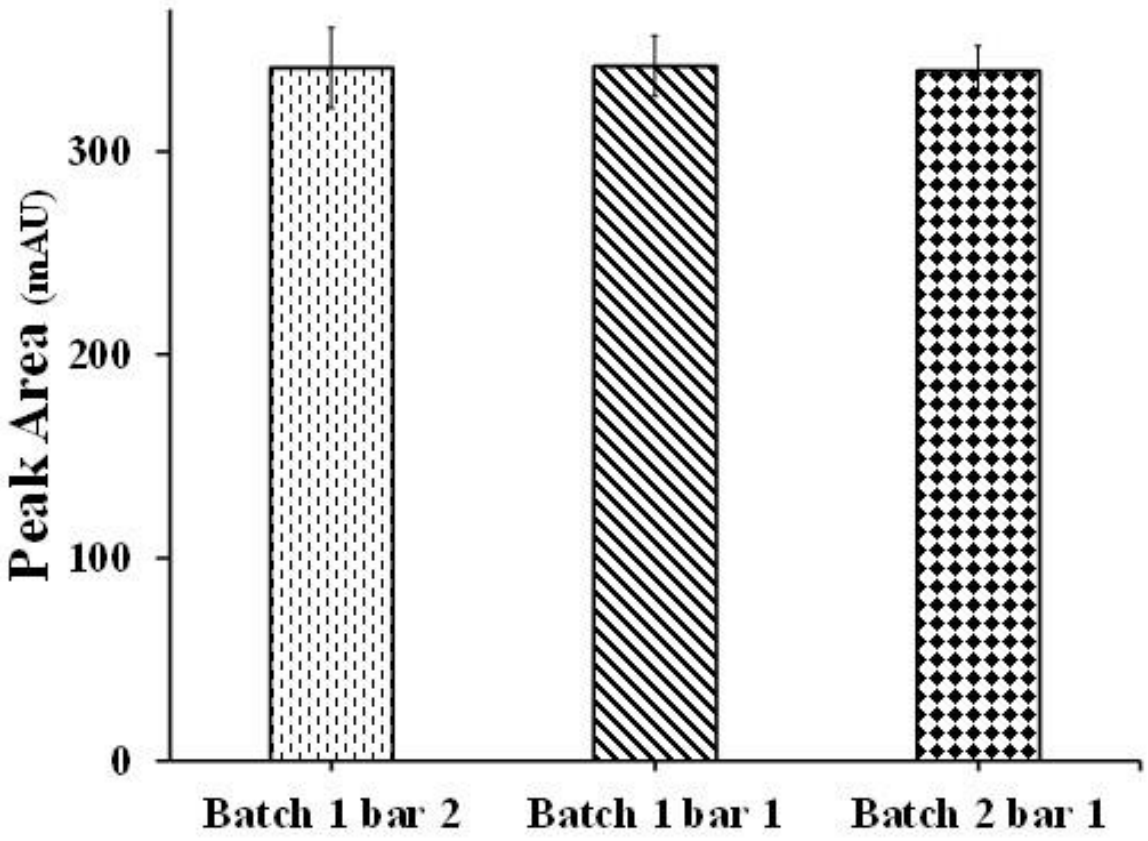


Fig 4. 15. Preparation reproducibility of PDMS/Ge coated stir bar, in the same batch (Batch 1 bar 1 & 2) in different reaction batches (batch 1 bar1 and batch 2 bar 1)

4.3.5. Comparison of PDMS/Ge with Laboratory prepared PDMS stir bar

Based on previous methods [23], we prepared a PDMS coated on glass stir bars, and compared its extraction efficiency with our newly developed PDMS/Ge coated stir bar. However, the extraction results in Fig 4.16. (Chromatogram in Fig 4.17.) presented that PDMS/Ge coated stir bar exhibited approximately four times more extraction as compared to PDMS coated stir bars.

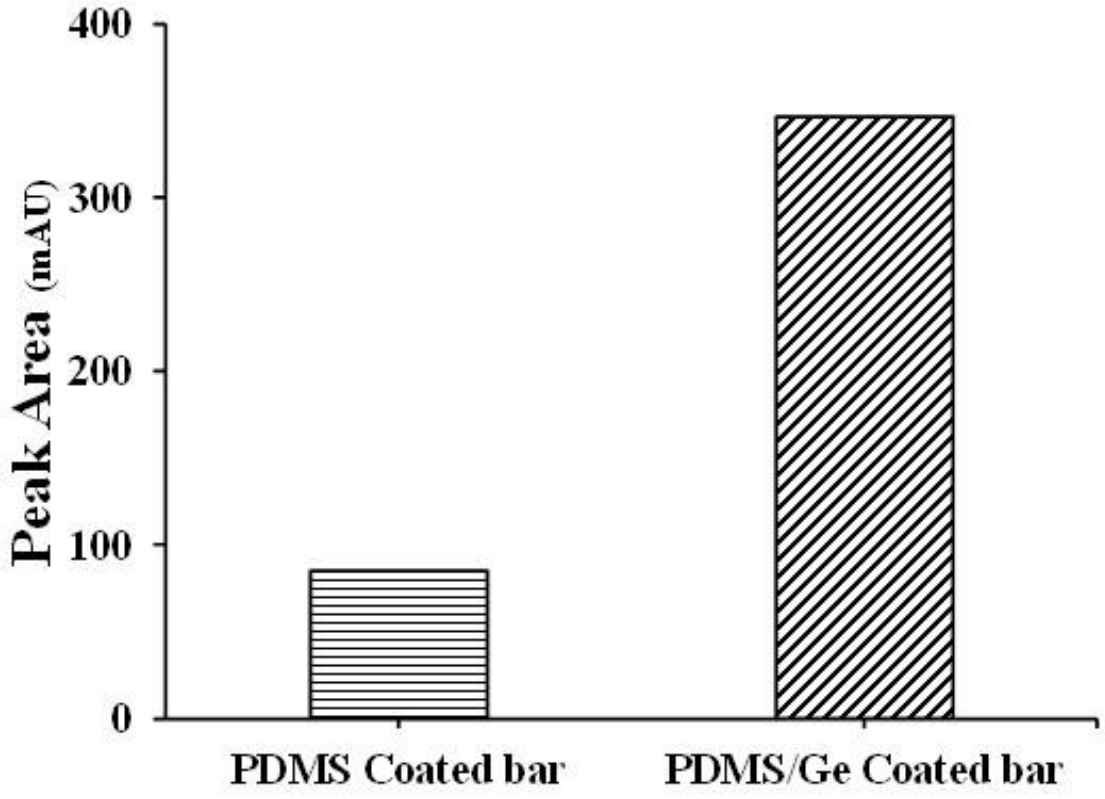


Fig 4. 16. Comparison between in-lab prepared PDMS coated bar vs PDMS/Ge coated stir bar for the extraction efficiency for 4-CN

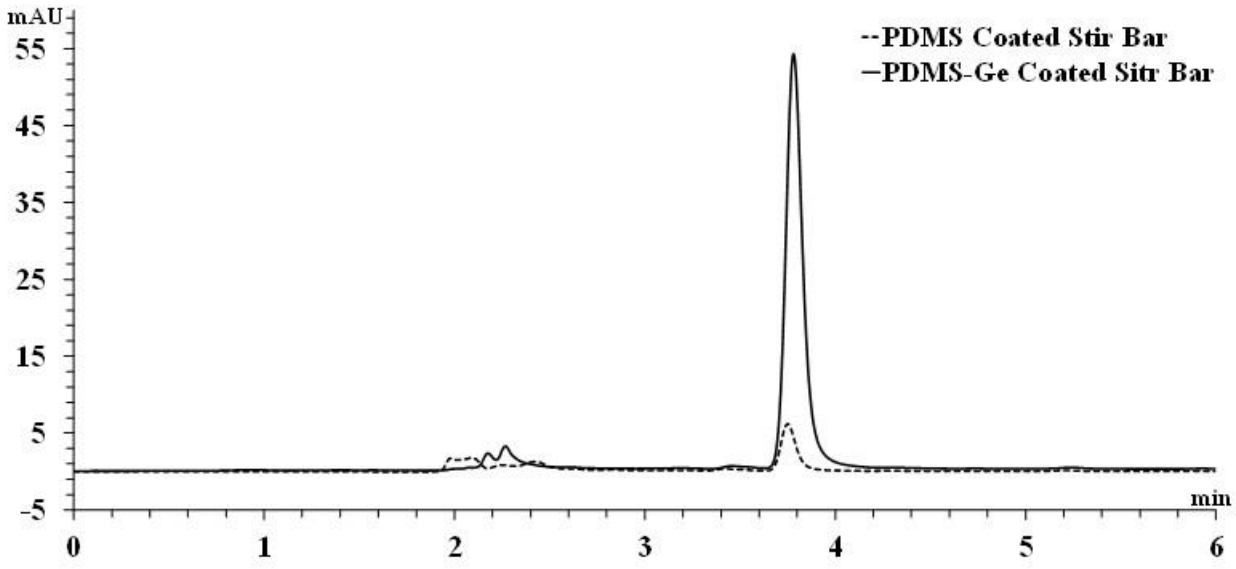


Fig 4. 17. Chromatogram showing the comparison between the extraction of the laboratory prepared bar vs PDMS/Ge bar

4.3.6. Wide-ranging Applicability of Method

In addition to various factors taken into consideration, method was tested for applicability and selectivity. For this purpose, 1-naphthol and 2-naphthol were taken as an interfering agent in the same extraction media because the chances of its existence in the same media are very high. The peaks of 1-naphthol and 2-naphthol were baseline separated from 4-CN using our developed method. Fig 4.18. shows the chromatogram of 4-CN in the presence of 1-naphthol and 2-naphthol. This also proves that our SBSE-HPLC-UV method is widely applicable for various naphthols.

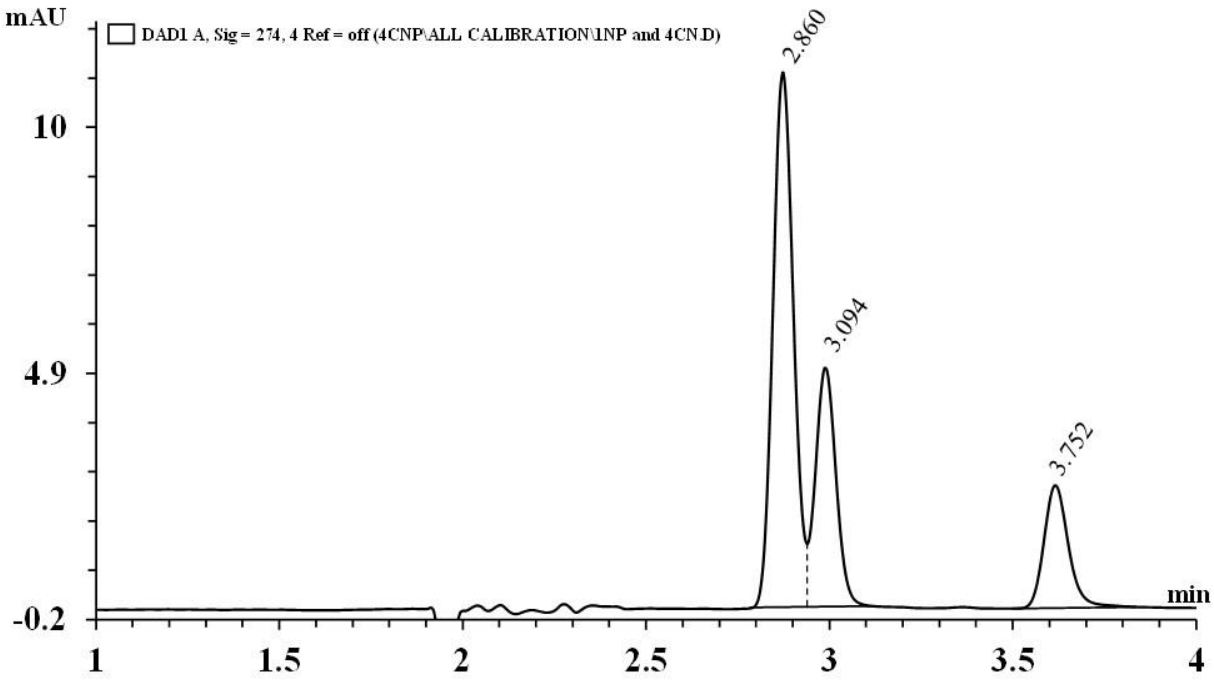


Fig 4. 18. Chromatogram of 4-CN (t_R: 3.752) in the presence of 2-Naphthol (t_R: 2.860), 1-Naphthol (t_R: 3.094)

4.3.7. Real Sample Analysis

The performance of the method was determined by the analysis of 4-CN in wastewater, swimming pool water, and urine. The extraction was done from the real sample and from a spiked sample. However, 4-CN concentrations were found to be 0.38 ng mL^{-1} in urine, 0.13 ng mL^{-1} in wastewater, and 0.39 ng mL^{-1} in pool water. Each sample was also spiked with 4-CN to have a concentration of 0.80, 150, and 500 ng mL^{-1}. Fig 4.19. – Fig 4.21 present the 0.80 ng mL^{-1} spiked and unspiked real sample analysis. Recoveries were estimated and showed in Table 4.2. All the extraction recoveries were ranged between 87.1 to 97.2 % with acceptable % RSD between 4-11 %.

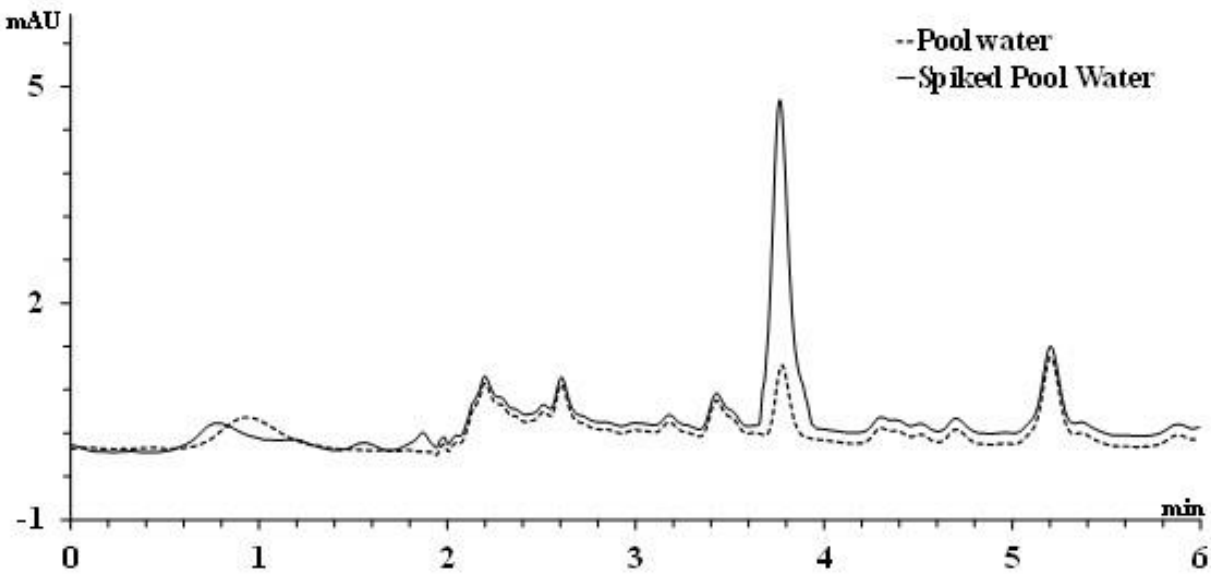


Fig 4. 19. Chromatogram showing the 4-CN in pool water, unspiked (dotted lines) and 0.8 ng mL^{-1} spiked (solid lines)

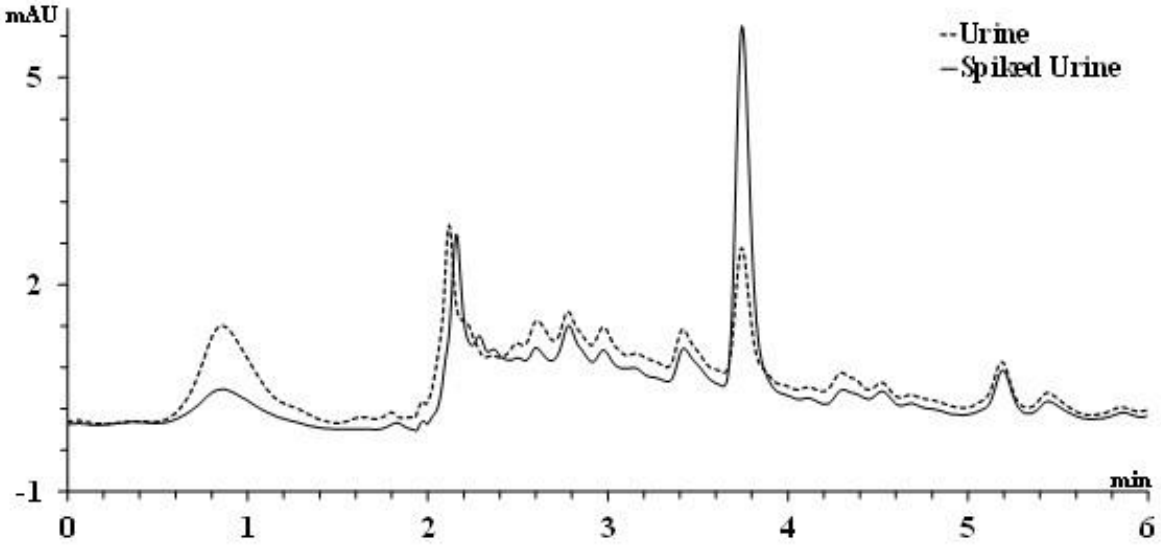


Fig 4. 20. Chromatogram showing the 4-CN in urine, unspiked (dotted lines) and 0.8 ng mL^{-1} spiked (solid lines)

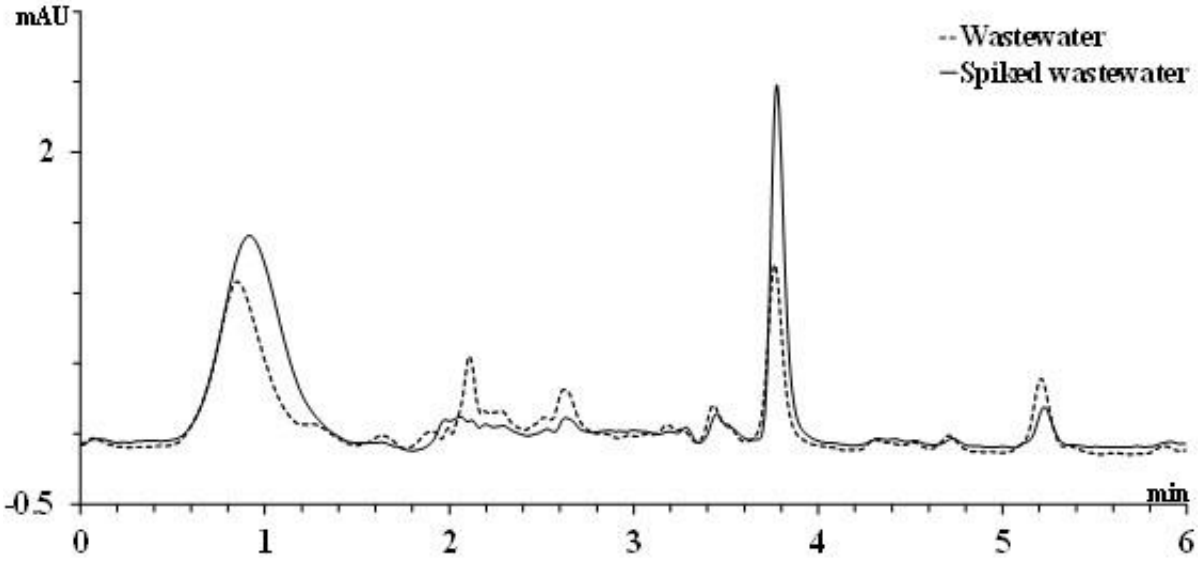


Fig 4. 21. Chromatogram showing the 4-CN in waste water, unspiked (dotted lines) and 0.8 ng mL^{-1} spiked (solid lines)

Table 4. 2. Quantitative analysis based on PDMS/Ge-SBSE-HPLC method in the real samples.

Real sample type	Unspiked	Spiked 0.80 ng mL^{-1}			Spiked 150 ng mL^{-1}			Spiked 500 ng mL^{-1}		
	4-CN Concentration ng/mL	4-CN Concentration recovered ng/mL	Recovery %	RSD %	4-CN Concentration recovered ng/mL	Recovery %	RSD %	4-CN Concentration recovered ng/mL	Recovery %	RSD %
Urine	0.38	0.71	88.8	10.4	130.71	87.1	8.5	455.25	91.1	6.5
Wastewater	0.13	0.75	93.8	6.1	145.82	97.2	4.4	475.35	95.1	5.2
Pool water	0.39	0.74	92.5	7.4	141.52	94.3	6.5	480.5	96.1	5.3

4.3.8. Summary of the work

This study presented a simple, fast, low-cost, widely applicable and sensitive method for the detection of 4-CN based by PDMS/Ge-SBSE-HPLC. This work illustrates remarkable linear ranges for detection, LOD, selectivity, and robustness in terms of preparation reproducibility. The current approach not only presents potential to work for complex matrices, but also has acceptable recoveries and precision.

Chapter 5
Conclusion

Considering the importance of sample preparation step, there is a need to introduce new sorbent surfaces that may play a promising role and present their application in real sample analysis. This work has used the beauty of sol-gel chemistry to introduce the new functional groups in to the polymeric network of the sorbent material and making them reliable for the extraction of almost every type of analyte with varying polarities. Three sorbent materials were synthesized including urea functionalized-[bis(hydroxyethyl)amine] terminated polydimethylsiloxane (BHEA-BPU), yttria surfaced-[Bis (hydroxyethyl) amine] terminated polydimethylsiloxanes (BHEA-Y), and germania-based polydimethylsiloxane (PDMS/Ge). The former two coatings were used for capillary microextraction and germania based coating used for stir-bar sorptive extraction. The characterization all the materials was successfully done by using X-ray photoelectron spectroscopy, thermogravimetric analysis, field emission scanning electron microscope, and energy dispersive X-ray spectrometer. BHEA-BPU and BHEA-Y coated capillaries were used for online extraction and high-performance liquid chromatographic analysis of amides, phenols, alcohols, ketones, aldehydes, and polyaromatic hydrocarbons. The PDMS/Ge based coating on the stir bar presented high preconcentration for the detection of 4-chloro-1-naphthol. All the extraction devices have proved their excellent potential by providing reliable, reproducible, and sensitive data in-terms of %RSD, LODs, and linearity. Moreover, real sample analysis is provided along-with extraction recovery and reproducibility. BHEA-BPU coating showed excellent overall sensitivity in terms of lower detection limits (S/N = 3) for the analytes (0.10 ng mL^{-1} to 14.29 ng mL^{-1}) with acceptable

reproducibility that is less than 12.0 %RSD (n = 3). Moreover, the capillary to capillary reproducibility of the analysis was also tested by changing the capillary of the same size. This provided excellent %RSD of less than 10.0 % (n = 3). BHEA-Y coating produced detection limits ranging from 0.18 ng mL^{-1} to 7.35 ng mL^{-1} (S/N = 3) with relative standard deviation (RSD) between 0.6% to 6.8% (n=3). The capillary preparation and coating method reproducibility was also considered by capillary to capillary extraction analysis with acceptable RSD between 4.1% to 9.9%. The PDMS/Ge coated stir bars showed a good preparation reproducibility of 1.7% (n=3) for one batch and 3.5% (n=3) for different batches. Under optimized experimental conditions, the method showed linearity in the range of 0.4-800 ng mL^{-1} with R^2 = 0.9992 and limit of detection (S/N=3) as 0.034 ng mL^{-1}. 4-chloro-1-naphthol was also extracted from wastewater, pool water, and human urine and showed relative recoveries between 87.1 - 97.2% with acceptable relative standard deviation i.e. 4-11%.

The current work is done using ultra-violet detector, however, the sensitivities of the presented analysis can be improved by hyphenating these extraction techniques with some highly sensitive detectors like mass-spectrometry. There is also a scope to develop and introduce sorbent materials that may be able to extract highly polar analytes.

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