THE APPLICATION OF SILICA MONOLITH FOR SOLID PHASE EXTRACTION

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TABLE OF CONTENTS

SUMMARY

Silica monolith had been in existence for more than a decade and the application of this technology for separation had been matured over time. The application of monolith had been extensively explored for separation in the form as columns. Although other areas of application have also been investigated, information of unmodified silica monolith as solid phase extraction is limited. In this thesis, application of silica monolith for solid phase extraction had been explored. Basically the thesis had been divided into three main areas:

1. **Application of the prepared silica monolith was realized as a sample preparation tool for extracting analytes from urine.**

The silica monolith was synthesized, characterized and finally tested for extracting catecholamines (epinephrine, norepinephrine), metanephrine, ketamine and opiates from urine. The classes of analytes represented different characteristics. The success in applying the silica monolith in extracting these analytes reflected the versatility in function of the tested monolith. For example, catecholamines and metanephrine represented compounds with highly polar group where as ketamine and opiates represented compound in the mid polar range. The testing of the silica monolith with the respective model analytes was described in separate chapters. Each chapter presented a progression from the previous one and a constant effort to further refine the process. The preliminary testing (Chapter 3) started with the extraction of catecholamines and metanephrine, the compounds with high pKa values (>11), and high hydrophilicity. A 2-cm

cartridge was used for extraction, taking urine as a biomatrix. The recoveries of these compounds after extraction ranged from 59-105% for the three analytes. The study proved the silica monolith to be effective for solid phase extraction and the results were encouraging. This led us to explore the potential of the silica monolith for extracting other compounds to confirm its diversity in application and the findings were described in Chapter 4. In this chapter, the batch to batch variation in the preparation of silica monolith was also investigated. Moreover, the effectiveness of miniaturization was realized and the cartridge length was reduced from 2 cm to 0.5 cm. The analyte was extracted from urine and showed recovery around 100%. Thus, a more extensive study was required to further demonstrate their effectiveness as solid phase extraction (Chapter 5). This led us to compare their performance with the commercial Oasis HLB in generating clean extracts. Opiates were used as a model analytes which again showed the recoveries around 100%. A full scan LC-MS and GC X QTOF analysis was carried out to demonstrate the effectiveness of the cartridge in reducing the matrix effect and the results were compared with the extracts generated from the commercial cartridge, the Oasis HLB. These studies demonstrated the successful application of unmodified silica monolith as solid phase extraction.

2. Application of silica monolith in desalination.

The mechanism behind the success of silica monolith as SPE was proposed to be due to ionic interaction with high surface area. This motivated us to realize the potential of silica monolith for desalination. Initially, the cartridge was tested with different concentration of sodium chloride and found effective in reducing 98% of salt in the samples. This encouraging result led to test the

cartridge for real samples. Thus, sample of seawater from the West Coast, Singapore was collected and tested for desalination capability of the silica monolith. Conductivity and osmolality were also determined to check the quality of water. The cartridge was able to be regenerated using either mild acid or high temperature at 60^{o}C.

3. **Finally an attempt was made to improve the surface characteristic of the silica monolith, especially surface area and pore structures.**

To achieve this, the silica monolith was compressed to the desired length during the aging period. The procured monolith was characterized for surface morphology using electron microscope, surface area and pore size distribution using nitrogen adsorption desorption and permeability using back pressure determination. The observed properties of the compressed silica monolith were compared to the non compressed monolith to demonstrate the effectiveness of the technique. The results showed that the surface characteristics were improved significantly with a compromise in permeability. Furthermore, the adsorption capacity of the compressed monolith was also compared to the non compressed one.

This study provided a novel concept of exploring unmodified silica monolith as a solid phase extractor. The finding in this study may be helpful to researchers in realizing the potential application of silica monolith in other areas of analysis, apart from being used as column alone.

LIST OF PUBLICATIONS

Journals

- Nema T, Chan ECY, Ho PC. 2010. Application of silica-based monolith as solid phase extraction cartridge for extracting polar compounds from urine. Talanta 82:488-494.
- Nema T, Chan ECY, Ho PC. 2011. Extraction of ketamine from urine using a miniature silica monolithic cartridge followed by quantification with liquid chromatography tandem mass spectrometry (LC-MS/MS). J Sep Sci. *Article in press.*

Conferences

- Oral presentation in PharmSci @ Asia 10 Symposium conducted by AAPS-NUS in NIPER, India.
- Poster presentation in 2nd Separation Science conference held in Singapore.
- Oral presentation in GPEN 2010 held in North Carolina, USA.

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

HPLC	High performance liquid chromatography
SPE	Solid phase extraction
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
ESI	Electrospray ionization
QTRAP	Hybrid triple quadrupole linear ion trap
FTIR	Fourier transformed infrared
MRM	Multiple reaction monitoring
CUR	Curtain gas
GS1	Ion source gas 1
GS2	Ion source gas 2
TEM	Source temperature
DP	Declustering potential
CE	Collision energy
EP	Entrance potential
CXP	Collision exit potential
SEM	Scanning electron microscope
TEM	Transmission electron microscope
XRD	X-ray diffraction
EDX	Energy dispersive X-ray
BJH	Barrett–Joyner–Halenda
BET	Brunauer–Emmett–Teller

CHAPTER 1

Literature Review

1.1 FUNDAMENTAL CONCEPTS OF STATIONARY PHASES

Stationary phase is the heart of each chromatographic system, whether employed as columns for separation or as extraction cartridges for sample preparation, and its performance determines the efficiency of the chromatographic process. Nevertheless, sorbents used for extraction, as in SPE, or separation, as in HPLC, work on the same principle but differ in chromatographic properties to achieve the respective goals. HPLC typically depends on numerous cycles of sorption and desorption in order to separate the analytes with good resolution. Whereas extraction depends on the sorption of analytes which is to be selective and strong in order to isolate the analytes from the interfering matrix and finally desorbing the analytes completely using a suitable solvent. The stationary phase is encased either inside an inert plastic or stainless steel holder in the shape of hollow straight rod, syringe barrel or disk depending on the desired application. Conventionally, chromatographic column is packed with porous silica microparticles, the size of which ranges from 2-10 μm when used for separation and from 50-60 μm when used for extraction. The column performance is commonly described by van Deemter equation which is given by:

$$H = A + B/\mu + C\mu$$

where,

H or HETP = height equivalent to a theoretical plate

μ = linear velocity

A term is a measure determined by eddy diffusion/interparticle channels; it is particle size dependent and velocity independent.

B term is a function influenced by molecular diffusion axially; it is inversely proportional to velocity.

C term is a function of the mass transfer kinetics; it is directly proportional to velocity and particle size.

These three terms A, B and C influence each other and give a function that can be represented by the typical van Deemter curve (Figure 1-1); and predicts the band broadening and the overall performance of the column. The HETP curve shows a minimum at a particular velocity, which is postulated to be the optimum velocity. At the optimum mobile phase velocity, the column will provide a maximum number of theoretical plates, i.e., the highest resolution power.

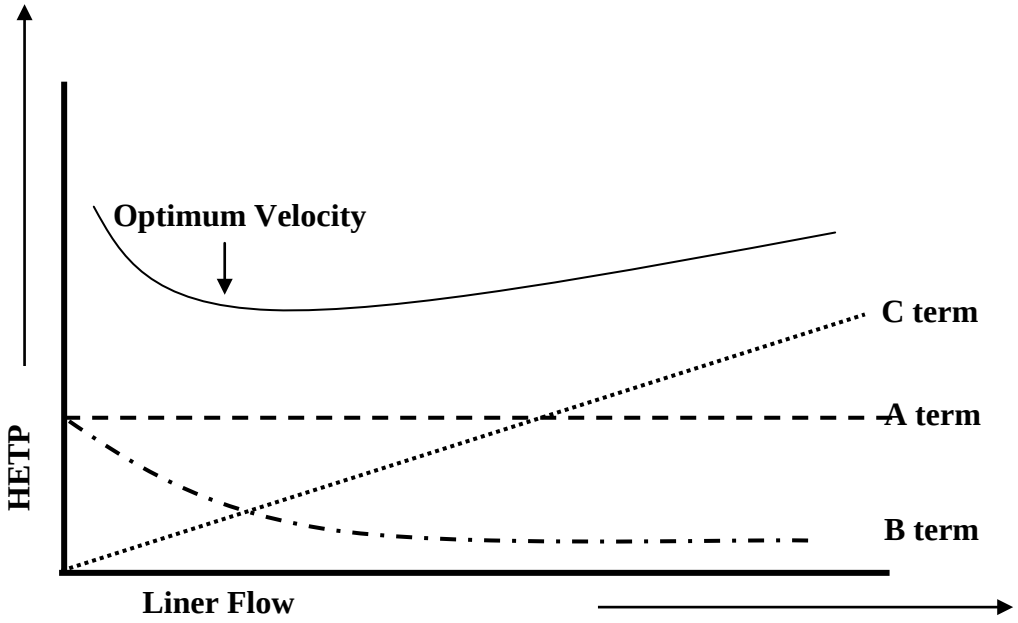


Fig. 1-1. van Deemter curve indicating the optimum velocity at maximum efficiency.

1.2 EMERGENCE OF THE MONOLITHIC CONCEPT

Because of the paramount importance of the column in the separation science, instrumentation of a separation system is designed and optimized around the column and aims at facilitating, preserving and enhancing the separation performance of the column. Plenty of research has been carried out in developing highly retentive and selective column with the prospects of resolving components in a short duration and cost-effective manner. Faster analysis time is the driving force in chromatographic

process and to achieve it, various approaches had been taken into consideration. One of the simplest approaches is the column operation at higher temperature. Increase in temperature decreases solvent viscosity effectively, allowing flow rate to increase markedly due to reduced back pressure which finally reduces analysis time. In addition, increasing temperature also enhances analyte mass transfer which contributes to increased separation efficiency. Although elevated temperature had been shown to have potential, it is limited by the thermal stability of analytes and stationary phases. Boiling point of solvents also limits the operation at elevated temperature. Another common approach is reduction in column length. It is acceptable until column efficiency remains satisfactory for separation. Column length is also directly related to backpressure, longer the column gives higher the back pressure and vice versa. Hence, to achieve higher efficiency with shorter analysis time, the approach of reducing column length is coupled with the reduced particle size. The particle size reduction is the significant approach which enhances efficiency manifolds due to the enhanced surface area which favors rapid mass transfer. However, it is limited due to increased backpressure as it is inversely related to particle size. Therefore traditional approaches to obtain column having ideal characteristics are still under progressive investigation. Table 1-1 depicts the relationship between approaches to enhance the performance against the properties of the column.

Table 1-1. Relationship between approaches undertaken to enhance performance versus the parameters affected.

Approaches → / **Affecting Parameters ↓**	**Particle size reduction (dp)**	**Column length (L)**	**Column temp. (T)**
Analysis time	Not related	$\propto$ L	$\propto$ 1/T
Column efficiency	$\propto$ 1/dp	$\propto$ L	Barely change
Backpressure	$\propto 1/dp^2$	$\propto$ L	$\propto$ 1/T

Furthermore, miniaturization in column is a trend in column technology in recent years. Miniaturization of column in analysis irrespective of the applications (e.g., proteomic, metabonomic, and environmental analysis) or any fields of science for separation and quantitation has the advantages of: (1) less solvent consumption, leading to lower cost of analysis in terms of lower cost of purchase of solvent and its disposals; (2) more environmental friendly and finally; (3) adding more sensitivity to the analysis [Saito et al., 2004; Legido-Quigley et al., 2002]. However, the limited loading capability and more sophisticated instrument requirements to achieve the desired flow rate and detection, limit their applicability.

Chromatographic resolution is based on the size and distribution of the particles along with the quality of packing. Higher column efficiency and shorter analysis time are the key factors that every chromatographer desires. Performance of particulate columns also depends on the frit that is placed at the end to retain particles within the column [Siouffi, 2003]. Ideally, the frits should be porous enough to allow uniform flow of mobile phase through the column which is difficult to achieve. This

leads to certain drawbacks in particle packed column. Conventional frits for microbore column, specially utilized in capillary electrochromatography (CEC) and micro HPLC, are usually prepared by sintering technique that utilizes very high temperature. Heat generated in the process can lead to destruction of stationary phase which can hamper the column performance and efficiency of separation. Bubble formation and analyte reaction with the frit material are some other problems associated with the end frits that cause deterioration of the column performance. Thus, a lot of skill and experience is required to reproducibly prepare a highly permeable and robust end frits.

Flow of mobile phase through the particle packed column depends on the permeability of the packed bed. This permeability is based on the size of particles and their distribution along with the quality of packing. Small particle (< 2 μm) packed columns result in faster separation and better resolution due to smaller eddy diffusion and shorter path length. Further decrease in particle size for enhanced performance at higher flow rates is restricted due to increased back pressure (as pressure is inversely proportional to square of particle diameter, according to Darcy's law) [Siouffi, 2003]. To achieve high separation efficiency with these columns, ultra high pressure liquid chromatography (UPLC) [MacNair et al., 1997; MacNair et al., 1999] and capillary electrochromatography (CEC) [Dittmann et al., 2000; Dadoo and Zare, 1998] have been employed. Although these instruments solve the problem to certain extent, their machine cost is high, for example UPLC requires high tensile strength expensive alloys. Design development, initial investment and maintenance cost are some of the major issues that limit their accessibility to the common users.

In light of the above concerns on particulate column, the need for efficient, uniform structured and porous fritless surface active column is desired, and it can be

prepared easily and is economical. These desired properties have been found to be the characteristic features of monolithic columns. The concept of monolithic columns was first conceived in the late 1970s when the scientists tried to use some organic monomers to prepare monolithic columns primarily to separate proteins [Kubin et al., 1967]. In recent years, monolithic stationary phase has gained high acclamation and myriad of research has been carried out. Figure 1-2 shows a schematic of monolith development in analytical science. The work has recently been reviewed by Cabrera [Cabrera, 2004]. It is because of their ease in preparation, efficient properties and excellent performance compared to conventional packed columns which make them an efficient tool in HPLC [Szumski and Buszewski, 2007]. According to Zou et al., monolithic stationary phase is a continuous unitary porous structure prepared by in situ polymerization of monomers (organic/inorganic) inside the column tubing [Zou et al., 2002; Gusev et al., 1999].

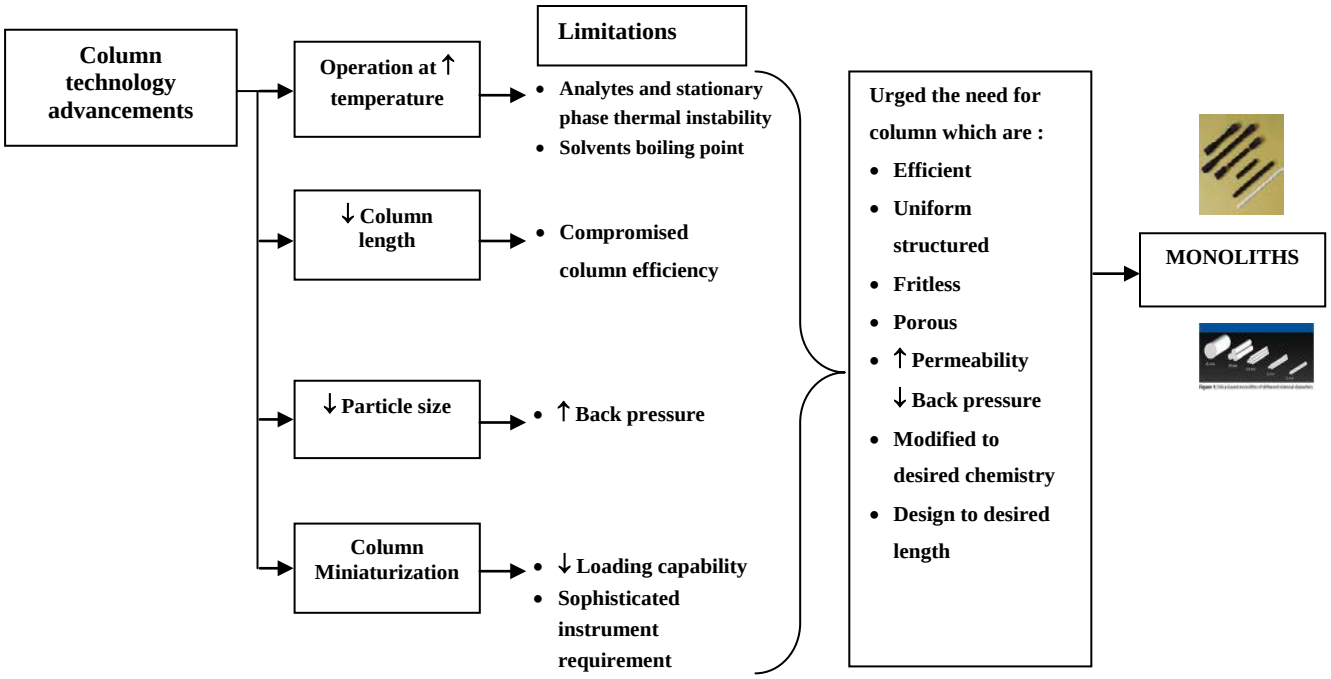


Fig. 1-2. Summary of monolith emergence.

Uniformity of bed with no end frits involved, higher permeability, convenient modification to desired chromatographic stationary phase (hence called as surface

active monolith) and fabrication to desired length are the main advantages of monolithic stationary phase. There are various ways of preparing a monolith. Some of the most common approaches for their preparation include (1) polymerization of organic/inorganic monomers of different chemical properties, (2) fusion of microparticles with the monolith inside the capillary by sintering and (3) usage of hybrid material [Siouffi, 2003]. Monolith based on organic monomers were the first and the most worked-on area in the chromatographic research, but the problems of swelling of polymers in some solvents and mechanical instability limit the use of organic monomers as monolithic stationary phase. These problems associated with organic monolith led to the introduction of inorganic based monoliths using monomers like, tetramethoxysilane, tetraethoxysilane and other functional monomers or combination monomers. These inorganic monoliths have the advantages of high mechanical stability and resistance to swelling in solvents when compared to organic monoliths [Motokawa et al., 2002; Minakuchi et al., 1996; Hjerten et al., 1989]. Another technique of fusing microparticles by sintering is one of the uncommon approaches for preparing monolithic columns. The approach had limited application due to the two major hindrances, first, difficulty in preparation and second, inconsistent column performance [Asiaie et al., 1998; Adam et al., 2000].

There are plenty of advantages associated with monolithic column which make them an efficient and promising tool in the separation technology, but due to some other disadvantages, the monoliths still has limited popularity as a stationary phase. Cracking and shrinkage of the formed rod inside column tubing and difficulty in housing the detached rod in suitable cartridge are the major drawbacks of monolithic column [Siouffi, 2003]. These pose as challenges in research on developing monolithic columns.

1.3 MONOLITHS: DEFINITION

In chromatographic terms, monoliths represent a continuous single rod of porous material [Tanaka et al., 2002]. It is characterized by high permeability due to uniform distribution of macropores and mesopores throughout the network enabling separation of many analytes. The macropores present provide the permeability for solvents to flow through, whereas mesopores provide the high surface area for separation. As the formed network fills the column volume completely, inter-particulate voids are absent, resulting in 100% flow of mobile phase through the column. For the preparation of monolithic column, the need for packing, as in particle packed column, is unnecessary, as the monolith can be prepared in situ by polymerization. However, this process of polymerization is restricted to capillaries (usually less than 200 μm in internal diameter, ID) due to the problem of shrinking of monolith in the capillaries or column of larger ID. A comparison of the physical and surface properties between a particle packed column and a monolithic column is shown in Table 1-2.

Monolithic columns are easier to prepare, to the desired porosity and pore diameter to suit different needs [Qin et al., 2006]. Specific selectors such as chiral selectors can be incorporated in the monoliths and kept in place through co-polymerization. The elution time can be reduced by a factor of 5 to 10 in comparison to particulate column [Ro et al., 2006]. No special skill is required in all these procedures, making inter-laboratory studies easy and comparable [Szumski and Buszewski, 2007]. There is also a decrease of risk of bubble formation or breakage of the capillary [Qin et al., 2006], as the column backpressure is lower under higher mobile-phase flow rates [Ro et al., 2006].

Monoliths are broadly classified on the basis of the nature of materials used for the preparation. Depending on this, there can be many types of monoliths but generally they are categorized into organic and inorganic based monolith. All other types of monoliths revolve around the chemistry of these two types of monoliths, either with certain modifications or by using combination of monomers. Organic monomers, like acrylamide [Hjerten et al., 1989; Palm and Novotny, 1997; Fujimoto, 1995] methacrylates [Peters et al., 1998; Peters et al., 1998; Peters et al., 1997] and others, are used for organic monoliths [Wang et al., 1993; Gusev et al., 1999]; whereas inorganic monomers, e.g., alkoxides of silicon [Minakuchi et al., 1998; Malik, 2002; Tanaka et al., 2002], titanium[Ren et al., 2006; Konishi et al., 2006] and zirconium [Randon et al., 2006], are used for inorganic monoliths. The two categories differ in their chemistry of preparation, in which polymerization is applied for the organic and hydrolytic polycondensation for the inorganic monolith. In our study, emphasis is given to the fabrication of inorganic based monolith with the context to silica monolith.

Table 1-2. Comparison of the physical and surface properties of a particle column (Symmetry C_{18}, Waters) and a monolithic column (Chromolith Performance RP-18e, Merck) [Cabrera, 2004]

Properties	**Monolithic column**	**Particle packed column**
Skeleton size (µm)	1.3-1.6	-
Particle size (µm)	-	5
Macropore size (µm)	2	-
Interparticle pore size (µm)	-	1.25-2
Mesopore size (A)	130	90
Total porosity	>0.80	0.65
External porosity	0.706	0.37
Surface area (m^2g^{-1})	300	340
Surface coverage, C_{18} ($\mu moum^{-2}$)	3.6	3.2
Total carbon (wt %)	19.5	18
Endcapping	Yes	Yes

1.4 MONOLITH ADVANCEMENTS

1.4.1. Silica Monoliths

Silica based monoliths are generally prepared by sol-gel process which offers a versatile means for their synthesis, as it provides an exceptional control over the composition and morphology of the formed monolith. Generally, sol-gel technology for the preparation of monolith involves sequential hydrolysis followed by the polycondensation of the hydrolyzed product to form a macromolecular porous structure, possessing a bimodal pore structure (macropores and mesopores). Figure 1-3 demonstrates a general mode of their preparation. Sol-gel technology has its existence since late 1800s but it had gained interest in early 1970s when inorganic gels were formed [Quigley et al., 2003]. It was in 1996 when Tanaka et al.

[Minakuchi et al., 1996] first used reversed phase porous silica monoliths for liquid chromatography. They prepared silica monoliths based on hydrolytic polycondensation of tetramethoxysilane in aqueous acetic acid in the presence of polyethylene oxide (PEO) to form a silica network structure in a mould. The detached rod was subsequently washed with water and treated with ammonium hydroxide solution that introduces mesopores in the range of 5-25 nm [Szumski and Buszewski, 2007; Minakuchi et al., 1996; Ishizuka et al., 1998]. Finally, it was encased in a PTFE (poly tetrafluoroethylene) tubing using Z-module. Later Nakanishi et al. [Nakanishi et al., 2000] modified this method and used urea instead of ammonium hydroxide as a source of ammonia. This improvement eliminated the need for post treatment of the monolith in a capillary with ammonium hydroxide as ammonia is generated by hydrolysis of urea at 120^0C which results in the formation of mesopores. The surface of the monolith formed in both cases was modified using conventional silane chemistry to attach different stationary phases through siloxane bond linkage.

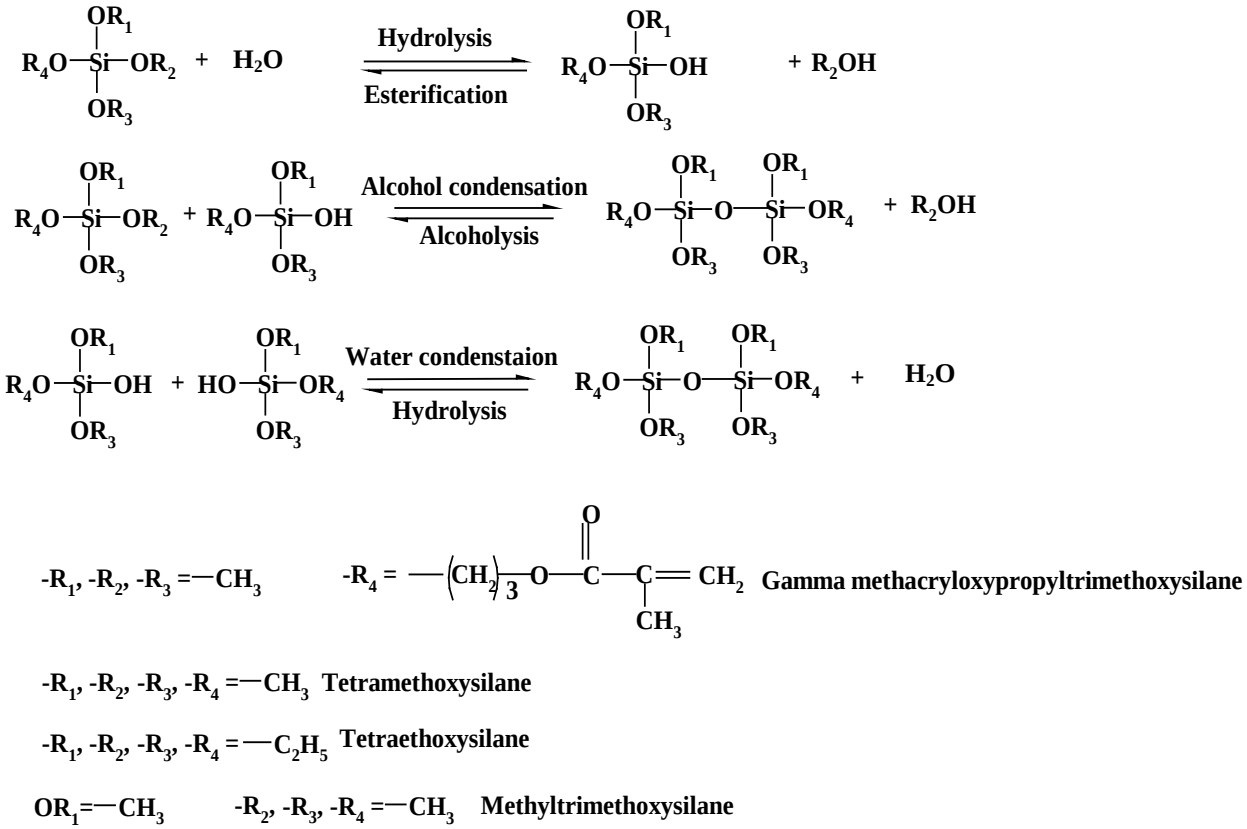


Fig. 1-3. General scheme of monolith preparation via sol-gel method.

Although the monoliths formed with these methods possess an excellent mechanical stability, the preparation was laborious. Furthermore, shrinkage and cracking within monolith are some of the disadvantages associated with the silica monoliths. Cracks are formed due to generation of high stress during evaporation of liquid from the pores when the gel contracts. Shrinkage results during the phase separation of the polymerizing monomer because the gel formed squeezes water out from the formed matrix [Rieux et al., 2005].

To overcome the problems of cracking and shrinkage, modification to desired stationary phases with various approaches with minimum skill and time have been developed. These methods proved to be effective with some compromises either in performance or properties. The first approach which marked the development was the preparation of particle loaded monoliths in capillary format with the intention to

prepare crack free column [Dulay et al., 1998]. Dulay et al. introduced this approach of preparation with the concept of embedding ODS particles inside the pores or cavities created within the formed matrix. Monoliths were prepared by embedding ODS particles (3-5 μm) in tetraethylorthosilicate (TEOS) via sol-gel technology. The solution was filled in a 75 μm capillary of 40 cm length and the formed monolith was used in CEC. The performance of the column was evaluated with a mixture of aromatic and non-aromatic compounds. Columns exhibited efficiencies up to 80000 plates/m and 33000 plates/m with 3 μm and 5 μm embedded ODS particles, respectively. Different efficiency is attributed to non-homogeneity in packing ODS particles and shielding of the particles form the analyte because of the deep imbedding of the particles. To counteract these problems, Bakry et al. [Bakry et al., 2006] modified the above procedure and encapsulated silica particles within polymeric backbone (poly styrene divinylbenzene). According to their approach, pretreated silica capillary was packed with silica particles using slurry packing method followed by introducing an immobilizing mixture comprising of styrene, divinyl benzene, azobisisobutyronitrile (AIBN) and decanol. After polymerization, the formed monolith was tested for polyphenols, peptides and proteins and efficiencies in the range of 120,000-200,000 plates/m were achieved for protein separation using 3 μm ProntaSIL C-18 particles.

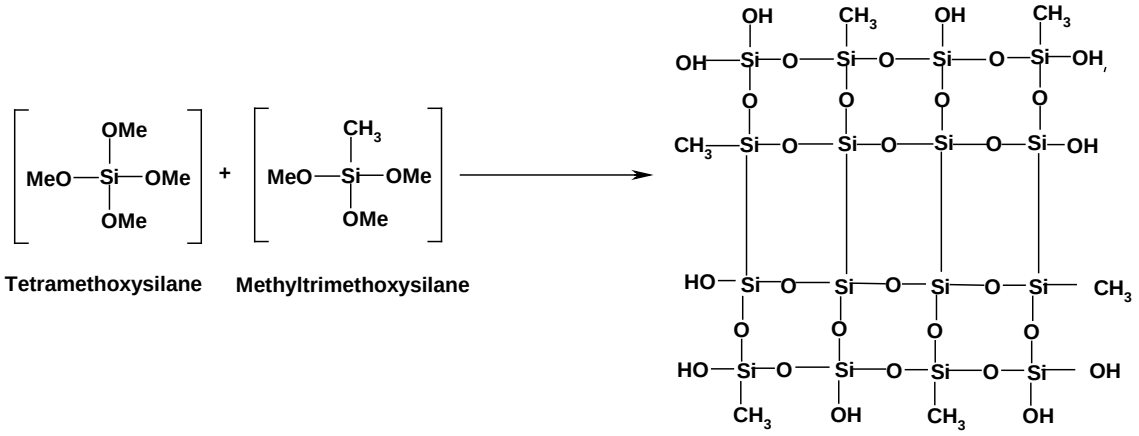


Fig. 1-4. Monolith preparation using mixed monomer via sol-gel method.

Monoliths used in various separation modes are prepared both in larger diameter column (as large as 4.6 mm which is fabricated in PEEK tubing after detachment from the mould) and smaller diameter column (as small as 50 μm capillary). In some cases monoliths prepared in capillaries are easier to synthesize then larger bore columns as they can bind covalently with the inner capillary wall which imparts more stability to the monolithic bed. It also eliminates the further fabrication of the detached rod from the moulds. Motokawa et al. [Motokawa et al., 2006] have successfully prepared monolithic column using mixed alkoxysilanes (tetramethoxysilane and methytrimethoxysilane) within the confinement of 530 μm capillary and compared it with the columns packed with 5 μm and 3 μm ODS particles. Figure 1-4 demonstrates its preparation. The monolith formed was free from gaps which were shown by SEM photographs that show the attachment of the monolith with the capillary walls. The monolith was tested on reversed phase HPLC system for the separation of alkylbenzenes which was equivalent in performance with the column packed with 3 μm particles but 2.5-4 times higher permeability. The column has shown to withstand higher flow rate (100 μl/min) when the formed capillary monolith was tested for the separation of proteins.

In another study, Kato et al. [Kato et al., 2002] prepared photopolymerized sol-gel monoliths (PSG) using γ-methacryloxypropyltrimethoxysilane (γ MAPS) due to the pore size dependency on temperature variation [Svec and Frechet, 1996; Svec and Frechet, 1992]. γ MAPS has a bifunctional nature, containing both methacrylate and alkoxysilane groups, which favors polymerization/polycondensation to prepare sol-gel monolith in a single step avoiding the need for final functionalization. In addition it is also used to vinylize the inner surface of the capillary to ensure gap free binding of the monolith to the sides of the capillary. Monolith was synthesized by acid catalyzed hydrolysis followed by condensation of γ MAPS and finally a photoinitiator (Irgacure 1800) in toluene was added. After stirring for 2.5 hr at room temperature solution was filled in capillary and allowed to polymerize by irradiation in a photochemical reactor at 350 nm for 20 min. The formed monoliths were tested on reversed phase CEC mode for the separation of alkylbenzenes. In the subsequent study, Kato et al. [Kato et al., 2003] modified the PSG monolith using dimethyloctadecylchlorosilane (DMOS) followed by end capping with chlorotrimethylsilane to mask the residual silanol groups and compared it with non end capped PSG modified with DMOS. The monolith was used as a reversed phase mode in CEC mode for the separation of amino acids from rat cerebrospinal fluid after derivatization with 4-fluoro-7-nitro-2,1,3-benzoxadiazole. Endcaping helps to improve the column performance based on reproducibility, stability and peak symmetry and eliminates secondary interactions like peak broadening and low performance [Yang et al., 2006]. Later Zhang et al. [Zhang et al., 2007] introduced a novel approach where they prepared methacryloxypropyltrimethoxysilane monolith based on sol-gel chemistry but using γ irriadiation for polymerization instead of photochemical technique [Svec and Frechet, 1996]. This imparts mechanical

stability to column which was not sufficient enough with the photochemical technique and also eliminates the need for initiator as radicals are directly generated on the monomers. Monolithic column was prepared with two modifications in the above method [Svec and Frechet, 1996]: (1) addition of sodium dodecyl sulfate to enhance the solubility of alkoxysilane in water and (2) elimination of photoinitiator. The formed column exhibited reversed phase character and was evaluated in a CEC and low pressure driven separation modes.

1.4.2 HYBRID MONOLITHS

Hybrid monolith synthesized via sol-gel technique using hybrid materials has been introduced as an alternative for the existing stationary phases and proved to be promising as it provides greater advantages over conventional silica monolith. Sol-gel hybrid materials are specially designed to possess desirable properties and eliminate the undesirable ones to improve column efficiency, stability and selectivity. The monoliths prepared using hybrid materials possess advanced properties which are superior and difficult to achieve with pure organic or inorganic materials. Furthermore, hybrid monoliths can be directly designed by combination of organic-inorganic monomer for the desired chromatography which eliminates the need for functionalization of stationary phases that is more common with the conventional method which involves preparing the monolith and then functionalizing it. Hence, in a search for an alternative to combine preparation and functionalization of silica monolith in a single step which reduces the time of preparation and laborious task of derivatization, Haynes and Malik [Hayes and Malik, 2000] prepared the monolithic column using solution without utilizing particles as well as avoiding the need of frits. They presented a single step process to prepare functionalised porous monoliths which is chemically bonded to the inner walls of the silica capillary. N-

octadecyldimethyl[3-(trimethoxysilyl)propyl]ammonium chloride was used as the sol-gel precursor which imparts chemically bonded ODS ligands to porous monoliths useful for CEC analyses. Later on, Laschober et al. [Laschober et al., 2007] prepared, in a single step based on sol-gel method, a capillary monolith with single alkyltrialkoxysilane, methyltrimethoxysilane (MTMS), as a precursor which has an advantage of having enhanced hydrolytic stability of Si-C bond and they have studied the variation of various parameters on the morphology, characterized by pore and skeleton diameter and the surface area of the monolith. These monoliths can be used in extended pH range. The monoliths resulted from MTMS however, exhibited higher tendency for spinoidal decomposition in comparison to tetraalkoxysilanes, due to their reduced compatibility with polar solvents. Synthesis of monoliths with this approach does not require hydrophilic polymer, polyethyleneglycol, as in case with tetraalkoxy silanes and also eliminates the need for functionalization of the capillary walls. The reaction was carried out at pH 1 to synthesize monolith so as to possess bicontinuous morphology. Surface area was low in comparison with tetralkoxysilane which could be attributed to the maximum of three valences (out of total four) of silicon involved in establishing bond with other organo-silica tetrahedrons. The chromatographic performance of monolith was tested with individual components and predicted to have separation based on their different retention times. The mechanism involved seemed to be complex and further investigation was warranted as suggested by the authors. In another study, Yan et al. [Yan et al., 2006] synthesized C_8 funtionalized hybrid silica monoliths via two step acid base catalyzed sol-gel chemistry. Monolith was prepared by co-condensation of tetraethoxysilane (TEOS, as matrix monomer) with C_8-TEOS (octyl TEOS as functional monomer) in presence of methanol, water and 0.5 M HCl. Mixture was stirred for 3 min and was allowed to hydrolyze for 6 hr

followed by dodecylamine addition. The final mixture was filled to pretreated capillary and allowed to react at 40^0C for 12 hr. The formed rod was tested on a reversed phase CEC mode. Two-step catalysis method was proposed in that study which made separation of hydrolysis and polycondensation step possible. Initially 0.5 M HCl was used for acid catalyzed hydrolysis to produce silanol groups followed by dodecylamine for base catalyzed condensation.

Simplicity of sol-gel technique and the mild conditions used in its preparation allow the incorporation of dopants like organic or biomolecules. The introduced molecule can either be used as spectroscopic probes to study the physical and chemical changes taking place in sol-gel chemistry or can be implied in the development of stationary phases possessing enhanced characteristic depending on the property of the entrapped molecule. Recently, Dunn and Zink [Dunn and Zink, 2007] have reviewed the properties and applications of molecules entrapped in a silica matrix prepared by a sol-gel method. Entrapped molecules functioning as a spectroscopic probe provides the insight of sol-gel chemistry which is useful to study the effects of changes of various parameters like solvent composition, polarity, viscosity, pH and rates of chemical reaction. Various organic molecules, functioning as probes, have been utilized to monitor the changes in sol-gel chemistry. For example, pyranine was used to study the effect of solvent chemistry on the aluminosilicate sol-gel [Winter et al., 1990]. Changes in the luminescence of pyranine were monitored from the initial sol phase to the finally dried aluminosilicate gels which were found sensitive to solvent chemistry and the surrounding pH. Various molecules have been used for the probing and depending on the characteristics of the dopants, various spectroscopic probing methods such as fluorescence anisotropy experiment (monitor the rotation of a molecule in a sol-gel matrix) [Narang et al.,

1994; Winter et al., 1990] and rigidochromism (property to measure reorientation of solvent dipoles) [McKiernan et al., 1989] can be used. Biomolecules can also be dopped inside the sol-gel silica matrix which finds its application in biosensing, high throughput screening and chromatography. The main concern for the encapsulation of the biomolecules within the confines of the silica matrix is the ability to retain their activity and function in the environmental conditions used for its preparation. Biomolecules are proteins and are active as long as the synthesis conditions are favorable to avoid protein denaturation but the conventional sol-gel method uses solvents like alcohol and the variable pH conditions that are necessary for network formation, proved to be detrimental for the biomolecules. This led to the introduction of diol or polyol modified silanes as substitutes for the conventional precursors (tetramethoxysilane or tetraethoxysilane) which proved to be more compatible with the released diol- polyol with the entrap biomolecules, which have been recently reviewed by Hartmann et al. [Hartmann et al., 2007]. Initially hydrolytic instability was the main concern in their preparation, but later on with the identification of polyol esters of silicates and siloxanes, encapsulation of biomolecules while retaining their activity was proved to be feasible. After this success, a number of scientists have studied the preparation using various materials for different applications. Various modifications of silanes via diol/polyol modification are represented in Figure 1-5.

Another approach uses molecular imprinting technology which demonstrates specific selectivity for the analyte (target) to be separated from the mixture. The predetermined target is used as the template in the preparation which after removal from the formed matrix leaves a site that is complementary in both shape and functionality to the target (Figure 1-6 depicts its preparation). Generally, preparation of molecularly imprinted polymer (MIP) involves two steps: (1) entrapment of guest

molecule in the formed matrix, (2) removal of the template to generate cavities which possess memory for guest molecule [Liu et al., 2007]. Such a concept of MIP can be potentially used in the separation of chiral compounds in CEC or low pressure driven chromatography. Wang et al [Wang et al., 2006] prepared porous silica based hybrid monolith selective for (S)-naproxen, using room temperature ionic liquid (RTIL) via non-hydrolytic sol-gel methodology (NHSG). RTIL is used as solvent which has unique properties, like low vapour pressure, high ionic strength and has a tendency to act as pore templates. NHSG eliminates the drying and aging step and improves selectivity by reducing/eliminating the number of residual silanol groups. The novel concept of using RTIL mediated NHSG eliminates the problems like shrinking and cracking associated with hydrolytic sol-gel technology, as no water is involved. The monolith was tested in CEC mode and racemic separation of naproxen was successfully carried out with varying acetonitrile concentration. Previously, Acosta et al. [Acosta et al., 1994] have reported the preparation and characterization of monolithic alumina gels by non-hydrolytic sol-gel technique. The gel formed was amorphous and non-hydrated with the scope directed towards its utility in catalysis field.

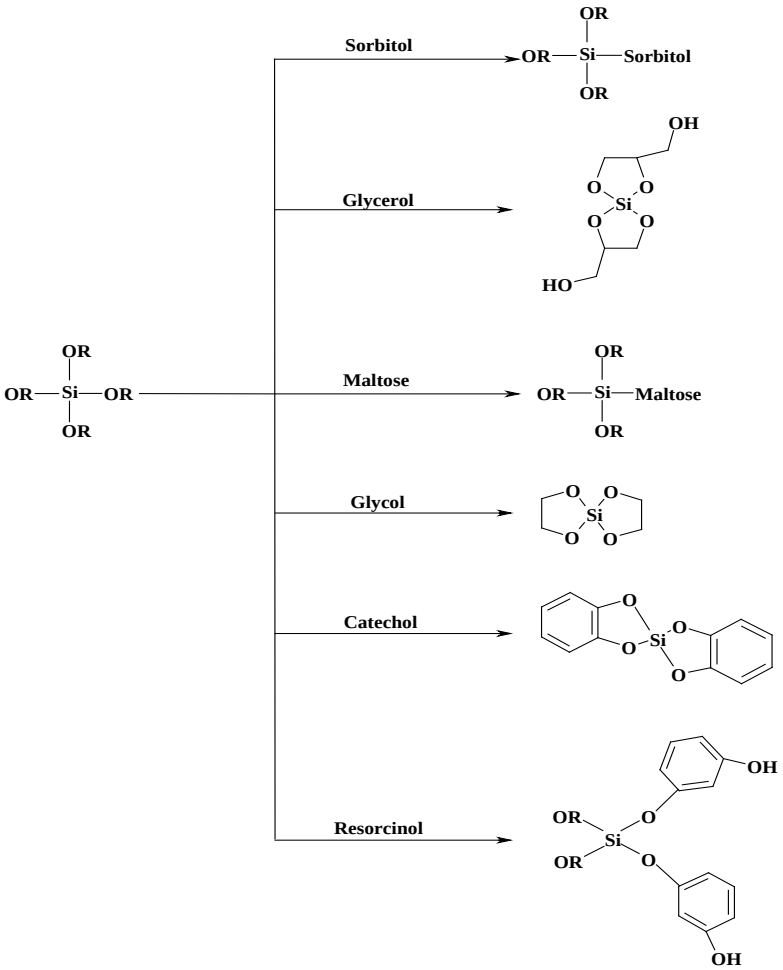


Fig. 1-5. Schematic representation of alkoxysilane modification to be used in materials syntheses [Hartmann et al., 2007].

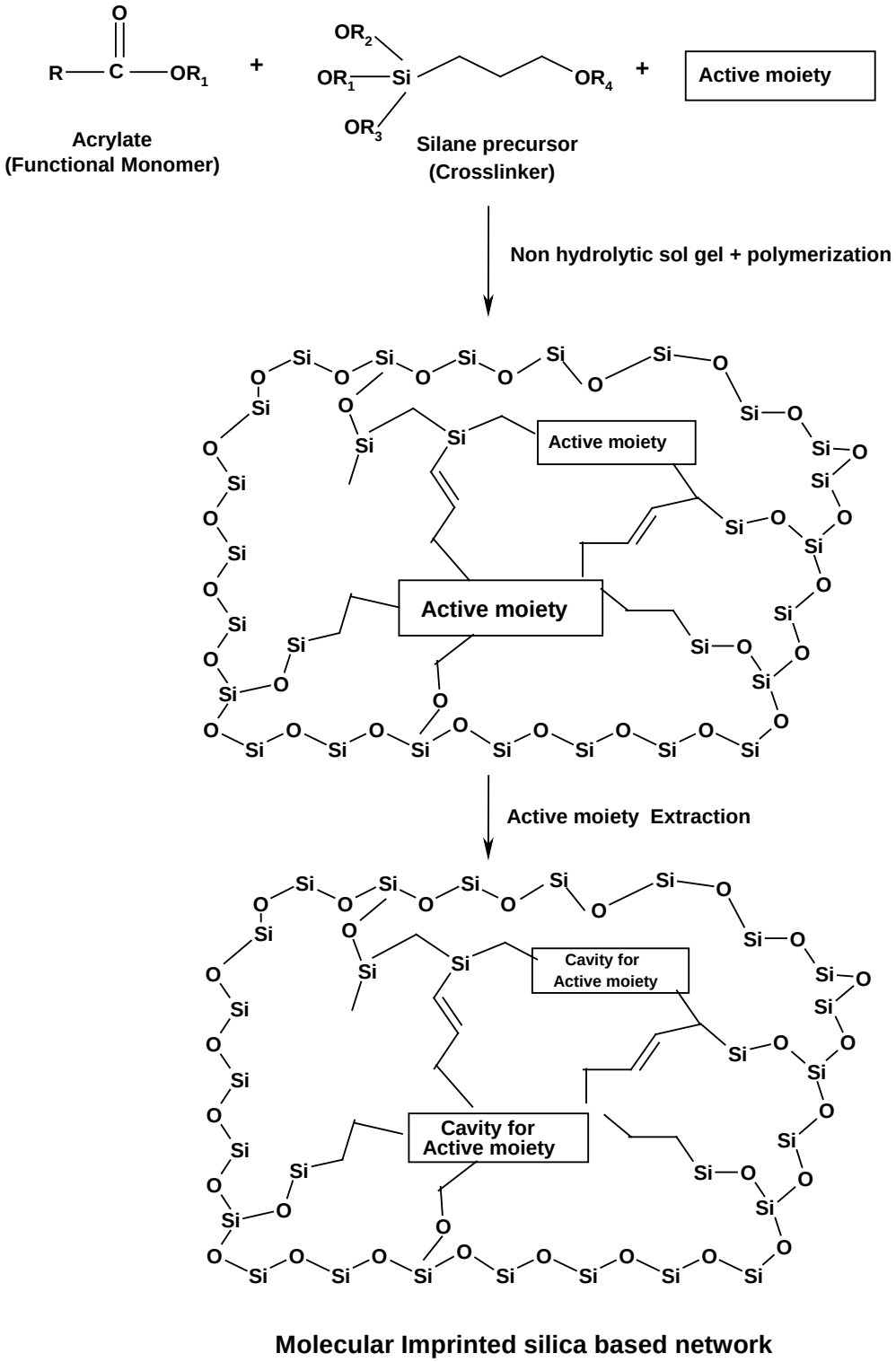


Fig. 1-6. General scheme for preparation of molecular imprinted monolith.

Jakschitz et al. [Jakschitz et al., 2007] reported the preparation of monolithic poly [(trimethylsilyl-4-methylstyrene)-co-bis(4-vinylbenzenzyl) dimethylsilane] stationary phases by thermal initiated in situ polymerization of trimethylsilyl-4-

methylstyrene and bis (4-vinylbenzyl) dimethylsilane in a pretreated capillary for the separation of proteins and oligonucleotides by ion-pair reversed phase liquid chromatography. The capillary column was filled with the mixture of varying ratio of monomers along with microporogen (2-propanol), mesoporogen (toluene) and the radical initiator (azoisiobutyronitrile) and allowed to polymerize at 65^0C for 24 hr for the separation of proteins and oligonucleotides. The prepared monoliths when tested in reversed phase ion pair mode showed good separation and high stability.

1.5 APPLICATIONS OF MONOLITH

Since their introduction in chromatographic field they have become synonymous to columns for various separation applications. However, less is explored about the variety of potential applications which could diversify their scope of application apart from being used as column alone. These include supports for solid-phase and combinatorial synthesis [Tripp et al., 2001; Pflegerl et al., 2002; Vlakh et al., 2004], scavengers [Tripp et al., 2001; Tripp et al., 2000], carriers for immobilization of enzymes [Krenkova and Foret, 2004; Josic and Buchacher, 2001; Svec, 2006], static mixers [Rohr et al., 2001], thermally responsive gates and valves [Yu et al., 2003; Luo et al., 2003], as well as solid-phase extractors and pre-concentrators. The reason for deficit application is attributed to their unique characteristics and advantages which they offer created more interest to chromatographers to maneuver more in column technology. Moreover, the exploration of technology in other areas has seemed to be in its infancy and much of the effort has to be put in to completely demonstrate its efficiency and ability. The present study dealt with the solid phase extraction and this thesis is dedicated to explore the potential of monolith as a solid phase extraction cartridge.

1.6 MONOLITH: AS A SOLID PHASE EXTRACTOR

Sample pretreatment prior to analysis is essential in analytical process especially when the sample matrices are complex. Samples free from interfering matrices not only simplify analysis but also maximize the sensitivity of detection by concentrating analyte within the detection limit as well as eliminating interfering contaminants. Liquid-liquid (LLE) and solid phase extraction (SPE) are the two major methods used widely for this purpose. While both methods are applied for sample clean-up, SPE has the advantage of ease in operation and environmental friendliness. In SPE, the use of toxic organic solvents is minimized. Therefore based on these considerations, SPE was found useful to extract analytes from complex mixtures like environmental [Koester and Moulik, 2005; Bulut et al., 2007; Wang and Zhang, 2006] and biological samples [Thabano et al., 2007]. The basic principle underlying the retention of analytes on SPE sorbent is based on reversible hydrophobic, polar and ionic interactions. Such interactions depend on the chemistry and nature of the sorbent and analyte. The availability of numerous solid-phase chemistries is the major advantage that SPE has over other extraction techniques. Sorbents used in SPE can be broadly classified into organic and inorganic sorbents. Organic sorbents are polymer-based whereas inorganic sorbents are silica-based. The physicochemical properties like, polarity, acidity and basicity of the sorbent and the analytes have great impact on the extraction efficiency. Of special significance is the extraction of polar analytes from the sample matrices. Polar analytes are difficult to isolate and preconcentrate as it is difficult to retain them selectively using SPE for accurate quantitation. For the sorbent to retain polar compounds efficiently, it should have a large and highly specific surface area to provide large number of hydrophilic interaction sites to retain the polar analytes. The polarity of the sorbent has to be competitive to the sample

matrix in order to have high affinity for the polar analytes. Numerous sorbents for polar analyte extraction, either as offline and online solid phase extractor, have been extensively reviewed [Raynie, 2006; Fontanals et al., 2007]. One of the techniques is solid phase microextraction (SPME) which has shown great potential in extracting polar analytes from liquid samples [Basheer et al., 2005; Djozan and Ebrahimi, 2008; Kloskowski et al., 2009]. Nonetheless, SPME suffers from limited sites for interaction due to low volume of the stationary phase as well as the limited number of coating fibers.

In recent years, polymer based sorbents have found increasing attention in the extraction of polar compounds and various modifications in the preparation of polymer-based sorbents have been explored for the purpose. Many of these sorbents also showed great potential with regards to their reusability but for limited extent. Polymer-based sorbents for extraction of polar compounds have been extensively reviewed by Fontanals et al. [Fontanals et al., 2007]. Their advantage of being able to be used over extended pH range makes them more popular in SPE. In spite of all the potential advantages, the major drawback of the polymer-based sorbent is its tendency to swell in organic solvents which leads to undesirable changes in its pore structure and makes them mechanically unstable. This instability can lead to their collapse or may result into run to run variability after multiple usages [Chaisuwan et al., 2008]. It is because of these limitations, polymer-based sorbent cannot be used repeatedly and has to be disposed after single or few usages. The silica-based sorbent on the other hand provides good organic solvent resistance and mechanical stability. However, the limited pH working range (2-8) of silica renders it less amenable to certain applications when compared to polymer-based sorbents. Nonetheless, if the pH values of the reagents used during SPE are compatible with the working range of silica,

silica-based cartridge may be explored for repeated polar compound isolation. The existing SPE cartridge based on silica are mostly particle packed with a particle size in the range of 30-60 μm and requires the filling of particles in the holder (syringe barrel) between porous frits. The efficiency of extraction depends on the quality of packing, i.e., more uniform packing will give less variation in the recoveries of samples. In addition, particle size also has significant effect in the quality of packing and performance of the cartridge. It is known that smaller the particle greater is the surface area that will favor rapid mass transfer. Although, reduction in particle size can facilitate the efficiency of the sorbent material for extraction of analyte(s), it is limited by the increase in back pressure. As discussed in earlier section, back pressure is inversely proportional to the square of particle size [Siouffi, 2003]. Because of that, small particles will create high back pressure that will be detrimental to the vacuum pump used for suction during the SPE process. Nonetheless, if the small particle is desired then the bed length has to be compromised in order to counteract the increased back pressure. This in turn limits the number of interaction sites on the sorbent surface, and therefore the capacity of the sorbent [Poole et al., 2000]. Particle packed SPE sorbents could also be packed as hard cakes after multiple usages as the particles are progressively compressed under the applied pressure. This will further increase back pressure, causing undue stress on the vacuum pump. In addition to these, particle based SPE cartridges form channels after few usages. This led to rapid movement of the analytes through the channel before they get sufficient equilibration time to interact with the stationary phase. Henceforth, the repeatability of the cartridge for multiple usages was limited and result into its rejection. These limitations urge the need for more reliable and easy SPE material which can provide high surface area and efficiency in performance without any undue problems. This led to the exploration of

the monolith for SPE application. In comparison to packed particles, monolith offers high porosity and permeability, rendering them an attractive alternative for SPE. Furthermore, monolith does not require end frits like particle packed SPE to retain particles within the vicinity of the holder.

Monolith as stationary phase was first introduced in the field of separation science in early 1990s [Tennikova et al., 1988]. Subsequently, monolith was leveraged in diverse separation science applications involving high performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrochromatography (CEC) [Svec, 2004; Cabrera, 2004; Tanaka et al., 2001; Bedair and El Rassi, 2004; Fu et al., 2004; Svec and Kurganov, 2008; Hilder et al., 2004]. The details of the diverse applications are reviewed in a series published by Svec [Svec, 2006; Svec, 2006; Krenkova and Svec, 2009]. While monolith is a popular material for column fabrication and it appears intuitive that it may play a role in SPE and protein/enzyme immobilization, it is surprising that the latter applications are broadly overlooked. Recently, monolith began to gain recognition as a sample preparation tool which is evident in a number of publications [Raynie, 2006; Fontanals et al., 2007]. The ease of modification of the surfaces of monolith for desired extraction renders it an amenable tool for offline and online sample preparation.

1.7 HYPOTHESIS

It is well known that secondary interactions with underivatized silica provide hindrances for the effective elution of selected analytes especially basic compounds. Silanol groups which have the tendency to exist as $Si\text{-}O^-$ above pH 4 are the potential sites for such secondary interactions. As a result, they contribute to cation-exchange secondary interaction [Buckenmaier et al., 2002]. Therefore, an extra effort has to be

put in to successfully elute the analytes. On the other hand for a sorbent to be effective as a solid phase extractor, it should have high surface area which can provide high rates of mass transfer with a consistent flow rate at low applied pressure. These requirements comply with monolithic stationary phase as they are considered to be a single particle which does not contain inter-particle voids. Therefore, solvent can flow through the stationary phase via the interwoven network structure of the monolith. In light of these observations and attributes of monolith, we hypothesize that the silica silanol groups of monolith can be explored as weak cation exchangers for the selective retention of polar basic analytes. The aim of this study is to explore the applications of silica monolith as offline solid phase extractor. Moreover the sample cleaning efficiency was also evaluated to render the analytes free from the matrix interferences. The overall presents a simple but robust method for the extraction of analytes from urine using high surface area silica monolith acting as a weak cation exchange cartridge.

1.8 RESEARCH OBJECTIVES

The objective of this thesis is to explore the potential of monoliths as a solid phase extraction cartridge. Firstly, the prepared silica monolith will be tested for bio-sample processing owing to their higher extraction capability because of their high surface area. The tolerability of the silica monolith in performing in higher organic solvent content make them more robust and rugged for extended duration of time. Secondly, the application of silica monolith was extended for desalination that could provide a useful tool in generating fresh water from the sea water. As the efficiency of reverse osmosis membrane is the major limitation in current desalination process. Furthermore improvement in the method of preparation of silica monolith in order to generate high surface area is another scope of this study. These kinds of high surface

area materials are desirable in many applications which include separation, extraction and catalysis.

1.8.1 PLAN OF WORK

- Preparation of silica monolith
- Characterization of the prepared monolith
    - Surface morphology
        - Scanning electron microscopy (SEM)
        - Transmission electron microscopy (TEM)
    - Surface area and pore size distribution
        - BET analysis
    - Chemical functionality
        - Fourier transmission infra red technique (FTIR)
- Application of the silica monolith as solid phase extraction cartridge for the extraction of epinephrine, metanephrine and normetanephrine from urine
- Application of the silica monolith as solid phase extraction cartridge for the extraction of ketamine from urine
- Application of the silica monolith as solid phase extraction cartridge for the extraction of morphine, codeine and cocaine from urine
- Application of silica monolith for desalination
- Improvements in the method of preparation in order to enhance the surface area

There was no intention to modify the surface of the prepared monolith to various functionalities. Therefore, surface modification was beyond the scope of this study. However, this opens the further prospects of the silica monolith to be tested for their efficiency with or without modification for solid phase extraction.

CHAPTER 2

Preparation and Characterization of Silica Monolith

2.1 INTRODUCTION: OVERVIEW OF SOL-GEL TECHNIQUE

As discussed earlier in section 1.2, sol gel technology has existed for a long time but the diversity of the process had only been realized in the last decades. It provides a versatile tool for the preparation of monoliths in almost any form: films, fibers, disks, particles, etc. The general schematic of their preparation is depicted in chapter 1 in Figure1-3. Understanding of the general chemical reactions involved in sol-gel process is important for proper design and production of stationary phases, as it allows controlling the whole process from start to end. The sol-gel process is a series of process which include a suitable precursor, generally a metal alkoxide $M(OR)_x$, which undergoes sol gel transition. The process includes catalytic hydrolysis of precursor followed by catalytic polycondensation of the hydrolyzed product, all occurring simultaneously. The process begins with the aggregation of particles into fractal clusters, which subsequently interpenetrate to certain extent and finally link together to form an infinite network. This results into gel formation which after gelation develops a co-continuous (sponge-like) domain structure and remains intact for a substantial period of time. The domains continuously increase in size, leading to coarsening and finally result in fragmented domains and continuous matrix [Siouffi et al., 2006; Tanaka et al., 2001; Vanbeek et al., 1992]. When phase separation and gel formation occur competitively, various transient co-continuous structures can be permanently trapped in the network. The system undergoes phase separation to generate micrometer range heterogeneity composed of gel and fluid phases. The fluid phase can be removed easily to leave pores of few micrometers. In cases of thermally induced phase separation the kinetics can be externally controlled through temperature. The structure formation is more or less spontaneous in nature and the onset of phase separation and sol-gel transition are governed by the chemical bond

formation. Figure 2-1 demonstrates the steps involved in the preparation of monolith based on sol-gel technique. The sol-gel process allows molding of arbitrarily shaped silica monoliths [Tanaka et al., 2001; Minakuchi et al., 1997; Nakanishi et al., 1997; Ishizuka et al., 2000]. The advantages of sol-gel processing are high homogeneity and purity of the resulting products, which is important in the preparation of monolithic chromatographic supports.

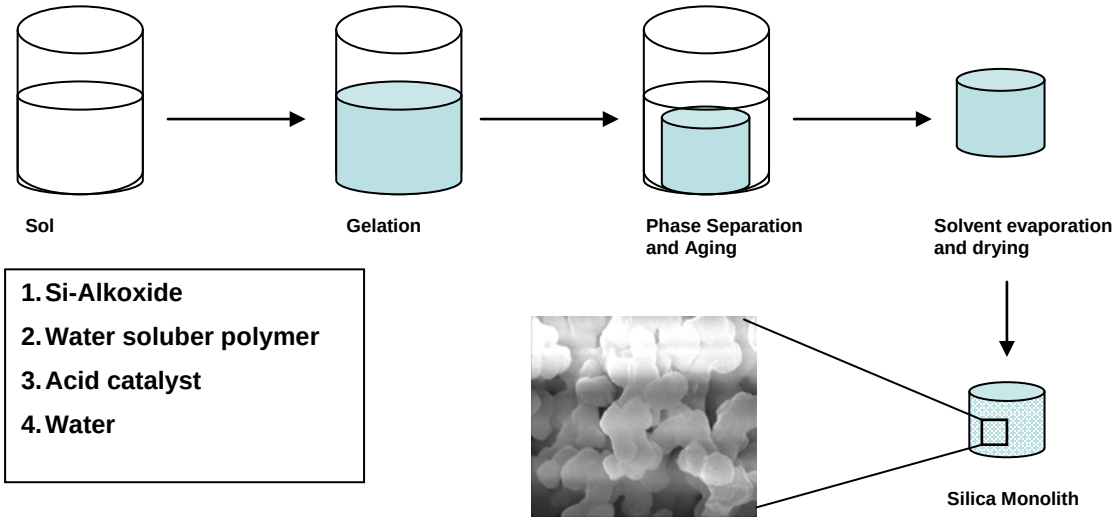


Fig. 2-1. Steps involved in Silica Monolith preparation.

The basic components of the reaction mixture are precursor, solvent and catalyst. The precursors used for sol-gel process are mainly silicon alkoxides which can be obtained in a high degree of purity. Among the many precursors available, tetramethoxysilane (TMOS) has been used frequently because it undergoes a more rapid hydrolysis than others [Nakanishi et al., 1997; Nakanishi et al., 1998; Minakuchi et al., 1997; Minakuchi et al., 1996; Ishizuka et al., 1998]. In a study by Wagh et al. [Wagh et al., 1999] three different precursors, namely TMOS, tetraethoxysilane (TEOS) and polyethoxydisiloxane (PEDS), were compared. The study claimed that TMOS yields narrow and uniform pores and higher surface area then the other two

precursors. Moreover, the factors that influence the kinetics of the process are the type of catalyst, its concentration and temperature. Thus, catalysts also play an important role in structure formation. The catalysts utilized in the reaction are mainly acids [Vanbeek et al., 1992; Agren et al., 2000] and bases [Harris et al., 1990; Friggeri et al., 2001], however, other catalyst like fluorides, acetates, etc have also been used [Tilgner et al., 1995; Boury et al., 2002; Murakami et al., 1999; He et al., 2001]. In acid catalyzed reaction, the rate of hydrolysis is significantly faster than condensation thereby leading to the formation of linear polymers. On the other hand, in base catalyzed reaction the condensation rate is faster than hydrolysis which results in the formation of highly branched structure and particle formation [Tilgner et al., 1995]. In addition to this difference, acid catalyzed materials are highly porous and possess high surface area as compared to low porosity and reduced surface area in case of base catalyzed materials [Siouffi, 2003]**.** All these features enable researchers to manipulate experimental conditions to facilitate the formation of monolith with desired characteristics**.**

2.2 OBJECTIVE

Based on the above discussion of the preparation steps involved in monolith synthesis, the objective of the study described in this chapter is to ensure that the prepared monolith meets the required standards. The SPE cartridge investigated in this study is a silica based monolith created with a sol-gel method reported by Motakawa et al. [Motokawa et al., 2002] The purpose of this chapter is thus to describe the preparation of the silica based monolith and the characteristic features of the prepared monolith which could be effectively used to demonstrate their capability as solid phase extraction cartridges.

2.3 MATERIALS AND METHODS

Tetramethoxysilane and polyethylene glycol (PEG) were purchased from Sigma Aldrich (Milwaukee, WI). Acetic acid was procured from Merck (Darmstadt, Germany). Milli-Q water (Millipore Bedford, MA, USA) was used throughout the experiment. All other chemicals used were of analytical grade.

2.4 EXPERIMENTAL

2.4.1 Preparation of Silica Monolith

Monolithic cartridge was prepared by sol-gel technology [Motokawa et al., 2002]. Briefly, 2 mL of tetramethoxysilane was mixed with a solution of 0.44 g polyethylene glycol (Mw 10,000) and 0.45 g urea in 5 mL 0.01 M acetic acid. The mixture was stirred for 45 min at 0^0C. The solution was kept at 40^0C where it gelled within 2 h and subsequently aged for 18 h at the same temperature. The aged gel was treated at 120^0 C for 3 h which led to formation of mesopores due to the liberation of ammonia generated by the hydrolysis of urea [Tanaka et al., 2001]. After cooling it down to room temperature (25^0C), the cartridge was washed with 50:50 water:methanol for 12 h. Finally the washed cartridge was calcined at 550^0C for 8 h inorder to remove any organic mater.

2.4.2 Characterization of silica monolith

The prepared cartridge was characterized with regards to its physical and chemical attributes. The microstructural properties were analyzed based on nitrogen adsorption-desorption isotherm using gas adsorption analyzer (ASAP 2420 Micromeritics, Norcross, GA). Surface areas were calculated in accordance to Brunauer, Emmette and Teller (BET) theory and the pore size distributions were calculated using Barrett, Joyner and Hellenda (BJH) model applied to desorption branch of the isotherm.

Surface morphological and chemical composition analysis was monitored using field emission scanning electron microscope (FESEM, JEOL JSM-6701F, Japan) equipped with energy dispersive X-ray detector (EDX). Transmission electron microcopy (TEM, JEOL JEM 3010, Japan) images were obtained to reveal the magnified morphological features of the prepared silica monolith. In order to get the images the prepared monolith was fixed in the stub which was placed in a sample holder of SEM. The sample was directly loaded without any prior coating because of the self-conducting nature of silica monolith. On the other hand for TEM images, the monolith was grounded to powder. A small portion was put over the grid and the excess was removed by tapping. Finally the grid was loaded onto TEM to capture the images.

The prepared silica monolith was also analysed using X-ray diffractometer (D5005 Bruker, Germany). X-ray diffraction (XRD) patterns were recorded using Cu Kα radiation under constant instrumental parameters. The sample was scanned between 1.4 and 80^0 at the rate of 0.02^0/2 second at 25^0 C. The silica monolith was grounded and the powder was placed in the sample holder for scanning.

Infrared (IR) analysis of the monolith was recorded on a Fourier transform infra red (FTIR) spectrometer (Spectrum 100 FTIR, Perkin Elmer, USA) in order to monitor the surface functionality over the monolithic structure. Sample preparation involved mixing 1% sample with potassium bromide (KBr) and compressed into a disc shape. This disc was analyzed in a transmission mode in the range 450-4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.5 RESULTS AND DISCUSSION

Bimodal pore structure is the characteristic feature of the monoliths which is characterized by the presence of mesopores and macropores. Mesopores are

responsible for the desired surface area for the interaction with the analytes whereas macropores provides the required permeability for the solvents with low back pressure. The high surface area mesopores facilitate the mass transfer between the phases efficiently within a short duration. The nitrogen adsorption/desorption isotherms of the synthesized silica cartridge is shown in Figure 2-2A. The isotherm exhibited type IV curve which had a characteristic hysteresis loop of H_2 type with a sharp step in the P/P_0 range from 0.6-0.9 which is particular for mesoporous sorbents. This agreed well with the previously reported characteristics of mesoporous materials [Sing et al., 1985; Jaroniec et al., 1998]. Figure 2.2B shows the derived mesopore size distribution based on Barrett–Joyner–Halenda (BJH) method which exhibited a mean pore diameter of 8.4 nm. The surface area and pore volume was found to be 505.88 m^2/g and 0.95 cm^3/g, respectively, using Brunauer–Emmett–Teller (BET) method.

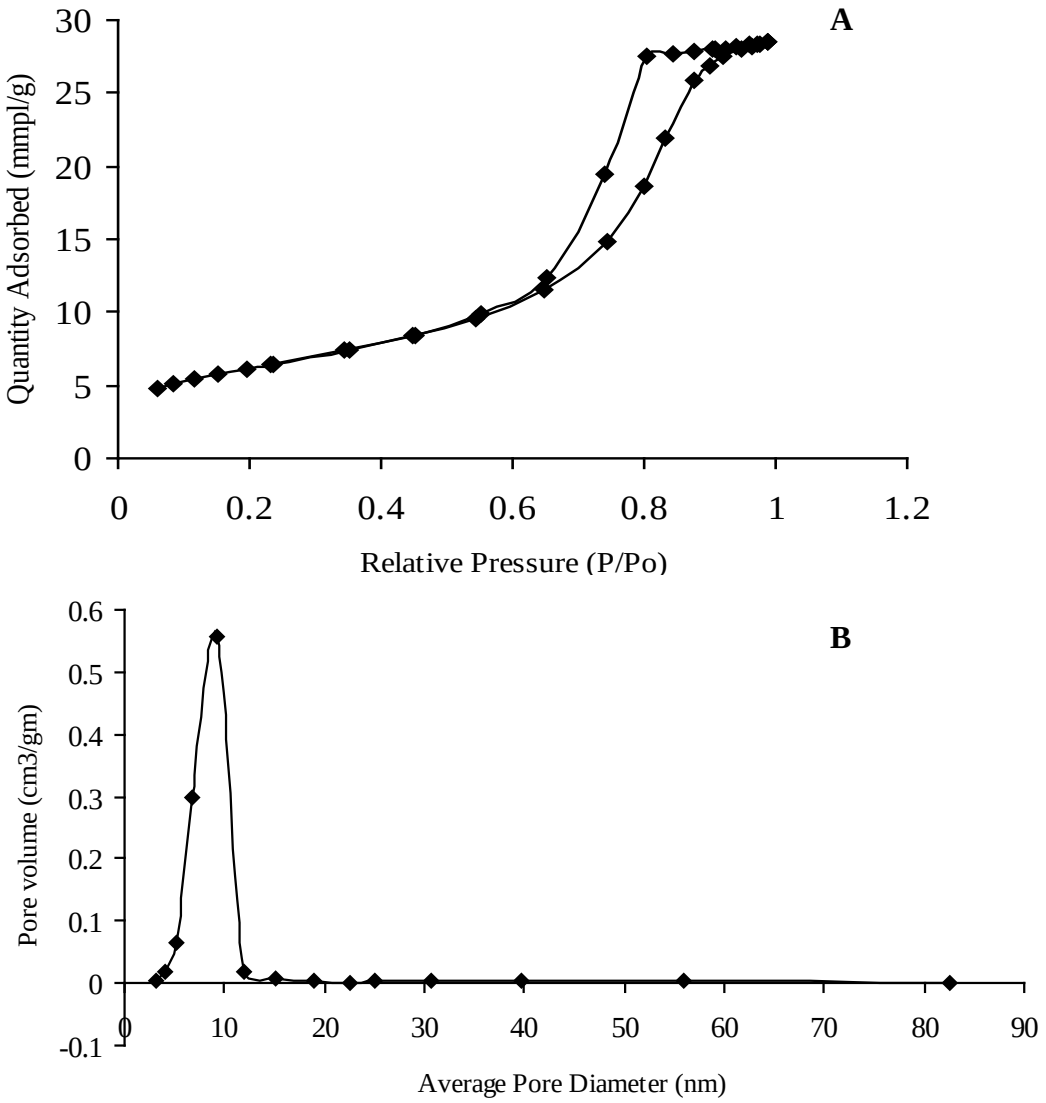


Fig. 2-2. Nitrogen adsorption-desorption isotherm (A) and pore size distribution (B) of the prepared silica monolith.

Figure 2-3 shows the XRD pattern of the in house prepared silicon monolith. A broad peak hump in the range of 15-30 in 2θ confirmed the amorphous nature of the monolith. As shown in figure, the broadness of full width at half-maximum (FWHM) denote that the amorphous material had a disordered structure.

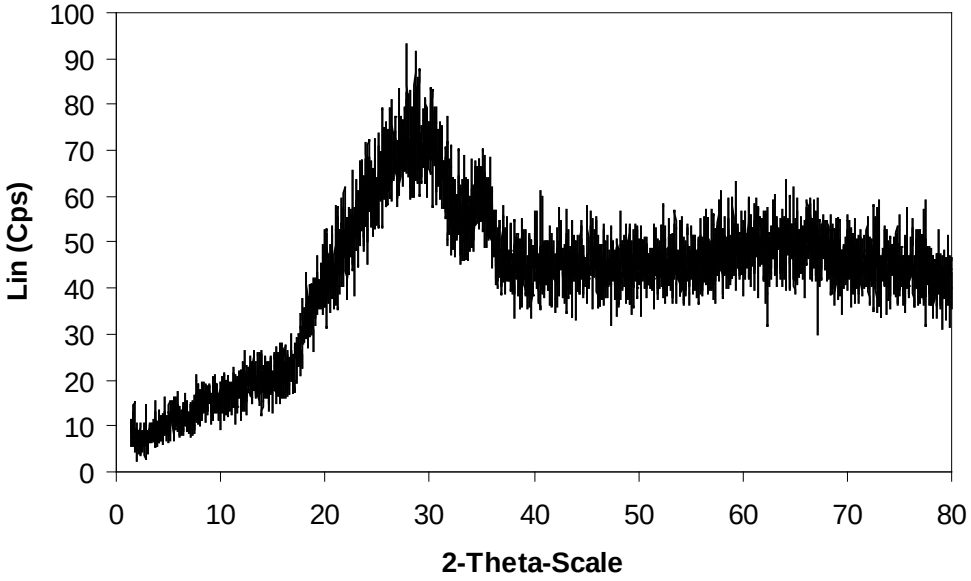
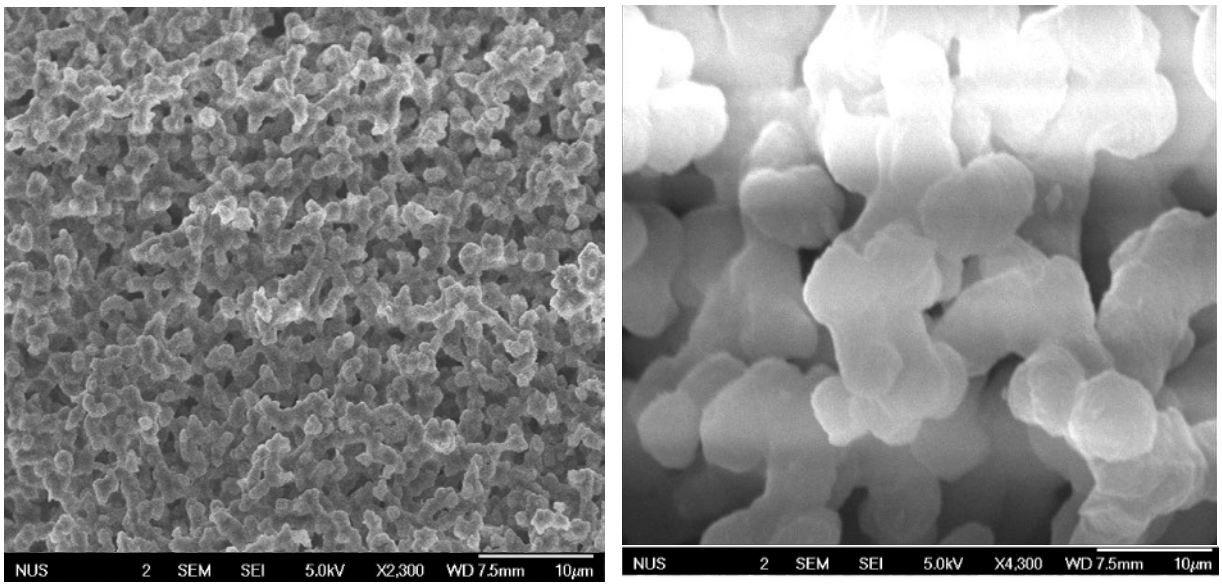


Fig. 2-3. XRD pattern of the prepared silica monolith.

The macroporous structure present in the prepared silica monolith was pictorially demonstrated using SEM photograph (Figure 2.4). The Figure exhibited well defined macropore. Moreover, SEM equipped with EDX detector provides an added advantage of demonstrating the elemental purity of the silica monolith. The spectra clearly indicate the presence of only two elements which are silicon and oxygen and absence of other elements in the structure.



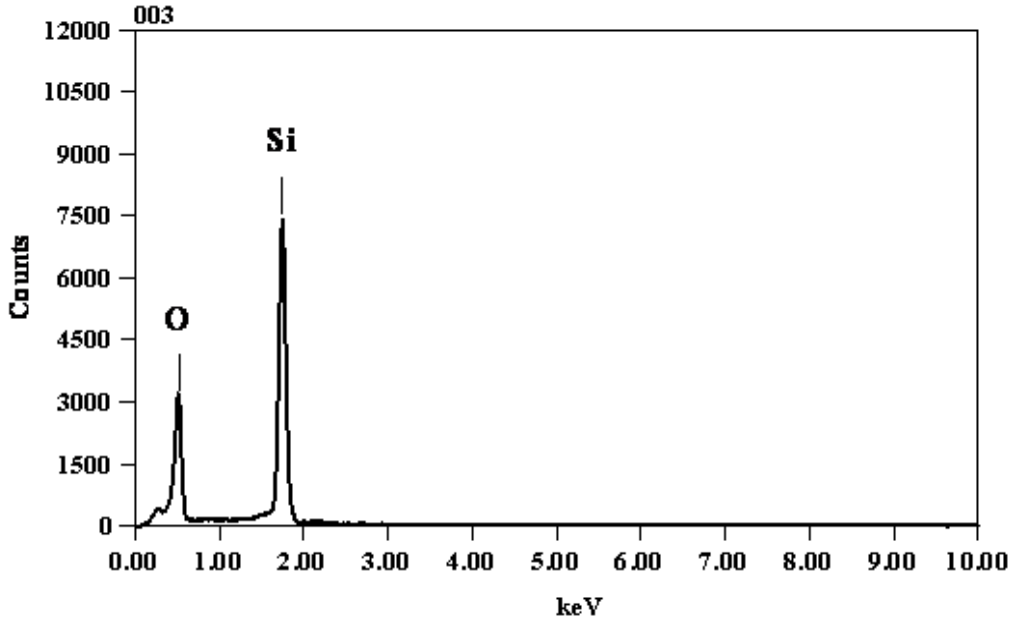


Fig. 2-4. SEM images and EDX spectra of the prepared silica monolith

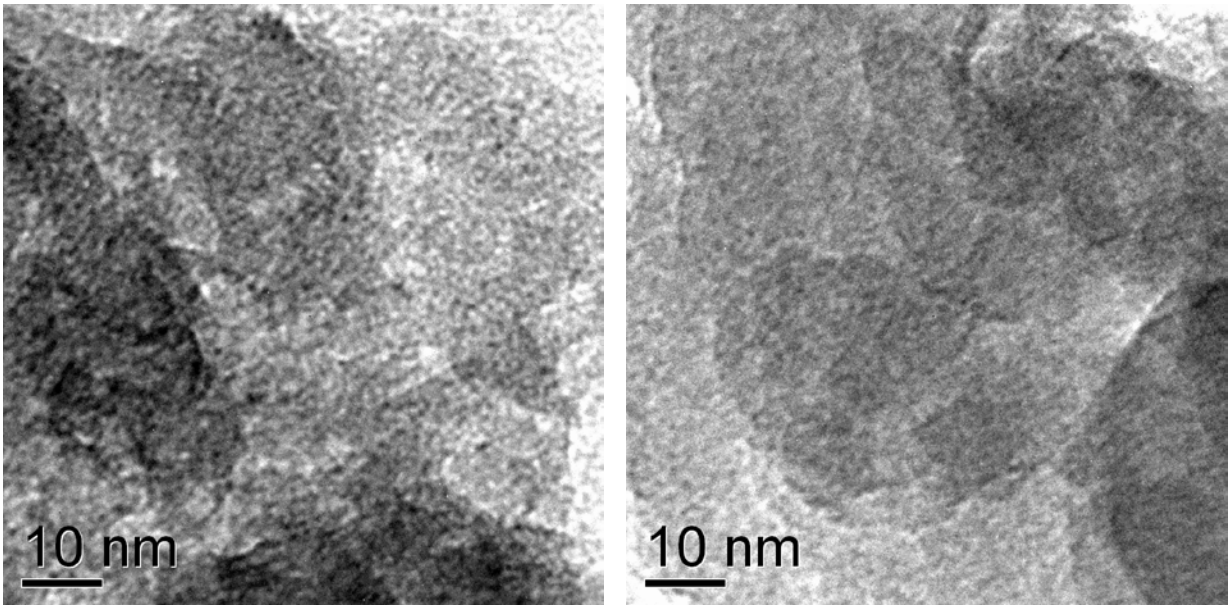


Fig. 2-5. TEM photographs of the prepared silica monolith.

Figure 2.5 represents the TEM images of the synthesized monolithic silica. The Figure demonstrated the presence of wormlike channels arranged in random fashion and distributed homogenously throughout the bulk phase. The material possess worm hole porous framework with poorly defined crystallographic symmetry. This is consistent with the XRD results and confirms the disordered structure present in mesoporous structure. Thus, the confirmed porous framework is expected to be suitable for HPLC application.

IR analysis (Figure 2-6) confirmed the distribution of hydroxyl groups over the cartridge. The presence of band in the range between 3800-3000 cm^{-1} was attributed to Si-OH stretching bands. Strong band in the region of 1100-1250 cm^{-1} corresponded to Si-O-Si stretching bands [Ma et al., 2008] and the peak at 1650 cm^{-1} was characteristic of SiO_2. These results suffice the applicability of the prepared cartridge for SPE.

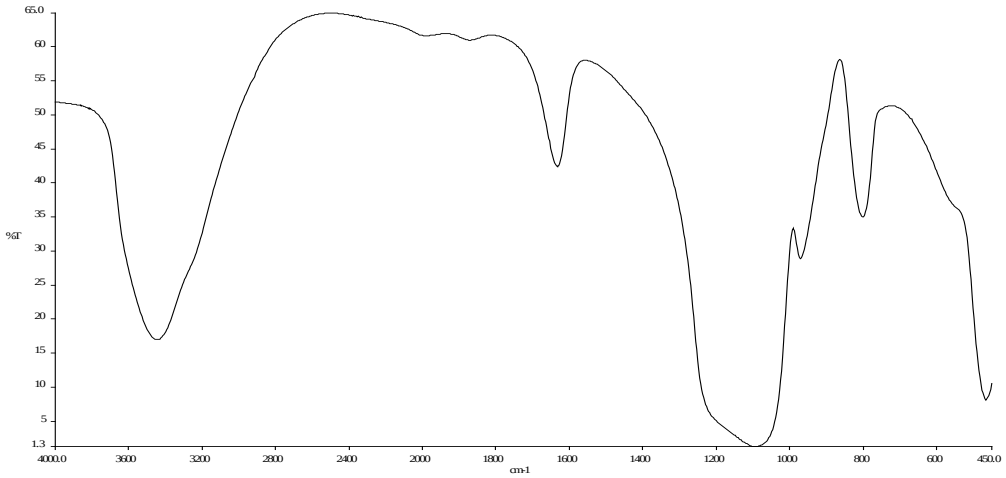
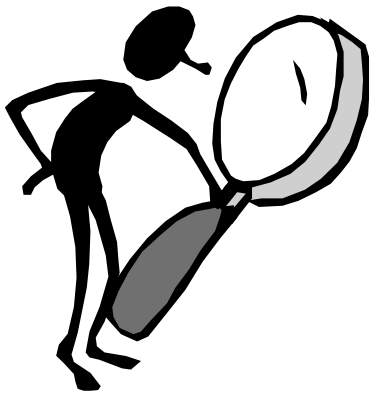


Fig..2-6. FTIR spectra of the prepared silica monolith.

2.6 CONCLUSION

SEM and TEM images of silica monolith demonstrated the presence of well defined macropores and mesopores which remarked their characteristic feature as a suitable stationary phase. The presence of these structures imparts high permeability which allows the use of high flow rates at low back pressure. The absence of interparticular voids allows all the solvent to flow through the sorbent for effective interaction of analytes with the sorbent material. This results in a positive effect on the mass transfer. The reactivity of the prepared silica was known to be due the presence of silanol groups which exist in $Si\text{-}0^-$ at pH above 4. The characterization results showed that the prepared silica monolith had the characteristics that made it suitable for various chromatographic applications. The prepared cartridge was subsequently tested for its suitability for solid phase extraction and the findings were reported in the subsequent chapters.

CHAPTER 3



Application of Silica Monolith for Catecholamine Analysis

3.1 INTRODUCTION

A catecholamines analysis measures the amount of the hormones like epinephrine, norepinephrine, metanephrine, and dopamine in the urine. These catecholamines are synthesized by nerve tissues, brain, and adrenal glands. They help the body to control stress or fright and prepare the body for various reactions. The main catecholamines are epinephrine, norepinephrine, and dopamine. They metabolize into vanillylmandelic acid (VMA), normetanephrine and metanephrine, which get excreted in urine. An excess amount of catecholamines can increase heart rate, blood pressure, breathing rate, muscle strength, and mental alertness. Certain rare tumors (such as a pheochromocytoma) can increase the amount of catecholamines in blood and urine. Thus by measuring these endogenous compounds the person can be diagnosed for pheochromocytoma and appropriate measures can be taken to combat this rare tumor.

3.2 OBJECTIVE

The aim of the study in this chapter was to explore the application of silica monolith as an offline solid phase extractor of polar basic analytes (epinephrine, normetanephrine and metanephrine). Moreover the sample cleaning efficiency was also evaluated, using osmolality study, to render the analytes free from the matrix interferences. This study presented a simple but robust method for the extraction of polar analytes from urine using high surface area silica monolith acting as a weak cation exchange cartridge.

3.3 MATERIALS AND METHODS

Epinephrine, normetanephrine and metanephrine were purchased from Sigma (St. Louis, MO, USA). Formic acid was from Fluka (Buchs, Switzerland). Acetonitrile (ACN) was of HPLC-grade and all other chemicals used were of

analytical grade. Milli-Q water (Millipore Bedford, MA, USA) was used throughout the experiment. Standard stock solutions of normetanephrine, metanephrine and epinephrine were prepared in 0.1 N HCl to give a final concentration of 1 mg/mL. The working standard solutions were prepared by appropriate dilution of stock. The prepared samples were stored in the dark at -20^{0}C.

3.4 EXPERIMENTAL

3.4.1. Solid Phase Extraction

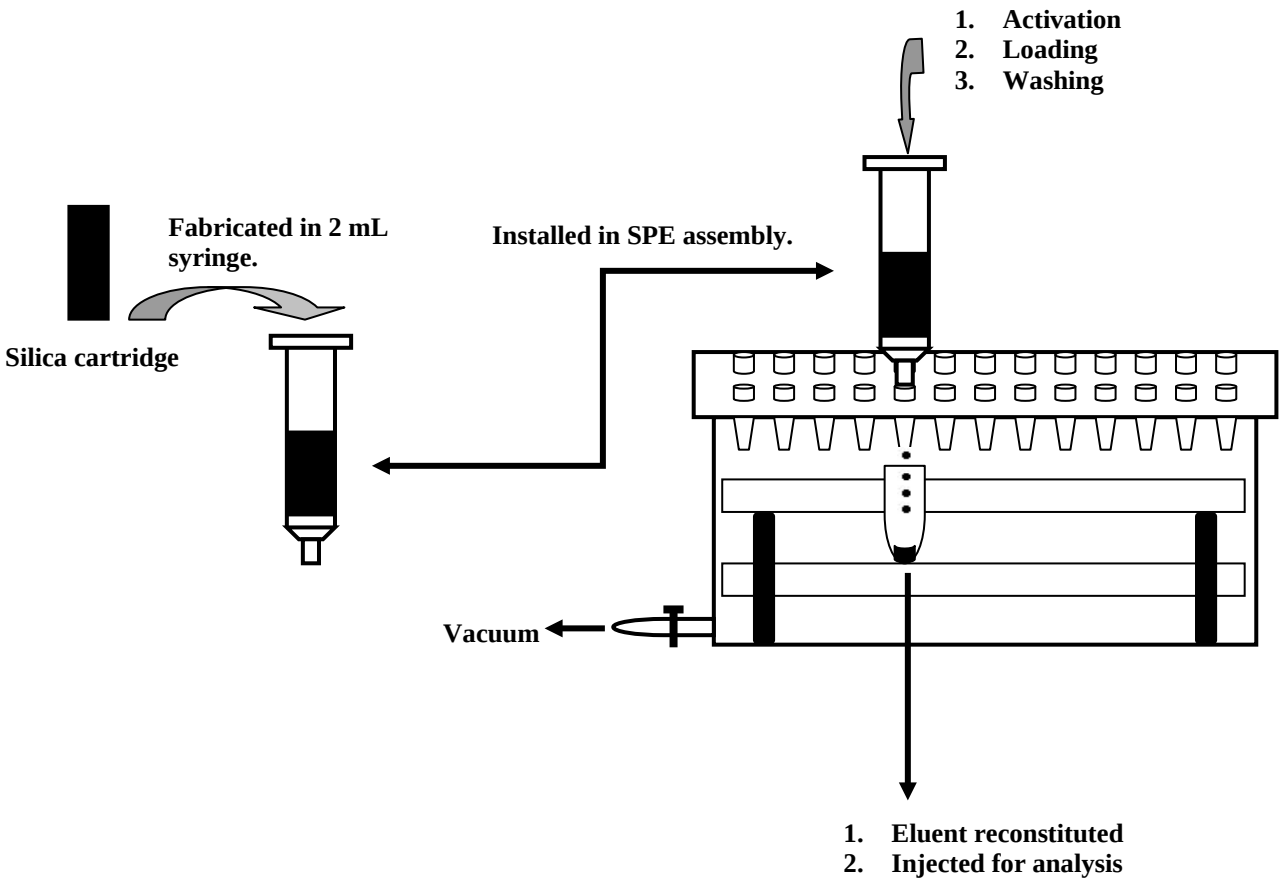


Fig. 3-1. Extraction steps using the prepared underivatized silica cartridge on SPE manifold.

The extraction (loading, washing and elution) was carried out after installing the prepared cartridge in a 2-mL syringe. The extraction was initiated with the activation of cartridge using 1.5 mL methanol followed by 1.5 mL of milli-Q water. A

1-mL aliquot of sample matrix (water or urine) was directly transferred over the preactivated cartridge and allowed to flow through under applied vacuum (40-45 kPa). No prior treatment of sample matrix was done before loading on to the cartridge. The cartridge was washed with 2 mL of water and drained completely before adding the elution solvent. Finally, the adsorbed analytes were eluted with 5 mL of 0.1% formic acid. The eluted analytes were collected and lyophilized. The residue was reconstituted with 500 µL of 0.1% formic acid. 5 µL of the reconstituted sample was injected into HPLC coupled to a triple quadrupole/linear ion trap mass spectrometer. Figure 3-1 depicts the schematic of steps involved in SPE.

For consecutive use the cartridge was washed with 1 mL of 0.1% formic acid and finally, with 5 mL of water before loading the subsequent sample. In order to test the efficiency and carryover effect of the cartridge, 50 ng/mL of each of the three analytes was spiked in a urine sample that was subsequently divided into five portions. Each urine portion was extracted consecutively on the same cartridge. Extraction of each spiked urine sample was followed by extraction of an unspiked urine sample. After each extraction, the cartridge was washed again as described above.

The sample cleaning efficiency was evaluated based on the osmolalities of the samples measured using the Vapro Osmometer 5520 (Wescor, Inc, USA) to verify that the analytes were free from interfering ions. For this, three spot urine samples collected in the morning, afternoon and at night were cleaned using the silica cartridge. The osmolalities of the urine samples before SPE and their corresponding extracts were recorded to measure the cleaning efficiency. The results were analyzed statistically by two-way ANOVA to verify the level of significance in the difference between the osmolalities of extracts before and after SPE.

3.4.2. Chromatographic Conditions

The Agilent 1200 series HPLC system comprised of a binary pump (G1312A), an online degasser (G1379B), an autosampler (G1329A) and a system controller. The data were analyzed using Analyst software. The separation was performed in the isocratic condition using a C_{18} column (150 mm X 2 mm ID X 5 µm particles) (Phenomenex, Torrance, USA) protected by a guard column (C_{18} ODS, 3.0 mm ID X 4 mm) (Phenomenex). The mobile phase consisted of 10% ACN in 0.012% formic acid and flow rate was set at 0.25 mL/min. The system was operated at ambient temperature. The column outlet was directly connected to the ESI probe with PEEK tubing.

3.4.3. Mass Spectrometry Conditions

LC was coupled to 3200 QTRAP mass spectrometer (Applied Biosystem, USA). The mass spectrometer and LC parameter were controlled using Analyst software 1.4.2 (Applied Biosystem, USA). The analytes were quantitated using multiple reaction monitoring (MRM) to study the transition of the parent to the product ions (Table 3-1). The MS was operated in positive ion spray mode with the parameters settings as follow: curtain gas = 10; collision gas (CAD) = medium; ion spray voltage = 5000 V; source temperature (TEM) = 300^0C; ion source gas 1 (GS1) = 45 psi; ion source gas (GS2) = 35 psi; and interface heater = on. Quadrupole 1 (Q1) and quadrupole 3 (Q3) were maintained at unit resolution and dwell time was set at 200 ms.

Table 3-1. Optimized parameters for LC/MS quantitation.

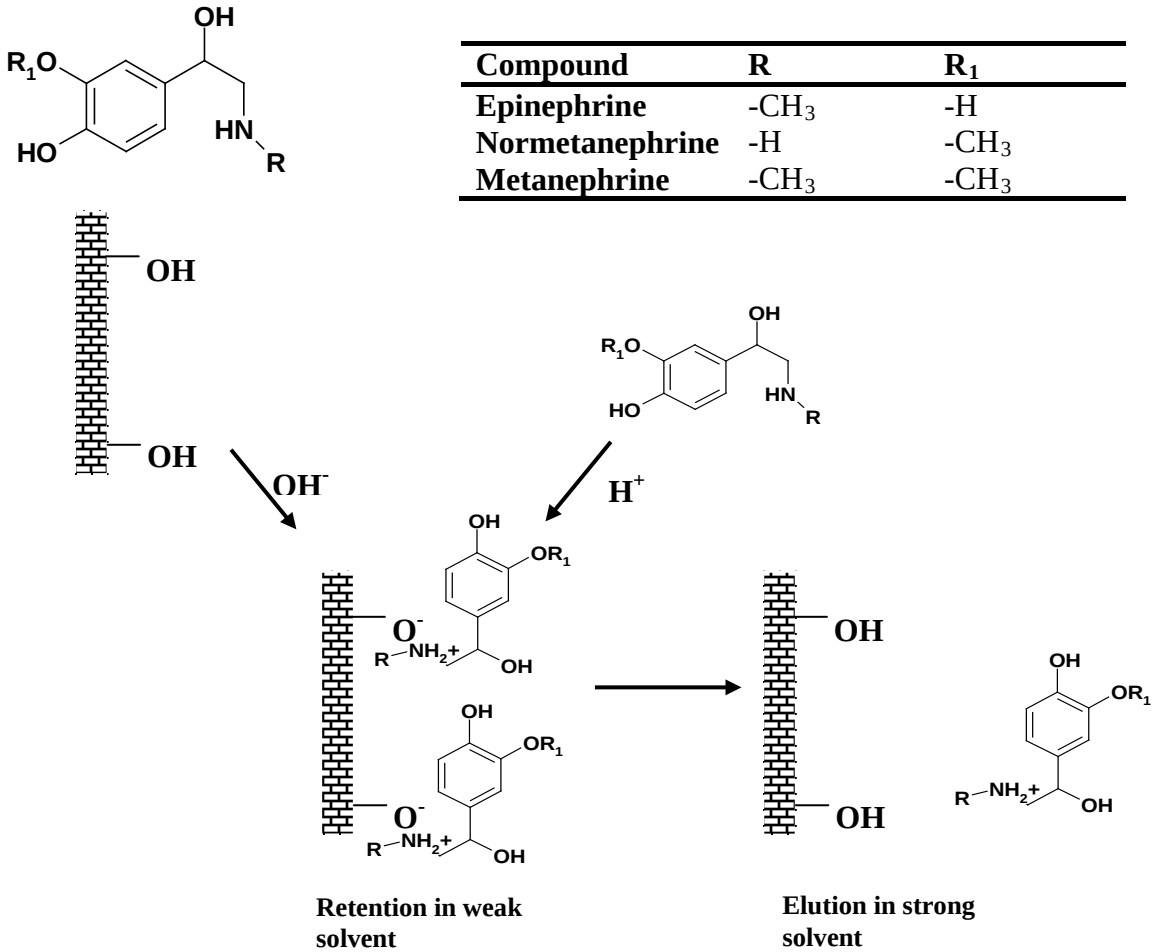
Analytes	Transitions	DP (V)	EP (V)	CE (V)	CXP (V)
Epinephrine	184→107	15	4	25	3
Normetanephrine	184→134	15	4	25	3
Metanephrine	198→148	15	4	25	3

DP - declustering potential; EP - entrance potential; CE collision energy; CXP – collision exit potential.

3.5 RESULTS AND DISCUSSION

3.5.1 Cartridge SPE conditions

Fundamental prerequisite for the extraction of polar compounds from the biological matrices demands highly polar stationary phase with high surface area. This will provide higher affinity for the analytes in competition to sample matrix. With this principle in mind, the performance of the prepared cartridge was evaluated based on its extraction efficiency of the model polar analytes. The underivatized silica cartridge contains siloxanes and silanols as the major functional groups. Silanols are responsible for the ion exchange whereas siloxanes provide the weak reversed phase binding characteristic [Naidong, 2003].



Compound	R	R_1
Epinephrine	$-CH_3$	-H
Normetanephrine	-H	$-CH_3$
Metanephrine	$-CH_3$	$-CH_3$

Fig. 3-2. Mechanism of adsorption and desorption of analytes in SPE.

The basic mechanism underlying the adsorption of analytes is based on polar ionic interactions. The schematic of the process is demonstrated in Figure 3-2. The underivatized silica materials contains hydroxyl group that is bonded to silica backbone. The silanol group (Si-OH) on the surface of cartridge is acidic and exist as $Si\text{-}O^-$ at pH above 4 and will retain cations if the selected pH maintains both entities in charged state. Thus, the cartridge acts as weak cation exchanger. Electrostatic interaction between the positively charged model analytes (quaternary amines) and negatively charged silica cartridge possessing number of hydroxyl group and high surface is the probable mode of adsorption. A routine SPE condition was applied based on previous literatures [Chan and Ho, 2000; Chan and Siu, 1988]. The retention based on ion exchange mechanism depends heavily on pH of the environment. The pH should be selected at which the analyte and the sorbent functional groups remain in charged state. Therefore, based on the nature of the sorbent and the analytes, pH was adjusted to 6.5 before loading the sample on to the cartridge. The cartridge was washed with twice the volume of the loaded matrix to eliminate unwanted impurities. Biological samples are known to possess polar ionic endogenous compounds which can be retained strongly on underivatized cartridge because of their affinity towards silanols. In order to remove these endogenous compounds, the polarity of the washing solvent has to be such that these interfering compounds find higher affinity towards them for their successful removal. Since the presence of organic solvent could have altered the polarity of water, the cartridge was washed with milli-Q water to eliminate the accumulation of the endogenous compounds. The analytes were then eluted out of the cartridge by regeneration of the free silanol groups (Si-OH) on the cartridge surface with mild acidified aqueous solution. In this study, the elution solvent was optimized by varying the concentration of formic acid in the elution solvent. It was

found that higher the percentage of acid used will increase the efficiency of elution. However, it will also require higher volume of washing solvent required to neutralize the cartridge for the subsequent uses. Therefore, 0.1% formic acid was selected for the elution of analytes from the sorbent after washing. The pH (found to be 3 with 0.1% formic acid) provided good recoveries without any deleterious effect on the sorbent. After rinsing the sorbent, the cartridge was reused again for SPE. The repeatability and the efficiency of adsorption are discussed in later section.

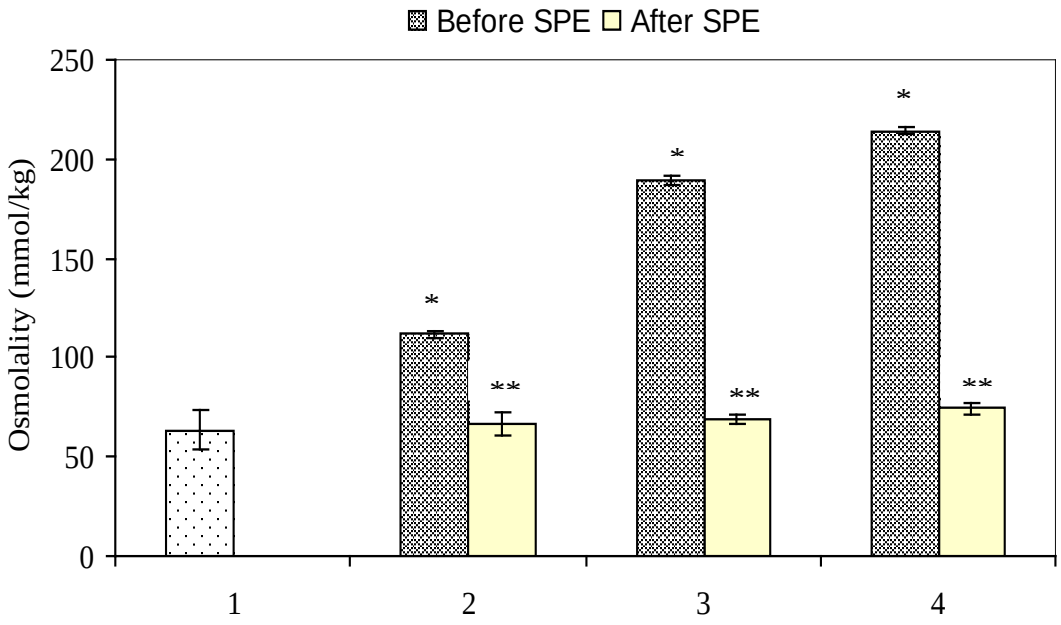
3.5.2 Adsorption

The adsorption capacity of the cartridge was evaluated based on the concentration of analytes before and after loading the cartridge. Water was mimicked as the sample matrix and was spiked with the three analytes simultaneously, in increasing concentration. The volume of 1 mL was always kept constant. The concentration of 100, 200 and 500 ng/mL of the three analytes was prepared in water and the mixed analytes were allowed to pass through the cartridge. As the matrix was water, the eluted solution was directly injected into HPLC/ESI/MS system to estimate the adsorption capacity. On analyzing the eluents, it was found that the three analytes were absent at all three concentrations. The result confirmed the fast and high adsorption capability of the cartridge. Further increase in concentration could be explored but the high sensitivity of the instrument limited the concentration to 500 ng/mL; furthermore, concentrations of biogenic amines higher than 500 ng/mL might not reflect the endogenous concentrations that could be encountered in clinical practice.

3.5.3 Osmolality

Molality is the number of particles dissolved in a mass weight of fluid (mmol/kg). Osmolality is a measure of the total number of osmotically active particles

in a solution and is equal to the sum of the molalities of all the solutes present in that solution. The osmolality of physiological fluids tends to be dominated by small molecules which are present in high concentrations. For instance in urine, sodium, potassium, chloride, bicarbonate and urea are the components present at high concentration which individually affects osmolality. The urine osmolality is the best measure of urine concentration. In this study, osmolality was measured using an osmometer. It works on the principle of measuring the vapor pressure of the solution (altered in proportion to the number of solute particles) with respect to pure solvent.



* Significant difference from the extracts ($p<0.005$)
** No significant difference from the pure extracting solvent ($p>0.1$)

Fig. 3-3. Comparison of the osmolality of the urine samples (n=3) before and after SPE: (1) Osmolality of the pure extracting solvent; (2), (3) and (4) are the osmolalities of the blank urine samples collected at different time points (morning, afternoon and evening) before and their extracts after SPE.

The purpose of SPE is to prepare samples free from the interfering endogenous molecules and ions. The presence of endogenous small molecules and ions in the sample can compete with the analyte ions in the detector, leading to ion suppression. Measurement of the osmolality of urine samples collected at different

times before and their respective extracts after SPE can be applied to determine the sample cleaning efficiency of the cartridge. Despite the various initial osmolality levels of the urine samples, it was found that the osmolality of the extracts after sample clean-up decreased significantly and was comparable to that of the original eluting solvent ($p < 0.001$, but no significant differences among the extracts from the respective urine samples, as tested by two way ANOVA) (Figure 3-3). The finding indicated the high efficiency of the prepared cartridges in cleaning up the endogenous ions in urine samples.

3.5.4. Method validation

Extracts were recovered via SPE method using an in-house weak cation exchange silica cartridge. The reconstituted samples after lyophilization were directly injected into HPLC/ESI/MS for analysis. Calibration curve in the linearity range of 20-200 ng/mL was constructed from triplicate measurements of each concentration level of the calibration samples in spiked water, following all steps of SPE. Figure 3-4 shows the typical chromatogram of a standard solution of epinephrine, normetanephrine and metanephrine. The linearity of the calibration curve was checked using least squared regression analysis taking peak area versus concentration (x) as the regression parameters with 1/x weighting factor. The correlation coefficient was found to be more than 0.99 for all analytes as shown in Table 3-2. The intra- and inter-day precision was expressed as the % relative standard deviation (%RSD) of the triplicate measurement at low (50 ng/mL), medium (100 ng/mL) and high (500 ng/mL) concentrations for each analyte. The %RSDs for intra- and inter-day variation at all concentrations were thereafter found less than 10% for the respective analytes (Table 3-3). The method sensitivity was established by examining the limits of detection (LOD) and limits of quantitation (LOQ). LOD was defined as the lowest

detectable concentration with a signal to noise ratio of at least 3 and the LOQ was defined as the lowest quantifiable concentration with a signal to noise ratio of at least 10. The LODs and LOQs for the analytes under these conditions are presented in Table 3-2.

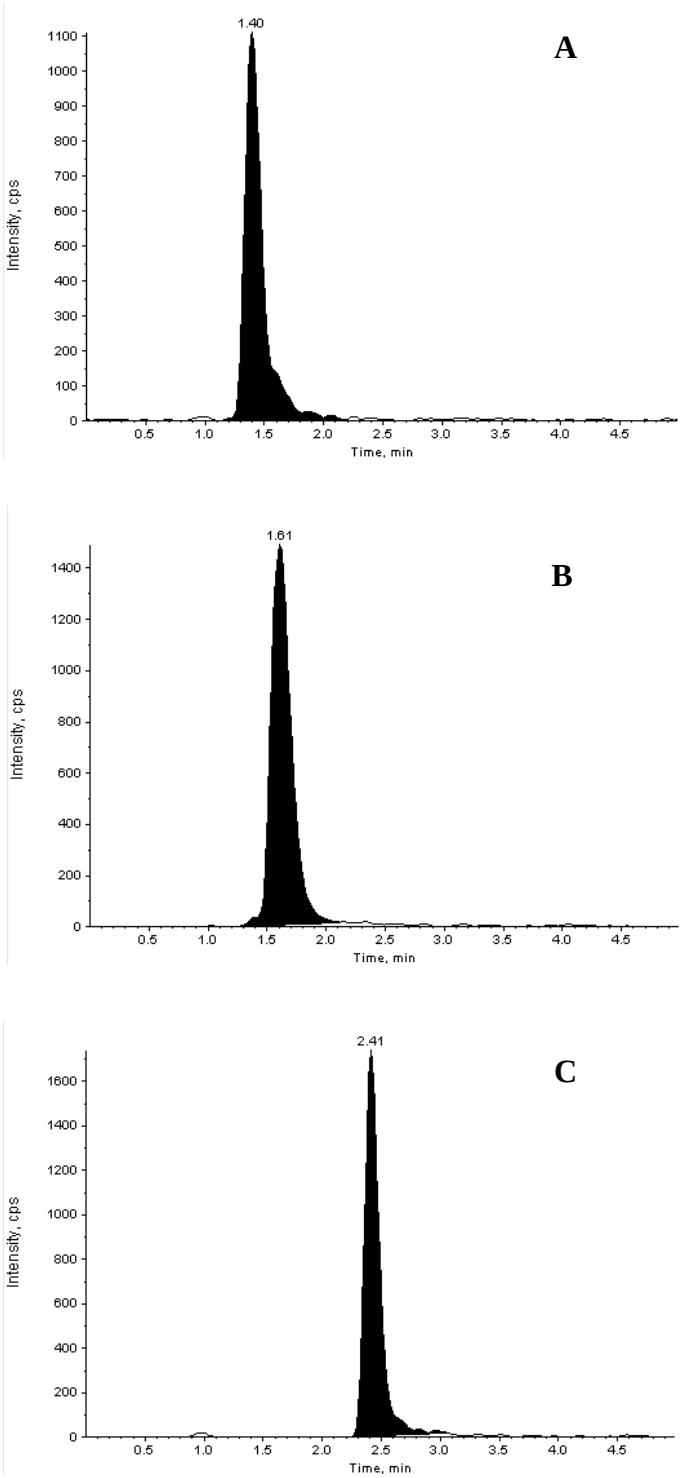


Fig. 3-4. LC MS/MS chromatograms of Epinephrine (A), Normetanephrine (B) and Metanephrine (C) at a concentration of 50 ng/mL.

Table 3-2. Linearity range, LOD and correlation coefficient of analytes in aqueous solution.

Analytes	LOD (ng/mL)	Linearity range (ng/mL)	Linearity	r^2
Epinephrine	5	20-200	y = 161.59x - 2413.4	0.9952
Normetanephrine	5	20-200	y = 266.09x - 536.33	0.9999
Metanephrien	3	20-200	y = 145.19x + 435.04	0.9920

Table 3-3. Interday and intraday variation of analytes in aqueous solution.

Analytes	Precisions (%RSD)					
	Intraday (n=3)			Interday (n=3)		
	Low	Medium	High	Low	Medium	High
Epinephrine	3.4	2.8	3.8	4.1	9.7	4.6
Normetanephrine	5.0	5.9	2.3	5.8	7.5	3.8
Metanephrine	4.7	4.0	5.5	3.4	5.2	7.2

3.5.5 Analysis of spiked urine

Epinephrine, normetanephrine and metanephrine are present in trace amounts in healthy urine samples.[Chan and Siu, 1988] Urine samples were spiked with analytes to the desired concentrations (20, 50, 100, 200 ng/mL) before they were subjected to SPE extraction. The SPE steps were identical to that applied for the water spiked samples. The relative standard deviations for intra and interday variation at all concentrations with spiked urine were within 10% (Table 3-4). The result of percentage relative recoveries (ratio of the peak areas of the spiked urine extracts to spiked water extracts) for urine samples is shown in Table 3-5. The results indicated that recoveries of normetanephrine and epinephrine were lower than that of metanephrine. Although 100% analytes were adsorbed onto cartridge but the interaction between epinephrine and normetanephrine with sorbent as compared to metanephrine was not sufficiently strong. This observation was possibly related to the differences in the competency of the three analytes with the sorbent in presence of many polar endogenous ions present in urine samples. The decreasing order of hydrophilicity of the three analytes is as follow: epinephrine> normetanephrine>

metanephrine (the order is predicted based on the elution of the analytes from the column). Since the polarity of epinephrine and normetanephrine was on the higher side as compared to metanephrine therefore, their competiveness to the binding sites on the sorbent with other ions in urine was higher. Thus, this reduces the chance of the two analytes (epinephrine and normetanephrine) to interact with the sorbent and accounts for their lower recovery.

Table 3-4. Interday and intraday variation of analytes in urine.

Analytes	**Precisions (%RSD)**					
	Intraday (n=3)			**Interday (n=3)**		
	Low	**Medium**	**High**	**Low**	**Medium**	**High**
Epinephrine	7.4	5.6	9.8	7.8	6.3	8.9
Normetanephrine	7.2	6.4	3.6	5.4	7.2	5.3
Metanephrine	4.6	6.4	5.4	4.3	5.2	6.8

Table 3-5. Relative recovery level of analytes in urine after SPE (n=3).

Spiked Concentration (ng/mL)	**%Relative recoveries (%RSD)**		
	Epinephrine	**Normetanephrine**	**Metanephrine**
20	60 (8.8)	55 (6.8)	101(6.7)
50	62 (7.5)	56 (5.5)	105 (6.2)
100	65 (6.9)	57 (4.9)	99 (5.7)
200	67 (6.5)	59 (3.1)	103 (4.9)

3.5.6 Extraction efficiency and cartridge carry over

The extraction efficiency of the cartridge for repeated use was evaluated based on the recoveries after multiple extractions on the same cartridge. A defined concentration (50 ng/mL) of the analytes was spiked in a urine sample and was extracted on the same cartridge. Extraction of each spiked urine sample was followed by one extraction of an unspiked urine sample to monitor the carryover effect. After each extraction, the cartridge was washed extensively and the last portion of the washed eluent from the cartridge was also analyzed to monitor the carryover. 5 mL of water was selected for washing based on its ability to neutralize the cartridge pH

before proceeding to the next step of sample extraction. The uniform pH condition was utilized for each extraction to ensure reproducible recoveries. Although extraction of 5 replicate samples was carried out to demonstrate the robustness of the prepared cartridge, it had been reused for many more times with minimal compromise on its reproducibility. Table 3-6 represents the relative recoveries of simultaneous extraction of three analytes with multiple extractions on the same cartridge.

Table 3-6. Relative recoveries of the analytes on a single cartridge after multiple extractions.

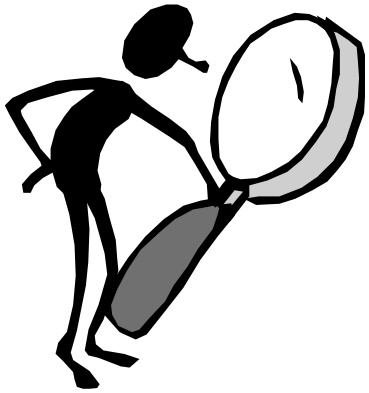
No. of extractions	**50 ng/mL concentration**		
	Epinephrine	**Normetanephrine**	**Metanephrine**
1st	62	55	101
2nd	64	57	99
3rd	61	52	104
4th	67	59	102
5th	65	54	106
% RSD	**3.7**	**4.8**	**2.6**

3.6 CONCLUSION

Our attempt to utilize the principle of underivatized silica monolith as SPE sorbent for extraction of polar analytes from urine was proven to be effective. Although the recoveries of epinephrine and normetanephrine from the silica monolithic cartridges were approximately 60%, the optimal recovery of metanephrine (100%) proved to be promising. In the urine samples, there are endogenous ions competing with the polar compounds for the binding sites in the cartridge; therefore, the recovery appeared to be lower for the polar compounds (e.g, epinephrine and normetanephrine) than for comparatively less polar compound (e.g., metanephrine). Nevertheless, the % of recoveries for epinephrine and normetanephrine are still consistently around 60%. Our results are encouraging, as the cartridges were effective in extracting epinephrine, normetanephrine and metanephrine simultaneously, from urine samples with high levels of reproducibility. Furthermore, the sample matrices

were directly loaded on to the cartridge without any prior treatment which was an added advantage of the prepared cartridge. With the application of the prepared cartridges, the developed assay was validated suitable for selectively extracting polar basic analytes even in the presence of complex matrix like urine. The finding of highly reproducible recoveries after consecutive multiple extractions on the same cartridge is particularly interesting. This indicates that it will be economical to use these cartridges as they can be reused for many times without significant carryover effects and loss of efficiency. This unique property is different from that of the conventional SPE system which has limited number of reusability [Taylor and Singh, 2002]. The observed robustness of the developed underivatized silica monolithic cartridge is accounted by its unique approach in sample extraction via weak rather than strong ion exchange interaction.

CHAPTER 4



Application of Silica Monolith for Ketamine Analysis

4.1 INTRODUCTION

Ketamine was selected to further diversify the applicability of the silica monolith as solid phase extraction. As compared to our previous study (chapter 3), ketamine is relatively less polar as compared to catecholamines. The pKa value of ketamine is around 7.5 whereas the pKa values for catecholamines were more than 11. For the analysis of ketamine, multiple reaction monitoring was carried out on three transitions which are 238 → 125, 238 → 163 and 238 → 179. The extraction steps (activation, equilibration, loading, washing and elution) were carried out on a 24 station vacuum manifold which allowed multiple samples to be processed simultaneously.

4.2 OBJECTIVE

In chapter 3 the silica monolith (2cm length) was installed in the syringe and this device was used for the extraction of catecholamines and metanephrine in urine [Nema et al., 2010]. However, the 2-cm cartridge was presumed to be long and would take long processing time over SPE vacuum manifold, making the overall process time consuming. In addition to this, the cartridge required higher volume of solvent in order to regenerate it. Therefore, the aim of this chapter is to overcome these problems and inorder to achieve this, the monolith was reduced to 0.5 cm length before being installed and ketamine was used to demonstrate its performance. In addition to the performance efficiency of the cartridge, matrix effect after sample preparation was also studied extensively to ensure that it is consistent even after multiple extractions. The smaller sized cartridge demonstrated the advantages of simple operation and faster equilibration due to its reduced length that reduced total analysis time and allowed low volume of eluent, making the overall process environmental friendly.

4.3 MATERIALS AND METHODS

Ketamine was purchased from Sigma (St. Louis, USA). Formic acid was from Fluka (Buchs, Switzerland). Acetonitrile (ACN) was of HPLC-grade (Mtedia, USA) and all other chemicals used were of analytical grade. Milli-Q water (Millipore Bedford, USA) was used throughout the experiment. Standard stock solution of ketamine was prepared in water to give a final concentration of 1 mg/mL. The working standard solutions were prepared by appropriate dilution of stock. The prepared samples were stored in the fridge until further use.

4.4 EXPERIMENTAL

4.4.1 Analytical conditions

The Agilent 1200 series HPLC system (Agilent technologies, Germany) comprised of a binary pump (G1312A), an online degasser (G1379B), an autosampler (G1329A) and a system controller was utilized for LC MS/MS analysis. This HPLC was coupled to a 3200 QTRAP quadrupole mass spectrometer (Applied Biosystems, USA) with electrospray ionization (Turbo Ionspray). The data acquisition, handling and analysis were performed using the Analyst software 1.4.2 (Applied Biosystem, USA). The separation was performed in an isocratic mode using a C_{18} column (150 × 2 mm ID × 5 µm particles, Phenomenex, USA) protected by a guard column (C_{18} ODS, 4 × 3.0 mm ID) (Phenomenex, USA). The mobile phase consisted of phase A as formate buffer (10 mM, pH 3) and phase B as 100% acetonitrile. The composition of mobile phase was 80/20 phase A/phase B; and the flow rate was set at 0.25 mL/min. The total analysis time was 5min. The system was operated at ambient temperature (23-25^0 C). Ketamine in urine was quantified using multiple reaction monitoring (MRM) experiments to monitor the transition of the parent to the product ions. In the present study three transitions were selected to monitor ketamine concentration in the

following order of intensity 238 → 125 > 238 → 179 > 238 → 163. The MS was operated in the positive ion spray mode with the parameter settings as follow: curtain gas = 10; collision gas (CAD) = medium; ion spray voltage = 5000 V; source temperature (TEM) = 300^0C; ion source gas 1 (GS1) = 35 psi; ion source gas (GS2) = 30 psi; and interface heater = on. Quadrupole 1 (Q1) and quadrupole 3 (Q3) were maintained at unit resolution and dwell time was set at 150 ms.

4.4.2 Solid phase extraction

The SPE steps in the order activation, equilibration, loading, washing andelution were carried out after installing the prepared cartridge in a 2-mL syringe. The cartridge was activated with 1 mL methanol followed by equilibration with 1 mL of milli-Q water. This was followed by loading the cartridge with 500 µL urine directly and allowed to flow through under applied vacuum (10 kPa). No prior treatment of urine was done before loading onto the cartridge. The cartridge was washed with 2 mL water and drained completely before adding the elution solvent. Finally, the adsorbed analytes were eluted with 2.5 mL of 1:1 mixture of isopropyl alcohol and methanol containing 1% formic acid. The eluted analytes were collected and evaporated to dryness at 40^0 C under nitrogen using the TurboVap LV (Caliper Life Science, USA). The residue was reconstituted with 500 µL of water. 5 µL of the reconstituted sample was injected into LC MS/MS system for analysis. For consecutive use, the cartridge was washed with 1 mL methanol and finally with 1 mL water before re-using the cartridge for the subsequent SPE. In order to test the efficiency and carryover effect of the cartridge, spiked urine with ketamine was extracted consecutively on the same cartridge. Extraction of each spiked urine sample was followed by extraction of an unspiked urine sample. After each extraction, the cartridge was washed again as described above.

4.5 RESULTS AND DISCUSSION

4.5.1 Batch to batch variation of synthesized silica monolith

The in house synthesized silica monolith could present an unsatisfactory repeatability when different batches of materials were used for SPE. Therefore, batch to batch variation of the synthesized material was taken into account with context to SPE. In order to achieve this silica monolith was prepared in the batch of three and used for extraction of ketamine. Table 4-1 represents the peak area of ketamine with the relative standard deviation (RSD) values to demonstrate the variation in different batches. The RSD value was lower than 7% which confirms that the prepared silica monolith presents consistent reproducibility with all batches.

Table 4-1. The peak area and RSD value of detected ketamine in concentration of 20 ng/mL at three different transitions with three different batches of silica monolith prepared independently.

Transitions	**Peak area**			**%RSD**
	Batch 1	**Batch 2**	**Batch 3**	
238→125	7040	7650	6780	6.2
238→163	1140	1190	1090	4.4
238→179	1390	1490	1350	5.1

4.5.2 Optimization of LC MS/MS

Before analyzing samples in MRM mode, system was tuned to establish parent and daughter ion mass transitions using the standard drug solution. Consequently, ionization source parameters were optimized to get the maximum sensitivity of detection and the optimum values obtained are as follows: declustering potential = 28, entrance potential = 10, collision energy = 30 and collision cell exit potential = 3. These parameters were optimized via direct infusion of standard drug solution. This was followed by optimizing other source parameters and their optimized values are mentioned in section 2.3. Based on above optimized parameters and maximum

sensitivity of the parent daughter pair the transitions selected were 238→125, 238→163 and 238→179 for analysis.

The properties of mobile phase such as pH and additives were investigated as they could potentially affect the resolution. The mobile phase composition may also affect the ionization efficiency. Different proportions of methanol-formate buffer (10 mM) and acetonitrile-formate buffer (10 mM) were tested and the results showed that the response using acetonitrile-formate buffer was significantly higher. Consecutively, pH adjustment using formic acid was also optimized and it was found that pH adjusted to 3 was optimum with the highest sensitivity and resolution.

4.5.3 Evaluation of matrix effect (ME)

The residual components present, even after sample preparation steps, can interfere with ionization process in MS leading to ion suppression or enhancement [Marchi et al., 2010; Marquet and Lachatre, 1999]. This phenomenon can lead to erroneous determination and identification of the analytes under investigation. It can potentially affect the method performance in terms of detection capability, accuracy, linearity of response and limits of detection and quantification [Annesley, 2003]. Thus, the success behind any sample preparation technique lies in their capability to minimize matrix effect to maximize the intensity of the analyte. The effect of endogenous compounds contributing to ME was evaluated by the ratio of peak areas from matrix sample spiked after the sample preparation versus standard solution. ME was calculated based on the study which utilized following formula for determination [Matuszewski et al., 2003]:

ME % = B/A * 100

Where A is the peak area of the analyte in pure solvent and B is the peak area of the analyte spiked after SPE in urine extracts. Based on the calculated values 100%

denotes absence of ME, where as <100% indicates ion suppression and >100% indicates ion enhancement. Therefore, taking into consideration above formula, it was found that ion suppression was the predominant phenomenon in our study. The values calculated for ME were all less than 100%. After confirming ion suppression, the % suppression was quantified using following formula:

% suppression = 100 – B/A * 100

Table 4-2 shows the quantitative measurement of ME at three different concentration of ketamine after multiple extractions on same cartridge. The results demonstrated consistency in ME of less than 26% after using the same cartridge 10 times. Although the performance of the cartridge was demonstrated for 10 extractions, cartridge could have used several times.

Table 4-2. Evaluation of matrix effect after multiple extractions on the same cartridge.

Concentration (ng/mL)	**% Suppression Number of times used of the cartridge**			**%RSD**
	1st	**5th**	**10th**	
20	21.6	21	22	2.3
100	25.6	25.3	26	1.4
200	24.6	24.9	24.1	1.6

4.5.4 Analytical evaluation

Ketamine was analyzed under the above optimized parameters. Figure 4-1 shows LC MS/MS chromatograms of a urine sample containing 50 ng/mL of ketamine monitored at their selected transitions. The calibration curve was constructed from the sample spiked with stock solution to get final concentration of ketamine in the range of 10-500 ng/mL. The results showed a linear response in the selected range with good correlation coefficients (r^2>0.99). The limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on the signal-to-noise ratios of 3 and 10 respectively. LOD and LOQ for ketamine were calculated for all the

three transitions selected and the lowest LOD and LOQ at the transition of 238→125 were found to be 0.5 ng/mL and 1.6 ng/mL respectively. The results for linearity, LOD and LOQ are shown in Table 4-3. The extraction recovery was determined by analysis of the spiked urine sample at three different concentrations, namely low (50 ng/mL), medium (100 ng/mL) and high (200 ng/mL). The recoveries along with their %RSD values are summarized in Table 4-4. The recoveries were found to be in the range of 89-107%. The recoveries were consistent and reproducible even after multiple extractions which demonstrated their robustness and stability. The reproducibility of the method was determined using intraday and interday precisions. Four extraction of spiked urine sample over a day gave the intraday RSDs whereas interday precision was determined by extracting spiked urine samples independently for three consecutive days. Table 4-5 showed good intra and interday precision with RSD values less than 13%.

Table 4-3. Linearity range, LOD and LOQ data of ketamine at three different transitions.

Transitions	**Linearity range(ng/mL)**	**Regression equation**	**r^2**	**LOD (ng/mL)**	**LOQ (ng/mL)**
238→125	10-500	Y = 4023 X + 55125	0.9945	0.5	1.6
238→179	10-500	Y = 797.7 X + 9956.7	0.9962	1	3.3
238→163	10-500	Y = 613.9 X + 7841.2	0.9953	3	9.8

Table 4-4. Recoveries with RSD values of ketamine after multiple extractions from urine samples.

Concentration (ng/mL)	**Recovery (%; Number of times used)**			**%RSD**
	1st	**5th**	**10th**	
50	105.1	97.9	89.3	8.1
100	106.8	100.6	92.5	7.1
200	96.7	95.4	89.9	3.9

Table 4-5. Intra- and inter-day variations in the determination of ketamine in urine samples.

Transitions	Intraday precision (%RSD; n=4)			Interday precision (%RSD; n=3)		
	50 (ng/mL)	100 (ng/mL)	200 (ng/mL)	50 (ng/mL)	100 (ng/mL)	200 (ng/mL)
238→125	8.8	8.1	7.0	15.3	12.1	11.3
238→179	10.1	9.1	8.0	13.3	12.0	12.2
238→163	10.8	8.5	8.2	9.9	11.9	12.6

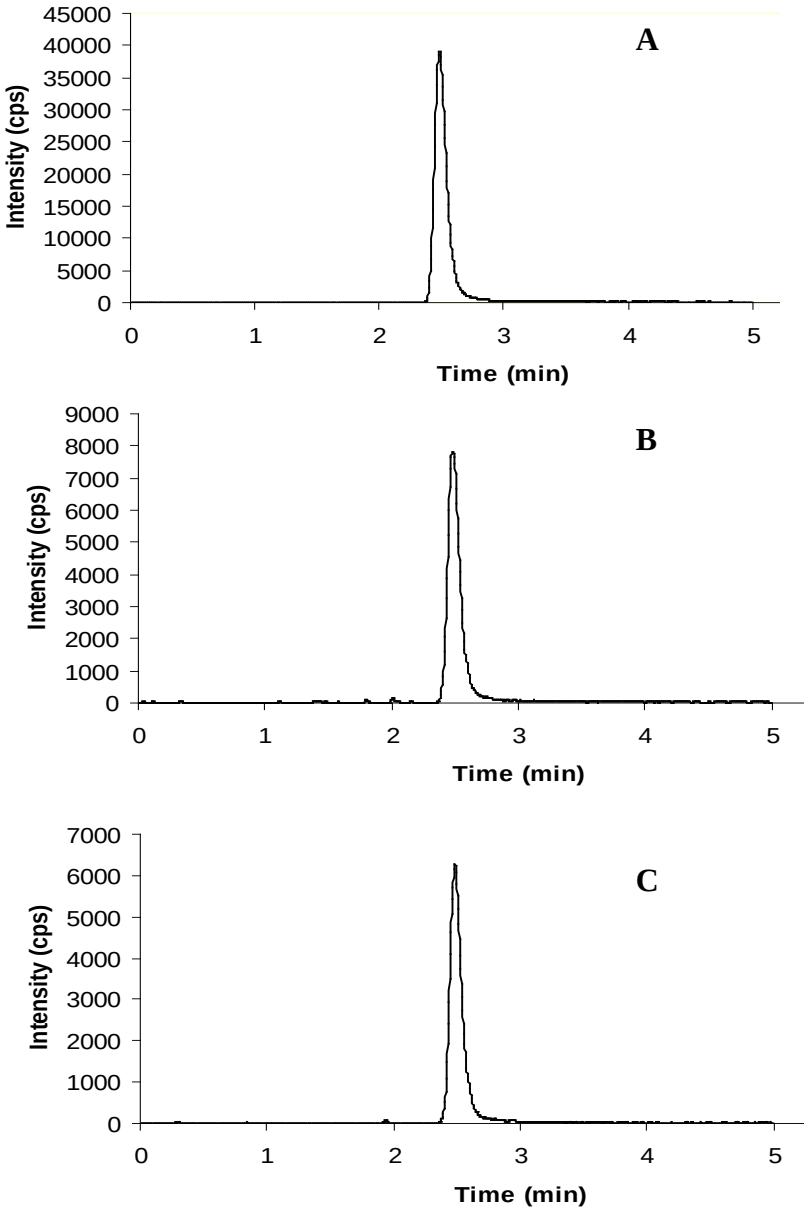
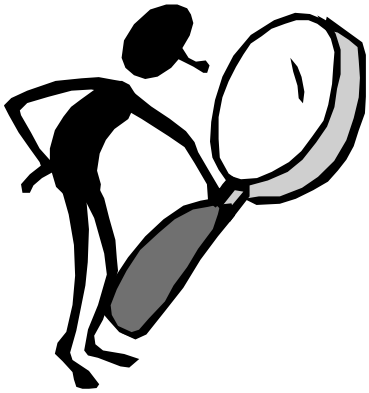


Fig. 4-1. LC MS/MS chromatograms of ketamine obtained at three different transitions, namely 238→125 (A), 238→179 (B) and 238→163 (C).

4.6 CONCLUSION

The applicability of the miniature silica monolith (0.5 cm) as SPE cartridge for processing urine samples containing ketamine was described in the study. The characteristic properties of the prepared silica monolith enabled extraction and desorption of the analyte efficiently. The SPE using silica cartridge demonstrated its capability to generate clean extract consistently. The silica cartridge could also be repeatedly used without any significant deterioration in its performance. High and consistent extraction efficiency was obtained with the prepared silica monolithic cartridge. The study provides another proof of application of silica monolith in bioanalysis, particularly for the sample preparation of complex matrices like urine. Silica based monolith could provide an alternative to polymer based monolith as they provide an advantage of better control in their synthesis, higher compatibility with various organic solvents and repeated applicability without compromising performance efficiency.

CHAPTER 5



Application of Silica Monolith for Opiates Analysis

5.1 INTRODUCTION

The prevalence of opioid and cocaine abuse is increasing substantially and globally.[Peindl et al., 2007; Hay et al., 2010] It is often very difficult and unreliable to determine their abuse through self-reporting by the addicts [Solbergsdottir et al., 2004]. The screening of drugs of abuse in the urine of drug addicts has become a conventional approach because of their non-invasive mode of collection. Several procedures based on liquid chromatography mass spectrometry (LC-MS) and LC-MS/MS techniques were developed for the determination of drugs of abuse [Marquet, 2002; Edwards and Smith, 2005; Klingmann et al., 2001; Wood et al., 2003; Hendrickson et al., 2004; Moeller and Kraemer, 2002]. Few attempts have been made through direct injection LC MS/MS of urine samples [Gustavsson et al., 2007]. Although the approach was straightforward and simple, the problem associated with matrix effect remains the point of concern. This could be attributed to the presence of salts, proteins and other interfering ions which could affect ionization in LC MS/MS [Matuszewski et al., 1998; Dams et al., 2003]. Therefore, sample preparation becomes necessary before subjecting them for analysis. Although many new techniques have been explored for sample extraction, SPE is considered to be the method of choice. The existing SPE cartridge based on silica are mostly particle packed and requires the filling of particles in the holder (syringe barrel) between porous frits. The efficiency of extraction depends on the quality of packing and the particle size distribution in order to attain consistent recoveries of sample.

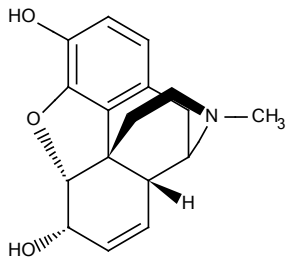
5.2 OBJECTIVE

The present study is an extension of our previous studies were the performance of the prepared monolith was demonstrated using catecholamines and ketamine as the model compounds and the results were found encouraging [Nema et

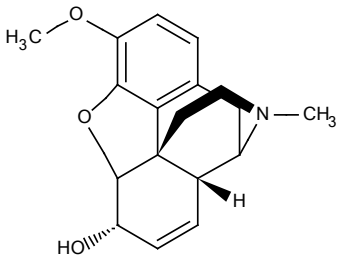
al., 2010]. Therefore, the aim of this progressive study was to further investigate extensively the ability of silica monolith as a SPE cartridge for extracting some most common drugs of abuse, such as, morphine, codeine and cocaine from the urine matrices. In addition to the LC-MS/MS analysis, the cleaning efficiency of the cartridge was further demonstrated in this study by measuring the osmolality, sodium content, LC/MS and the GC X GC/TOFMS scan noise level of the urine samples after SPE with the monolithic cartridge. In order to have a better understanding of the performance, this time Oasis HLB cartridge was used as reference standard for comparison. More emphasis was given in understanding the capability of silica monolith in generating clean extract as compared to the commercial cartridge.

5.3 MATERIALS AND METHODS

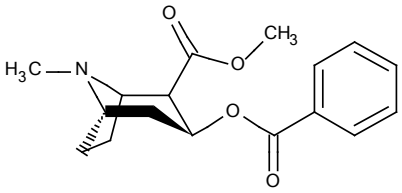
Morphine, cocaine and codeine were purchased from Pharma Science, (Montreal, Canada). Figure 5-1 shows the chemical structures of the three analytes along with their respective pKa values. Methoxyamine (MOX) and N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) containing 1% trimethylchlorosilane (TMCS) were purchased from Pierce (Rockford, USA), urease of sigma type III was procured from Sigma-Aldrich (St. Louis, USA). Formic acid was from Fluka (Buchs, Switzerland). Acetonitrile (ACN) was of HPLC-grade (Mtedia Company Inc., USA) and all other chemicals used were of analytical grade. Standard stock solutions of morphine, cocaine and codeine were prepared in water to give a final concentration of 1 mg/mL. The working standard solutions were prepared by appropriate dilution of stock. The prepared samples were stored at -20^{0}C until further use.



1. Morphine (8.2)



2. Codeine (8.2)



3. Cocaine (8.4)

Fig. 5-1. The structures of opiates along with pKa and compounds are numbered based on their elution order.

5.4 EXPERIMENTAL

5.4.1 Solid phase extraction

The extraction process (activation, equilibration, loading, washing and elution) was carried out after installing the prepared cartridge in a 2-mL syringe. The process started with the activation of cartridge using 1 mL methanol followed by equilibration with 1 mL of milli-Q water. A sample of 500 μL urine was aliquoted directly onto a preactivated cartridge and allowed to flow through under applied vacuum (10 kPa). No prior treatment of urine was done before loading onto the cartridge. The cartridge was washed with 2 mL water and drained completely before adding the elution solvent. Finally, the adsorbed analytes were eluted with 2.5 mL of 1:1 mixture of isopropyl alcohol and methanol containing 1% formic acid. The eluted analytes were collected and evaporated to dryness at 40^0 C under nitrogen using the TurboVap LV

(Caliper Life Science, Hopkinton, MA). The residue was reconstituted with 500 µL of water. 5 µL of the reconstituted sample was injected into HPLC coupled to a triple quadrupole/linear ion trap mass spectrometer (3200 QTRAP mass spectrometer, Applied Biosystem, USA). For consecutive use, the cartridge was washed with 1 mL methanol and finally with 1 mL water before re-using the cartridge for the subsequent SPE. In order to test the efficiency and carryover effect of the cartridge, 100 ng/mL of each of the three analytes was spiked in a urine sample that was subsequently divided into five aliquots. Each aliquot was extracted consecutively on the same cartridge. Extraction of each spiked urine sample was followed by extraction of an unspiked urine sample. After each extraction, the cartridge was washed again as described above.

5.4.2 Testing of the cleaning efficiency of the cartridge

The sample cleaning efficiency was evaluated based on the ability of the cartridge to yield clean extracts and demonstrate the absence of interfering ions which account for ion suppression and deterioration of separation column. In our study, osmolality, sodium content, LC/MS and gas chromatography/time of flight mass spectrometer (GC/TOFMS) analysis were carried out to validate the sample cleaning capability of the silica monolithic cartridge. The findings were compared to that of a commercially available cartridge, Oasis HLB cartridge Vac (Waters) which was claimed to be effective for extracting a wide range of compounds. Furthermore, the tendency of HLB cartridge to generate clean extract was the major focus of comparison of the prepared silica monolith. Among the four techniques employed to validate the cleaning efficiency of the cartridge, osmolality measurement is considered to be a less sensitive method towards total osmotic active particle measurement. In this study, osmolalities of samples were measured using the Vapro

Osmometer 5520 (Wescor, Inc, USA), sodium content was measured using Atomic Absorption Spectrometry (AAnalyst 400, Perkin Elmer, USA) and chromatograms were obtained using LC/MS (3200 QTRAP mass spectrometer) and GC X GC/TOFMS (LecoCorp., St. Joseph, MI) respectively. The study was conducted with the samples collected before and after SPE to demonstrate their efficiency in generating clean extract.

5.4.2.1 GC/TOFMS analysis

The GC/TOFMS analysis required sample derivatization before they could be subjected to analysis. The samples were prepared in accordance to a previous study [Pasikanti et al., 2010]. Briefly, 200 µL of each sample was obtained at various stages of the SPE process and 100U of urease was added to each of them. The samples were incubated at 37^0C for 45 min, after which 1.7 mL of methanol was added and vortexed for 10 min in order to precipitate proteins and degrade urea. The vortexed samples were centrifuged for 10 min at 10,000 rpm at 4^0C. The supernatants collected were evaporated to dryness at 40^0C under nitrogen using the TurboVap LV. The dried samples were vortexed with 100 µL toluene for 1 min and dried again under nitrogen. This was followed by derivatization with MOX for 2 hr at 60^0C. After 2 hr, a second phase of derivatization was carried out with MSTFA containing TMCS for 1 hr at 60^0C. The samples were allowed to cool down to room temperature (23-25^0 C) and 100 µL of the supernatant was transferred into a GC vial and subjected to GC/TOFMS analysis.

The two dimensional Pegasus 4D GC X GC/TOFMS (LecoCorp., St. Joseph, MI) analysis was performed in GC/TOFMS mode. A DB-1 30 m × 250 µm (i.d.) fused silica capillary column (Agilent J&W Scientific, Folsom, CA), with 0.25 µm film thickness, was used for separation. Helium was used as a carrier gas at constant

flow rate of 1.5mL/min. 1μL of the prepared sample was injected in a split ratio of 1:20 via CTC Combipal autosampler. The inlet temperature was maintained at 270^0C. The temperature program used starts at 70^0C with a hold time of 0.2 min, then increased at 10 ^{0}C/min to 270 ^{0}C and held at same temperature for 10 min. The MS was operated in an electron impact (EI) mode with a set temperature of 250^0C. Mass spectra were collected in the full scan mode from *m/z* 40 to 600 with an acquisition rate of 20 spectra/sec. Data were collected with the LECO ChromaTOF software v 3.32 (LECO, St. Joseph, MI, USA).

5.4.2.2 LC/MS analysis

The Agilent 1200 series HPLC system (Agilent technologies, Waldbroon, Germany) comprised of a binary pump (G1312A), an online degasser (G1379B), an autosampler (G1329A) and a system controller. The data were analyzed using the Analyst software 1.4.2 (Applied Biosystem, USA). The separation was performed in the gradient mode on a C_{18} column (150 × 2 mm ID × 5 μm particles) (Phenomenex, Torrance, USA) protected by a guard column (C_{18} ODS, 4 × 3.0 mm ID) (Phenomenex). The mobile phase consisted of phase A as 1 mM ammonium formate containing 0.15% formic acid and phase B as 100% acetonitrile. The flow rate was set at 0.25 mL/min. In order to resolve the analytes, a gradient system was applied which comprised of 15% phase B for 3 min and then increased to 30% over 7 min and then returning to 15% phase B where it was re-equilibrated for 5min. The total analysis time was 15min. The system was operated at ambient temperature (23-25^0 C). The column outlet was directly connected to the ESI probe with PEEK tubing.

HPLC was coupled to a 3200 QTRAP mass spectrometer. The mass spectrometer and LC parameter were controlled using Analyst software 1.4.2. For study of the cleaning efficiency of the cartridge, the blank urine sample and its filtrate

after SPE were subjected to full MS scan, while the analytes in the spiked urine samples were quantified using multiple reaction monitoring (MRM) experiments to monitor the transition of the parent to the product ions (Table 5-1). The MS was operated in the positive ion spray mode with the parameter settings as follow: curtain gas = 10; collision gas (CAD) = medium; ion spray voltage = 5500 V; source temperature (TEM) = 350^0C; ion source gas 1 (GS1) = 40 psi; ion source gas (GS2) = 30 psi; and interface heater = on. Quadrupole 1 (Q1) and quadrupole 3 (Q3) were maintained at unit resolution and dwell time was set at 150 ms.

Table 5-1. Optimized parameters for LC/MS quantitation.

Analytes	Transitions	DP (V)	EP (V)	CE (V)	CXP (V)
Morphine	286→165	55	10	45	3
Cocaine	304→182	55	10	45	3
Codeine	300→165	55	10	45	3

DP - declustering potential; EP - entrance potential; CE collision energy; CXP - collision exit potential.

5.5 RESULTS AND DISCUSSION

5.5.1 Conditioning of the cartridge for SPE

The performance of the prepared cartridge was evaluated based on its extraction efficiency of the model analytes namely morphine, codeine and cocaine. The underivatized silica cartridge contains siloxanes and silanols as the major functional groups along with the high surface area. Silanols are responsible for the ion exchange whereas siloxanes provide the weak reversed phase binding characteristic [Naidong, 2003]. The basic mechanism underlying the adsorption of analytes is based on polar ionic interactions and adsorption. The silanol group (Si-OH) on the surface of cartridge is acidic and exist as $Si\text{-}O^-$ at pH above 4 and will retain cations if the selected pH maintains both entities in charged state. Thus, the cartridge acts as weak cation exchanger possessing high surface area. Electrostatic interaction between the

positively charged model analytes and negatively charged silica cartridge is the probable mode of retention. A routine SPE condition was applied based on previous literatures [Chan and Ho, 2000; Chan and Siu, 1988]. The retention based on ion exchange mechanism depends heavily on pH of the environment and need to adjust accordingly. However, the pH of urine (6-7) was self sufficient to render both entities (sorbent and analyte) in charged state, thus, the need for pH adjustment was eliminated and the sample was directly loaded on to the cartridge. The cartridge was washed four times the volume of the loaded matrix with water to remove salts and other polar constituents. Biological samples are known to possess polar ionic endogenous compounds which can be retained strongly on silica monolithic cartridge because of their affinity towards silanols. In order to remove these endogenous compounds, the polarity of the washing solvent has to be such that these interfering compounds find higher affinity towards them for their successful removal. Since the presence of organic solvent could have altered the polarity of water, the cartridge was washed with milli-Q water to remove the accumulation of the endogenous compounds to the maximum extent possible. The analytes were then eluted out of the cartridge by regeneration of the free silanol groups (Si-OH) under acidic conditions. In this study, the elution solvent was optimized by varying the percentages of IPA and methanol containing formic acid. It was found 1:1 ratio of IPA and methanol containing 1% formic acid was most efficient in eluting the analytes out of the cartridge. The presence of acid neutralizes the charge on the sorbent surface whereas the two alcohols successfully remove the detached analytes from the sorbent. The use of the organic solvents in elution also complimented the regeneration of the cartridge for subsequent use. The cartridge was further regenerated using 1 mL methanol followed by 1 mL of water which was sufficient to neutralize the cartridge for consecutive

applications. The pH (found to be 2 with 1% formic acid) provided good recoveries without any deleterious effect on the sorbent.

5.5.2 Adsorption efficiency of the cartridge

The adsorption capacity of the cartridge was evaluated based on the concentration of analytes before and after loading the cartridge. Water was used as the sample matrix and it was spiked with the three analytes simultaneously, in increasing concentrations in a constant volume of 500 μL. The mixture with the respective concentration of 100, 200 and 500 ng/mL of the three analytes in water was allowed to pass through the cartridge. As the matrix was water, the eluted solution could be directly injected into LC MS/MS system to estimate the adsorption capacity. On analyzing the eluents, it was found that the three analytes were absent at all three concentrations after a single passage, confirming the fast and high adsorption capability of the cartridge. Further increase in concentration could be explored, but the high sensitivity of the instrument limited the concentration to 500 ng/mL for direct injection of the samples into the MS system. Furthermore, concentrations of opiates higher than 500 ng/mL might not reflect the concentration levels that are encountered in clinical practice.

5.5.3 Sample cleaning efficiency

In this study, osmolality, sodium content, LC/MS and GC/TOFMS analyses were performed to validate the cleaning efficiency of the cartridge; and the finding was compared to that obtained from the commercially available Oasis HLB cartridge. The blank urine samples were taken into consideration, as the purpose of the study was to demonstrate the efficiency of the cartridge in removing interfering ions.

5.5.3.1 Osmolality

In urine, sodium, potassium, chloride, bicarbonate and urea are the components present at high concentration which individually affects osmolality. The urine osmolality is the best measure of urine concentration. In this study, measurement of the osmolality of blank urine samples, in triplicate, collected before and after SPE was applied to determine the sample cleaning efficiency of the cartridge and the findings are shown in Figure 5-2. It was found that the osmolality of the urine samples after sample clean-up decreased significantly ($p < 0.001$) and was comparable to that of water, indicating the highly effective sample cleaning efficiency of the monolithic cartridge (Figure 5-2). The cleaning efficiency of our developed monolithic cartridge was found equivalent to the commercial Oasis HLB cartridge.

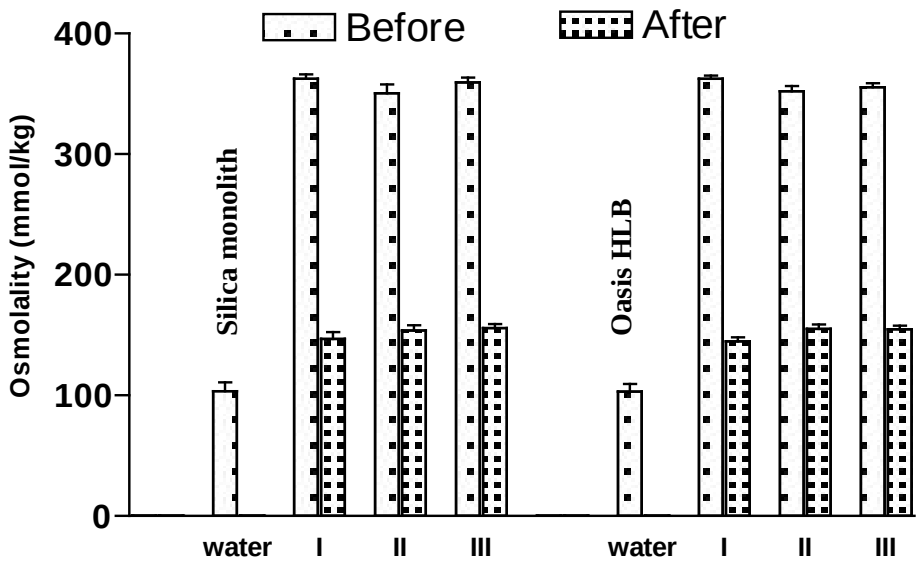


Fig. 5-2. Comparison of the osmolalities of water with urine samples before and after SPE with silica monolithic cartridge (Left) and Oasis HLB cartridge (Right); (I), (II) and (II) are the measurements from different experiments.

5.5.3.2 Sodium content

The presence of ions (like sodium, potassium, etc) in the samples often adds complexity in LC/MS quantification. Generally, ESI or APCI (Atmospheric pressure

chemical ionization) results in deprotonated $[M-H]^-$ molecules in the negative mode and protonated $[M+H]^+$ molecules in the positive ionization mode. However, several adduct ions such as $[M+Na]^+$, $[M+K]^+$, or $[M+NH4]^+$ were also formed in addition to $[M+H]^+$. Although many ions are present in biological matrices, sodium is our targeted ion because of its tendency to form stable adducts which could lead to variability in quantification. Thus, it becomes desirable to eliminate it as much as possible from the sample before analysis in order to procure reproducible results. Our results from AAS analysis showed that before sample preparation using SPE, the sodium content in the urine sample was 9.35 g/L. After SPE cleaning with the monolithic cartridge, the sodium content in the filtrate was 0.94 g/L, resulting in an approximate 90% reduction. The Oasis HLB cartridge also yielded a comparable reduction in sodium content (from 9.24 to 1.09 g/L) after SPE. This indicated that the prepared cartridge was equally efficient as the commercial product in reducing sodium level in urine sample.

5.5.3.3 LC/MS and GC/TOFMS analysis

The absence or reduction of endogenous interfering ions was further confirmed using LC/MS and GC/TOFMS analysis. In order to demonstrate this, full scan LC/MS of the samples obtained before and after SPE were analyzed. Subsequently, the chromatograms were compared for the reduction in the intensity of the interfering ions focusing at the retention times of the three analytes under study. The retention time of morphine, codeine and cocaine was 1.59, 2.09 and 9.03 min., respectively. Figure 5-3 shows the full scan chromatograms of the urine before and the eluent after SPE with the respective silica monolithic and the commercial Oasis HLB cartridge. On analyzing the chromatograms, obtained after SPE with the silica monolith cartridge, it was found that the noise levels were reduced by 4 times at the

retention times of the 3 analytes. In comparison, the noise levels after SPE with the Oasis HLB were only reduced by 1 time at the retention times of cocaine and codeine and by 4 times at the retention time of morphine. This indicates that the prepared silica monolithic cartridge could be more effective than the Oasis HLB cartridge in reducing the endogenous ions in urine samples for our application.

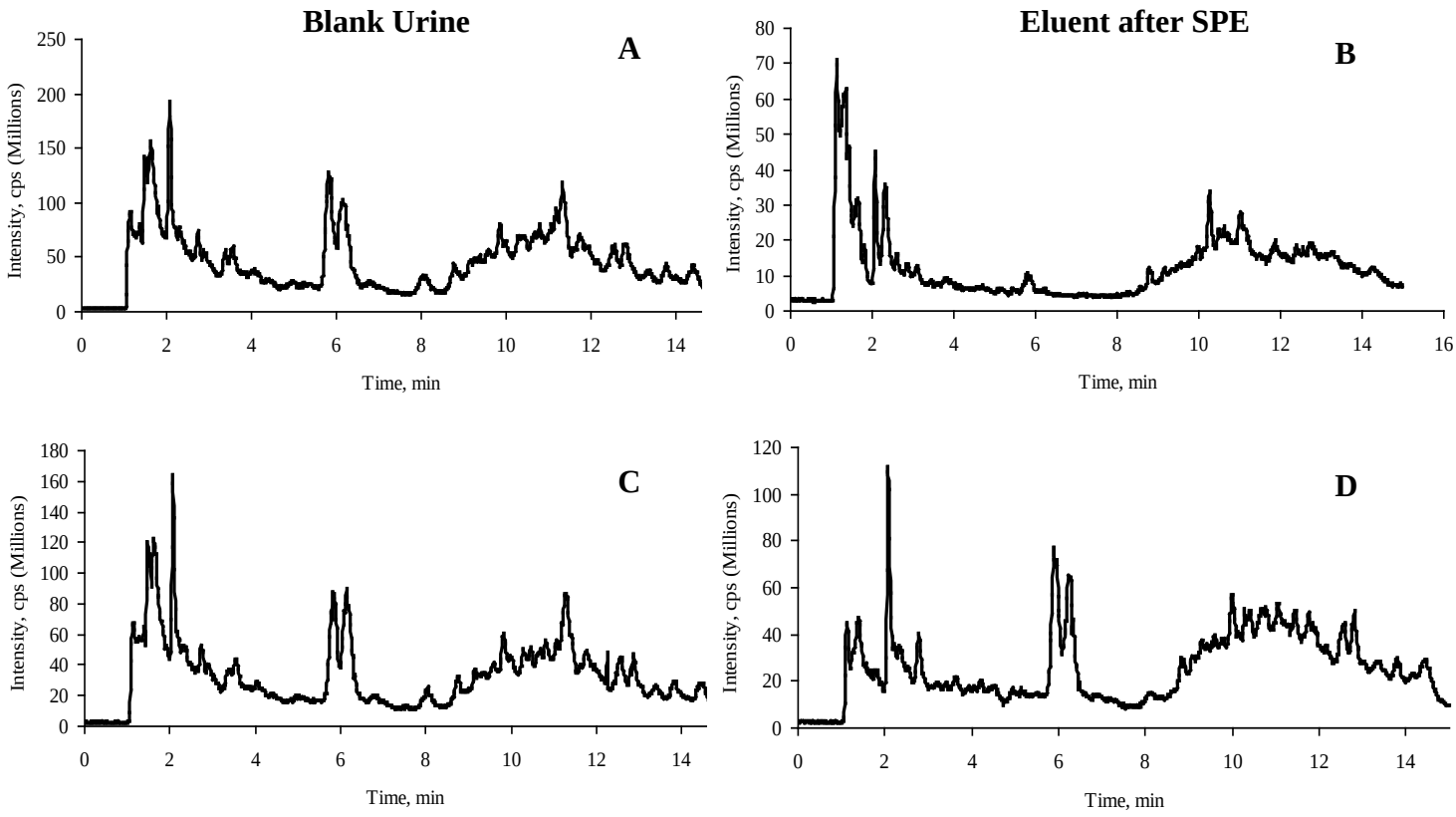


Fig. 5-3. Full scan LC-MS chromatogram of blank urine sample and extracted blank urine obtained from the silica monolithic cartridge (top, A & B) and Oasis HLB cartridge (bottom, C & D).

Similarly for the GC/TOFMS analysis, the samples obtained before and after SPE process were analyzed after derivatization. The obtained chromatograms were compared based on the number of peaks present in the samples after sample processing. Figure 5-4 illustrates the chromatograms of the urine sample before and the eluent after SPE. The comparison clearly indicates the decrease in the number of

peaks post-SPE. The chromatograms obtained from the silica monolithic cartridge were compared with the chromatograms of the extracts obtained from Oasis HLB cartridge and was found equivalent in delivering clean extracts. These experiments further strengthened our hypothesis that the silica monolithic cartridge could effectively reduce the background ion interferences.

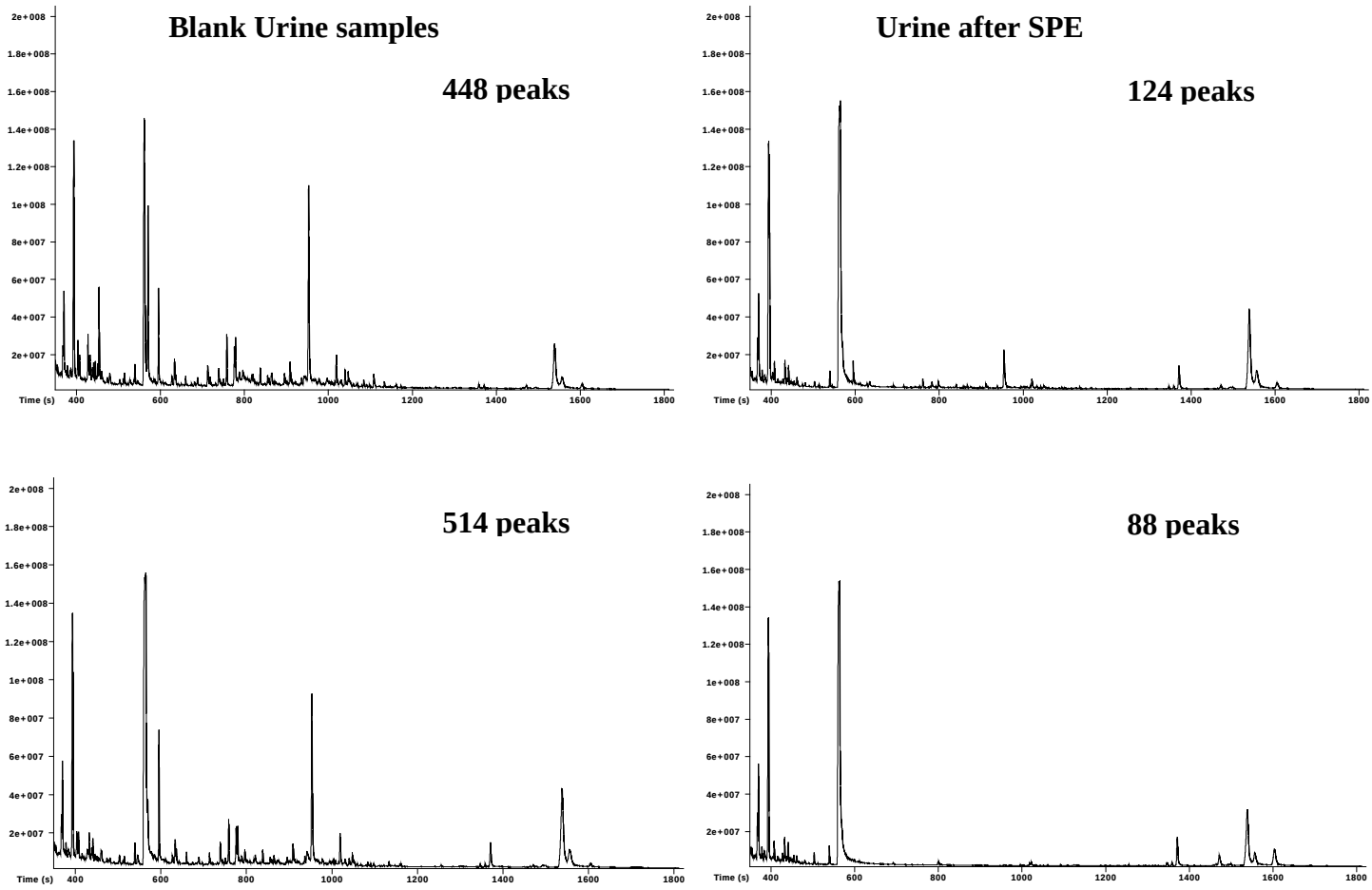


Fig. 5-4. GC/TOFMS full scan chromatograms along with the number of peaks present in urine samples before (left) and eluent after SPE (right) with the respective silica monolithic (top) and Oasis HLB cartridge (bottom).

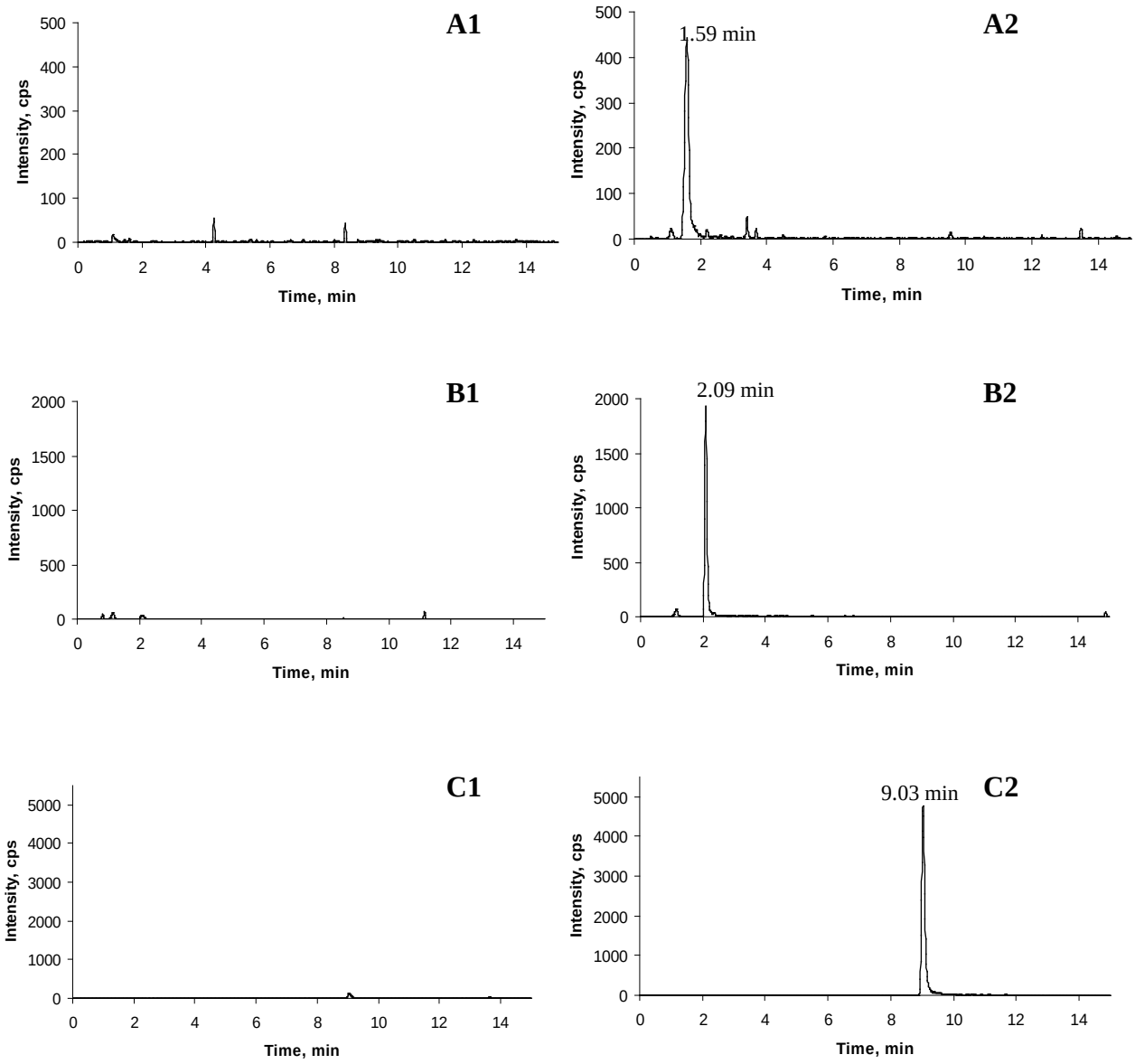


Fig. 5-5. LC/MS/MS chromatograms of the blank urine samples (A1), (B1) and (C1) and the corresponding urine samples spiked with 100 ng/mL of morphine (A2), codeine (B2) and cocaine (C2) following SPE with the prepared silica monolithic cartridge.

5.5.4 Method validation: specificity, linearity, precision and detection limit

The extracted, concentrated and reconstituted samples were injected directly into LC MS/MS for selective MRM analysis. Fig 5-5 illustrates the respective MRM chromatograms of the three opiates spiked in urine at 100 ng/mL and their respective blank extracts. The chromatogram demonstrated the absence of any interference at the

retention time of the analytes. Calibration curve in the linearity range of 50-500 ng/mL was constructed by making a series of dilution of standards in blank urine extract obtained after SPE. In the construction of the calibration curve, triplicate measurements of each concentration level of the calibration samples were performed; and the linearity of the calibration curve was checked using least squared regression analysis taking peak area versus concentration as the regression parameters with 1/x weighting factor. The correlation coefficient was found to be more than 0.99 for all analytes as shown in Table 5-2. The intra- and inter-day precision was expressed as the % relative standard deviation (%RSD) of the triplicate measurements at low (50 ng/mL), medium (200 ng/mL) and high (500 ng/mL) concentrations for each analyte. The %RSDs for intra- and inter-day variation at all concentrations were found to be less than 12% for the respective analytes (Table 5-3). The sensitivity of the method was established by examining the limits of detection (LOD) and limits of quantitation (LOQ). LOD was defined as the lowest detectable concentration with a signal to noise ratio of at least 3 and the LOQ was defined as the lowest quantifiable concentration with a signal to noise ratio of at least 10. The LODs and LOQs for the analytes under these conditions are presented in Table 5-2.

Table 5-2. LOD, LOQ, linearity range, and the correlation coefficient for the calibration curve of the respective analytes.

Analytes	**LOD (ng/mL)**	**LOQ (ng/mL)**	**Lineariy range (ng/mL)**	**Linearity**	**r^2**
Morphine	2	6.7	50-500	y = 185.6x +3318	0.9979
Codeine	1	3.3	50-500	y = 241.0x + 5565	0.9939
Cocaine	0.2	0.67	50-500	y = 462.6x + 2868	0.9973

Table 5-3. The precision of the assay in intra- and inter-day variation of the analysis in urine samples.

Analytes	Precisions (%RSD)					
	Intraday (n=3)			Interday (n=3)		
	Low	Medium	High	Low	Medium	High
Morphine	4.4	8.3	9.9	5.9	11.5	11.0
Codeine	5.6	4.8	8.0	5.2	3.5	8.7
Cocaine	5.7	10.8	1.6	11.7	6.8	6.7

5.5.5 Analytes recovery after SPE in urine

Urine samples were spiked with the respective morphine, codeine and cocaine to the desired concentrations (50, 100, 200, 300, 500 ng/mL) before they were subjected to SPE extraction. The absolute recoveries which were defined as the ratios of the peak areas of the spiked urine extracts to corresponding standard before SPE are shown in Table 5-4. The results indicate that recoveries of the three analytes were in the range of 85-105%. Although, the polarity of these analytes is not high and they have different degree of hydrophilicity (the order of hydrophilicity is morphine > codeine > cocaine as determined by their order of elution from the analytical column), the silica monolithic cartridge still held the analytes intact till the relevant elution solvent was used to completely elute them out from the cartridge. This indicated the strong adsorption capacity of the silica monolithic cartridge which can be attributed to high surface area and weak cation exchange capability.

Table 5-4. Recoveries of analytes from urine samples after SPE (n=3).

Spiked Concentration (ng/mL)	%Recoveries (%RSD)		
	Morphine	Codeine	Cocaine
50	109.5 (2.8)	114.5 (3.3)	103.3 (2.0)
100	96.4 (7.2)	110.1 (2.8)	109.6 (3.9)
200	88.8 (1.5)	90.3 (0.6)	87.2 (1.9)
300	88.9 (2.3)	86.2 (0.5)	91.4 (3.7)
500	92.3 (4.7)	98.9 (1.9)	86.9 (1.6)

5.5.6 Extraction efficiency and cartridge carry over

The extraction efficiency of the cartridge for repeated use was evaluated based on the recoveries of the analytes after multiple extractions on the same cartridge. A defined concentration (100 ng/mL) of the analytes was spiked in a urine sample and was extracted using the same cartridge. Extraction of each spiked urine sample was followed by one extraction of an unspiked urine sample to monitor the carryover effect. After each extraction, the cartridge was washed extensively and the last portion of the washed eluent from the cartridge was also analyzed to monitor the carryover. 1 mL of methanol followed by 1 mL of water was found sufficient to successfully regenerate and neutralize the cartridge pH before proceeding to the subsequent sample extraction. The uniform pH condition was utilized for each extraction to ensure reproducible recoveries. Although extraction of 1, 5, 10, 15 and 20 times of usage had been demonstrated in this particular study to demonstrate the robustness of the prepared cartridge, it could be reused for many more times with minimal compromise on its reproducibility. Table 5-5 represents the recoveries of simultaneous extraction of three analytes with multiple extractions on the same monolithic cartridge. As previously discussed, the ability to perform multiple SPE on the same cartridge was found to be the major limitation of particle packed cartridge. In order to compare the performance of particle packed cartridge with the monolithic cartridge, the former cartridge was prepared by packing silica particles (60-120 mesh size, BDH) in a 2 mL syringe. The recoveries from the particle packed cartridge ranged from 15-20% initially and the recoveries dropped further in the subsequent recycle uses (data not shown). The poor performance of the particle packed cartridge could be attributed to its lower surface area and non-uniform packing. Furthermore, the failure for subsequent usages could be attributed to channel formation due to particle

rearrangement while the solvent flowed through the system. All these factors possibly led to insufficient interaction time between the analytes and the packed sorbent. In addition to the comparison of silica monolithic cartridge with particle packed cartridge, the multiple extractions on the same cartridge was also performed on the commercial available Oasis HLB cartridge. It was found that the recovery deteriorated drastically after single use, following the sample protocol as the with silica monolith cartridge (recoveries dropped from 96% in the initial use to 55% in the subsequent use). However, if the Oasis HLB cartridge was washed further with double the volume of organic solvent (1 mL to 2 mL) the cartridge could be reused for 5-6 more times with good recovery.

Table 5-5. Recoveries of the analytes on a single cartridge after multiple extractions.

No. of extractions	**100 ng/mL concentration**		
	Morphine	**Codeine**	**Cocaine**
1st	105.6	100.9	88.6
5th	97.5	98.2	93.0
10th	104.6	102.8	93.5
15th	88.9	109.6	107.8
20th	102.8	106.8	106.5
% RSD	**6.9**	**4.4**	**8.9**

5.6 CONCLUSIONS

The attempt to utilize the silica monolith as SPE sorbent for extraction of morphine, codeine and cocaine from urine was proven to be effective. The recoveries of morphine, codeine and cocaine from the silica monolithic cartridges were in the range of 85-105%. Furthermore, the percentages of recovery of the three analytes were consistent after repeated usages. This confirmed that it is economical to use the silica monolithic cartridges as they can be reused for many times without significant carryover effects and loss of efficiency. The observed robustness of the developed underivatized silica monolithic cartridge was possibly accounted by its unique

approach in sample extraction via weak rather than strong ion exchange interaction. In addition, the sample matrices were directly loaded onto the cartridge without any prior treatment which was another advantage of our cartridge. The developed assay was finally validated to be suitable for the selective extraction of analytes (undertaken study) in a complex biological matrix such as urine.

CHAPTER 6

Application of Silica Monolith for Desalination

6.1 INTRODUCTION

According to U.S. Geological survey approximately, 96.5% of Earth's water is in seas and oceans, 1.7% is in the ice caps and 0.8 % is considered fresh water. The remaining percentage is of brackish water which is slightly salty and found as surface water in estuaries and aquifers [Gleick, 1996]. Continuous demand of fresh water has compelled to search for alternatives to the existing sources of fresh water to meet the increasing need. Various measures such as conservation, recycling and desalination are under study to find solution to meet the increasing demand for fresh water,. Desalination has been developed and used for centuries owing to the unlimited saline water supply from seas and oceans [Greenlee et al., 2009]. It is considered as capital and energy intensive process and typically requires conveyance and pretreatment of the feed water, disposal of the concentrated brine and maintenance. Advance systems are already in existence which either use thermal (distillation) or membrane based (reverse osmosis (RO)) technology. Although the cost and environmental concern has limited the popularity of desalination via thermal process, membrane based technology via RO polymeric membranes had shown great potential. Today, it accounts for the major portion in desalination process with less energy consumption and being economical [Veerapaneni et al., 2007] as compared to thermal technologies. But RO membrane alone cannot provide the safe drinking water. Among the major challenges of RO membrane, fouling is the major problem which results in decreased efficiency with the span of time [Meindersma et al., 2006]. Pretreatment provides the necessary steps before the feed water make contact with the RO membrane and it determines the membrane life time and minimizes membrane replacement frequency. However, added stages lead to increase plant expenses [Macedonio et al., 2007; Wolf et al., 2005; Sheikholeslami, 1999; Hilal et al., 2003].

Generally, membranes used in the pretreatment as well as RO membrane are organic based made of polyvinyl chloride (PVC), polyvinylidene fluoride (PVDF) and other polymers. Sea water contains high concentration of salts and organic foulants which leads to fouling of polymeric membranes. Thus, increased stability of the membrane with reduced energy consumption is desired for effective desalination. This problem can be addressed using inorganic based membranes owing to their superior mechanical properties, chemical inertness and thermal stability. Adsorption is an important phenomenon and found useful in sea water desalination as it can remove many of the substances present in raw feed. For example, activated carbons in the form of powder or granule [Gur-Reznik et al., 2008] have been used as a pretreatment method prior to RO but it was found that they have limited capacity and intensity of adsorption. Another study demonstrated the use of double walled carbon nanotubes for water purification and seemed to be promising [Hummer et al., 2001], but such membranes may be difficult and costly to manufacture, prone to defect formation, and might have a high propensity for fouling given their hydrophobic nature [Shannon et al., 2008]. Porous inorganic membranes such as zeolites and amorphous microporous silica are among the few more studies used for desalination [Lin and Murad, 2001; Li et al., 2004]. Although the two techniques were effective, the synthesis of controlled ordered structure of zeolite was tedious [Li et al., 2004] whereas amorphous silica is soluble in water which has a detrimental effect on structure [Duke et al., 2004; de Vos and Verweij, 1998]. Therefore, considering these facts and based on our observation on the efficiency of silica monolith in reducing the osmolality of urine samples, we propose the use of silica monolith for the sea water desalination. Since they are synthesized incorporating a carbonized template within the pores, performance in aqueous environment was significantly enhanced due to the

blocked movement of silica groups across the surface [Duke et al., 2006]. According to the study by Lint et al, [Lint et al., 2006] the mechanism responsible for the retention of ions was due to electrostatic interaction. They have studied the mechanism of retention of electrolyte on inorganic membrane. According to their study, membranes perform excellently at pH way beyond their iso electric point (IEP), which corresponds to greater membrane potential. Existence of the membrane in the charged state at the pH of sea water is considered to be a critical factor in desalination process. As discussed in chapter 1, silica exists as $Si\text{-}O^-$ at the pH above 4, and its ionic state is evident due to its low IEP value ($IEP_{silica} = 2$). The pH of sea water is around 7-8 which is sufficient to keep the monolith in charged state and thus facilitate the retention. The characteristic feature of silica monolith makes them a suitable candidate to be explored for the desalination process.

6.2 OBJECTIVE

Although, monoliths today are applied in many areas of science apart from separation, their potential and unique character can be exploited in other fields as well. Desalination is one such area where monoliths can stand alone in the form of a series of monoliths attached in tandem or can be used as a pretreatment method to enhance the performance and life expectancy of RO membrane. Their unique characteristic of high surface area, permeability and ease of preparation attracted us to explore its potential for desalination. Therefore the aim of the present study is to investigate the potential of lab scale prepared silica monolith for desalination. In this study, sodium chloride rejection at various concentrations was investigated to determine the capacity of the prepared silica monolith for desalination to the maximum extent possible.

6.3 MATERIALS AND METHODS

All the chemicals used are mentioned in Chapter 2 for the preparation of silica monolith. The sodium chloride (NaCl) used in the study was of analytical grade procured from Merck (USA). All other chemicals were of analytical grade. NaCl was dissolved in water to concentration of 50,000 ppm and this was considered stock and kept in a fridge until further use. From this stock solution, various dilutions were prepared to test the performance efficiency of the prepared monolith.

6.4 EXPERIMENTAL

6.4.1 Silica monolith preparation and characterization

The silica monolith was prepared and characterization following the same protocol discussed in Chapter 2. The chapter was devoted to a more detailed discussion on the preparation and characterization of the silica monolith. In this particular study the prepared silica monolith was used in the setup mentioned in Chapter 3, except that 1 cm length monolith was cut and used for desalination tests.

6.4.2 Membrane charge behavior

As the mechanism considered for retention was electrostatic interaction (section 6.1), it is desirable to determine the charge on silica monolith and also its behavior in different concentration of NaCl solution. Therefore, zeta potential measurement was taken as the representative experiment to understand the charge and the charging behavior. For this experiment the prepared monolith was grounded and suspended in water to measure the charge on the monolith. The stock suspension of grounded silica monolith was prepared by weighing 5mg of silica monolithic powder and suspending in 5 mL of water. From this stock, various representative samples of silica monolithic powder in water and also in aqueous solution with different NaCl concentrations in the range of 0.001-0.1% w/v were used for zeta potential

determination. The zeta potential was determined using Zetasizer nano (Malvern, UK).

6.4.3 Desalination test

Desalination test was carried out using the setup depicted in Fig 3-1 of Chapter 3. This setup requires low pressure operation as monoliths are known for their high permeability. Thus, vacuum used in the setup to allow samples to flow through was considered sufficient for effective performance. Since, sea water accounts for 3.5% w/v salinity which is due to the dissolved salts comprised of sodium, calcium, magnesium, potassium, chloride, boron and sulphate present in the form of ions. However, sodium chloride (NaCl) contributes 76% of the total dissolved salts to salinity. For this reason, NaCl solution was used as a feed water to represent seawater for the analysis to confirm its desalination ability.

The prepared silica monolith was tested at various concentrations of NaCl ranging from 10,000-40,000 ppm. The NaCl solutions were loaded on to the prepared monolithic cartridge and the cartridge passed samples were analyzed for sodium content, tested for chloride, conductivity and osmolality. The sodium content was measured using atomic absorption spectrometry. Chloride was tested qualitatively with silver nitrate solution of the cartridge passed water. The qualitative test (visual detection of precipitate with silver nitrate) could only confirm the presence, absence or reduction of chloride. Conductivity of the water before and after cartridge passed was also analyzed using the conductivity meter for purity testing. Osmolality study was also carried out to further demonstrate the purification efficiency of the cartridge. The eluents from the cartridge were compared in osmolality with the feed and pure water.

The efficiency of the prepared silica monolith was determined by measuring salt rejection (%SR) expressed as:

$$\%SR = \frac{Cf - Cp}{Cp} \times 100$$

where Cf and Cp are the concentration of NaCl in feed and permeate water (ppm), respectively.

The prepared silica monolith was used repeatedly after regenerating it using mild acid conditions. 1mL of 0.1% formic acid was used for regeneration of the cartridge. The acid treatment was followed by water washing in order to neutralize the low pH. The water washed out from the cartridge was constantly tested for pH using pH paper. 2 mL of water was used to neutralize the acid. 1 mL of NaCl solution was allowed to pass through the cartridge and the collected permeate was tested for its sodium and chloride, respectively. The collected permeate was tested following a single passage cycle through the cartridge.

6.5 RESULTS AND DISCUSSION

6.5.1 Surface charge determination

The zeta potential of the silica monolith was found to be -31.5 (mV) when suspended in water. This indicated the presence of negative charge at pH of water. Once the nature of the charge on the monolith was confirmed, the effect of NaCl concentration needs to be established. Figure 6-1 shows the trend of zeta potential at different NaCl concentration. The results clearly indicated that with the increase in NaCl concentration zeta potential decreases. This was expected as it was discussed that at pH > pH_{IEP} the negativity of the monolith increases and thus increases the retention. Although, the figure depicted the decrease in zeta potential with increasing NaCl, the curve attained plateau at the higher concentration above 0.05% (w/v). This

plateau could be attributed to the saturation of the charged interactive sites after an optimum level is reached.

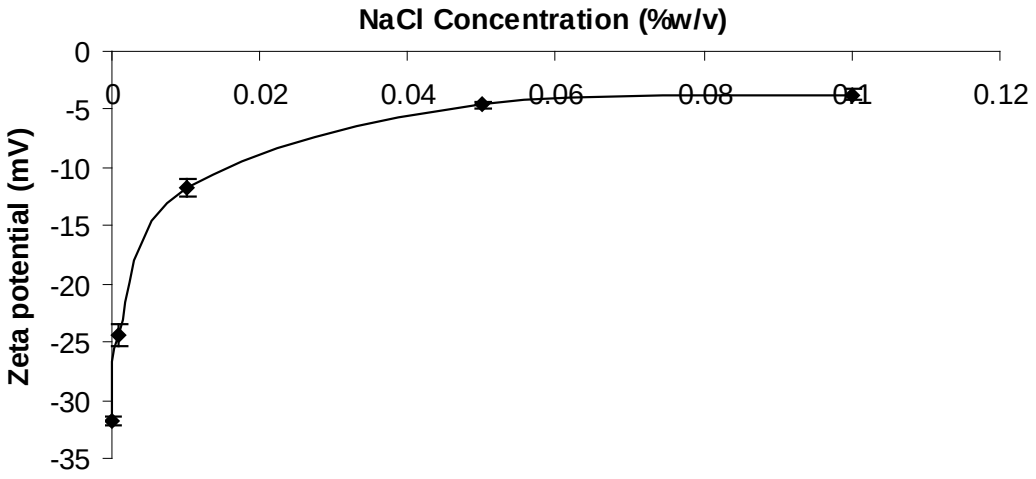


Fig. 6-1. Effect of NaCl on zeta potential of silica monolith.

6.5.2 Preliminary Study

Table 6-1 depicts the % reduction in the NaCl after single passage through the silica monolith. The silica monolith had shown to reduce the sodium content by almost 98% for the 10,000-30,000 ppm loads and reduce to 90% with 40,000 ppm salt concentration. The efficiency reduced from 98% to 90% at higher concentration and it could be attributed to the saturation of the interactions sites on the monolithic surface. Hence, silica monolith generated the highest salt rejection in the concentration range of 10,000-30,000 ppm. In addition, the cartridge was found effectively regenerated using 0.1% formic acid and pure water The variation in the desalination efficiency after multiple usages was well below 3% (as listed in Table 6-2), demonstrating their consistency in performance during experimental testing and hydrostability.

Table 6-1. Measurement of Sodium content before and after cartridge passed (CP), NaCl solutions in the concentration range from 10,000-40,000 ppm.

Before CP (ppm)	After CP (ppm)	% Reduction
10000	200	98
20000	380	98.1
30000	560	98.1
40000	3850	90.4

The presence of chloride was tested using conventional silver nitrate test ($AgNO_3$). The sample before and after cartridge passed was tested for chloride by visually detecting the presence of the precipitate after adding $AgNO_3$. It was found that no precipitate appeared in the permeate, whereas the feed water showed a thick precipitate immediately after adding $AgNO_3$.

Table 6-2. Efficiency in the desalination process indicated by the variations in sodium content measurement after multiple extractions with the same cartridge at different concentrations of the NaCl solution.

NaCl concentration (ppm)	% Reduction after triplicate extraction (ppm)			%RSD
	I	II	III	
10000	200	205	210	2.5
20000	380	370	390	2.6
30000	560	535	550	2.3
40000	3850	3800	3880	1.1

According to WHO standards, drinking water should have dissolved salts less than 600 ppm. The results have shown that the monolith had produced good quality water for the salt concentration in the range of 10,000-30,000 ppm. With the high surface area with good permeability and surface charge of the monolith, a single pass through the cartridge was sufficient for effective desalination.

6.5.3 Real sample analysis

The success of preliminary experiment led to testing the prepared silica monolith for desalinating sea water. The procured sea water was tested for sodium and was found to be 25,000 ppm. This concentration was well within the range (10,000-30,000 ppm) where cartridge demonstrated excellent desalinating capacity. The samples were treated in the same way as mentioned in section 6.3.2 and the finding indicated a 98% reduction in sodium level. Similarly, the samples were tested for chloride using $AgNO_3$ and shown the absence of chloride.

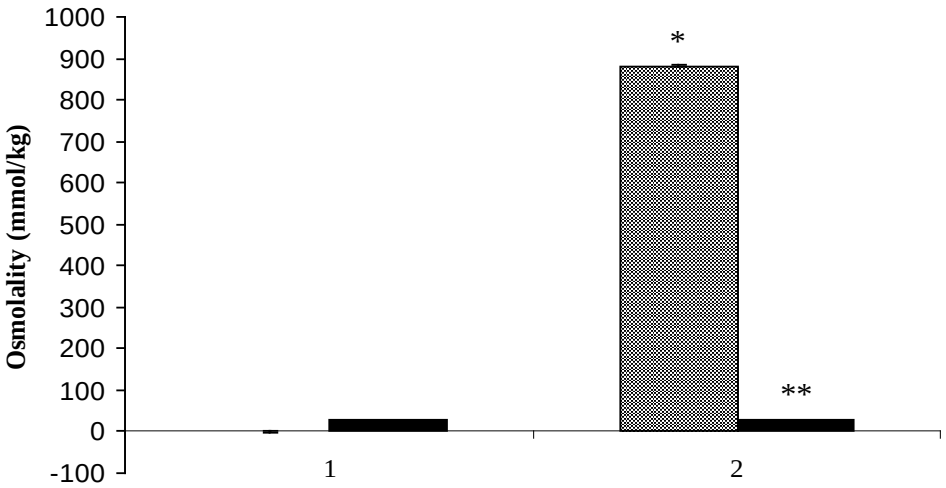


Fig. 6-2. 1. Osmolality of pure water; 2. Osmolality of seawater before (*) and after (**) cartridge passed.

Furthermore, the seawater samples before and after treatment were tested for their change in osmolality using osmometer. The test was performed to demonstrate the capability of the silica monolith in reducing the concentration of active ions. Based on the results depicted in Figure 6-2, it was found that the osmolality of saline water after cartridge filtration had almost the same osmolality as that of pure water. The findings reflected the high efficiency of the prepared silica monolith in treating seawater. Subsequently, the samples filtered through the cartridge were also tested for conductivity, another indicator for purification efficiency.

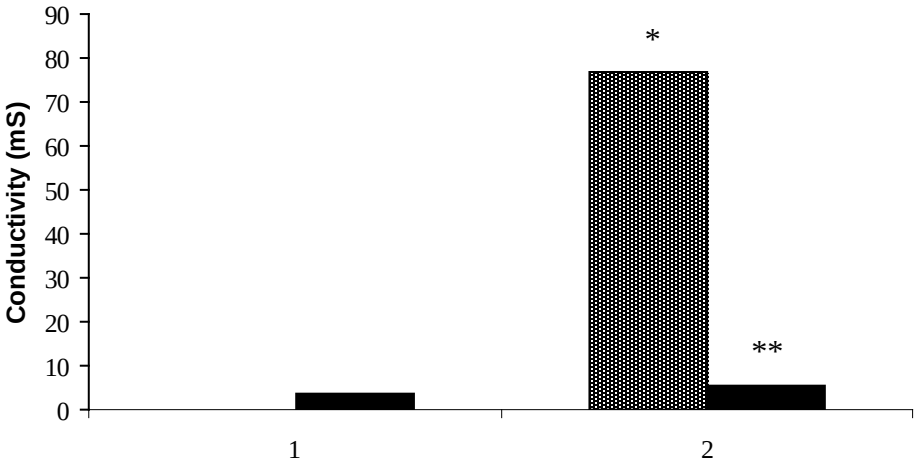


Fig. 6-3. 1. Conductivity of pure water; 2. Conductivity of seawater before (*) and after (**) cartridge passed.

Figure 6-3 showed a significant reduction in conductivity after cartridge passed and was comparable to the conductivity of pure water. This further strengthens our hypothesis of utilizing silica monolith for desalination.

The findings in the osmolality and conductivity studies demonstrated that the prepared silica monolith was not only effective in removing NaCl but it also had the capacity to remove other ions as well. The sample extracts from the silica monolith were also tested for calcium and potassium using Flame photometry and it was found that these elements were also missing in the permeate when compared with the feed water (data not shown).

6.5.4 Silica monolith regeneration

The prepared silica monolith after use was regenerated by washing it with 0.1% formic acid and finally with water. Although regeneration was simple and straight forward, there are two major limitations that need to be addressed. Firstly, the use of formic acid, though in small volume it could cause an environmental concern. Secondly, the water required to neutralize the acid wash was more than the volume that was actually generated by the cartridge, as mentioned in section 6.3.3. This made

the overall process insignificant and uneconomical. Therefore, the use of lower volume of water at higher temperature was predicted to regenerate the cartridge. With this, the process can be made more environmental friendly and more economical due to increased efficiency as less water would be required for regeneration. Thus, in order to demonstrate the effectiveness of using temperature, charging behavior of the monolith at various temperatures was investigated. This led to the measurement of zeta potential of the grounded monolith at different temperatures. Figure 6-4 demonstrated the effect of temperature on the charge of silica monolith. It was found that with the increasing temperature the charge (measured as zeta potential) on the monolith decreases in negativity and at 60^0 C the zeta potential drastically changes from negative to positive. The decrease in the negative charges resulted in the elimination of the retained ions and regeneration of the surface. Although temperature utilization was found economical but the mechanism behind this phenomenon still need to verified.

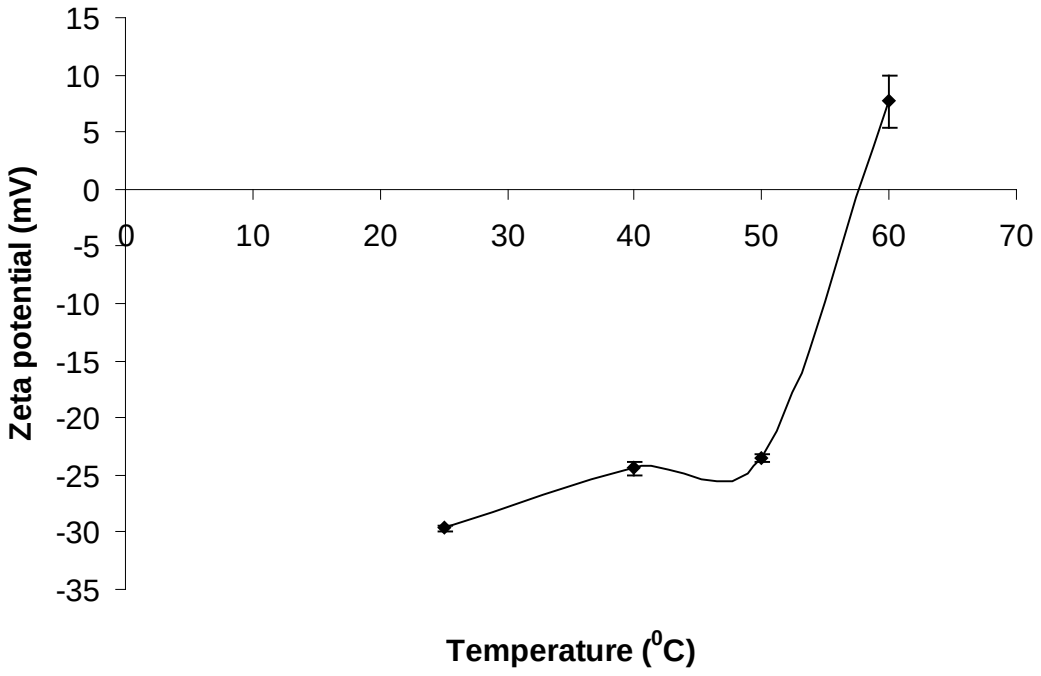


Fig. 6-4. Effect of temperature on the zeta potential of silica monolith.

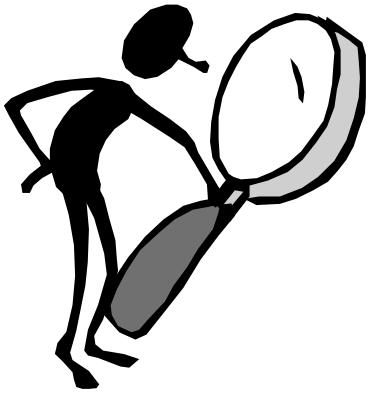
With the establishment on the effect of temperature on the zeta potential of silica monolith, elevated temperature was subsequently applied to regenerate the silica monolithic cartridge after desalination.. After production of 1 mL of pure water from NaCl solution, it was found that the cartridge could be effectively regenerated with 0.75 mL water heated to 60^0 C. The eluent was tested for sodium content to confirm the completion of the regeneration. Though the procedure gave a gain of only 25% of the effect, it could be further developed and optimized to improve the yield. Since, only water was used for regeneration, and formic acid was not required for this procedure, this made the overall process economical and environmental friendly.

6.6 CONCLUSION

The findings in this study have shown great potential application of the silica monolith for desalination. In addition to its remarkable desalination property, the cartridge can be regenerated, either by mild acid or warm water treatment; and the cartridge can be reused for many times. On the basis of these findings, we conclude that silica monolith can potentially provide simpler and affordable means in generating fresh water with reduced input and greater output.

With the introduction of silica monolith either alone or as a pretreatment cartridge, the desalination capacity can be enhanced along with greater affordability and lesser energy consumption. As a pretreatment step it can prolong the life of RO membrane without fouling or clogging which reduces the cost of the water production. The silica monolith protects the RO membrane by adsorbing salts and reducing the salinity of water before it reaches the RO membrane. The cost of monolith preparation is more affordable than RO membrane. Therefore, replacement of monolith is more cost effective than replacing the RO membrane. It warrants to further explore the use of silica monolith for desalination.

CHAPTER 7



Modification in the Silica Monolith Preparation to Enhance the Surface Area

7.1 INTRODUCTION

The synthesis of porous inorganic materials with high surface area and controlled pore sizes are highly in demand due to their inherent applications [Corma, 1997; Mezza et al., 1999; Mosquera et al., 2002; Baskaran et al., 2000; Davis, 1993]. Several attempts in the synthesis of these materials have been made but the preparation of porous inorganic materials with controlled morphology and high surface area is still a challenging task. The porous inorganic materials have been fabricated as spheres[Schacht et al., 1996; Boissiere et al., 2000; Zhao et al., 2000; Huo et al., 1997], crystals [Che et al., 2001; Guan et al., 2000; Yu et al., 2002], films [Zhao et al., 1998; Yang et al., 1996; Yang et al., 1996; Grosso et al., 2001], rods [Yu et al., 2002], fibers [Marlow et al., 2000; Yang et al., 1998], and monoliths [Yang et al., 2003; Huesing et al., 2003; Templin et al., 1997; Bagshaw et al., 1995; Feng et al., 1999; Melosh et al., 2000]. Among these, monoliths have attracted increasing attention because of their implied applications in separation and catalysis. These porous materials with advance properties have been synthesized from the calcinations of aluminosilicate gels in the presence of surfactants [Kresge et al., 1992] or using amphiphilic block copolymers as templates [Kramer et al., 1998]. For many applications, especially separation and catalysis, materials with high surface area and hierarchical pore structure are desirable. Nakanishi and Soga [Nakanishi and Soga, 1991] have prepared silica monolith with interconnected macropores and textural pores using inorganic precursor. The prepared material has been successfully applied in HPLC [Minakuchi et al., 1998; Minakuchi et al., 1998; Minakuchi et al., 1997; Minakuchi et al., 1996] and catalysis [Chiu et al., 2004], both of which require macropores for high permeability and mesopores for high surface area. Endeavor to improve the technique to adjust the sizes of macropores and mesopores

simultaneously is a major research focus recently. In an attempt, Smatt et al.[Smatt et al., 2003] prepared a silica monolith with hierarchical porosity through double-templating preparation route. Poly (ethyl glycol) (PEG) was employed as a phase-separation-inducing agent in order to produce macropores and cetyltrimethylammoniumbromide was applied as structure-directing agent to obtain mesopores. In another study [Wei et al., 1999] mesoporous silica monolith was synthesized using D-glucose as pore-forming agent. However, the resultant material obtained does not have characteristic features of monolith and no macropores coexisted. On the contrary, a study by Zhong et al. [Zhong et al., 2009] demonstrated the utility of inorganic salts in synthesizing silica monolith with well defined macroporous and mesoporous structure. Among the various salts (NaCl, $NaNO_3$ and Na_2SO_4) used, NaCl demonstrated the greater tendency in promoting the formation of ordered mesostructure. Despite focusing on improvising the surface characteristics, synthesis of crack free monolith was also taken into account in the above studies. The reason for achieving better outcome in this study could be attributed to the higher temperature (>500 ^{0}C) treatment in the process.

7.2 OBJECTIVE

In this preliminary study, compression was explored as a mode of enhancing the surface area of monolithic materials with ordered pore structure. The concept evolved during an attempt to develop a bigger diameter monolith. For the fabrication of the monolith, if the monolith was compressed after preparation but it would lead to the destruction of the monolith and thus its characteristic properties. Therefore, it was thought to compress the monolith during its process of aging. This resulted into the modification of the preparation steps described in Chapter 2. The aim of this study was to generate a high surface area silica monolith with ordered mesopores and bigger

diameter. The prepared cartridge was characterized and tested for its adsorption capacity as solid phase extraction cartridge.

7.3 EXPERIMENTAL

7.3.1 Modified silica monolith preparation and characterization

The silica monolith was prepared with the method discussed in Chapter 2 with modification. The steps involved in the preparation were same as discussed in Section 2.4.1 except that the monoliths were not aged in full 18 h stretch. The sol prepared after mixing the ingredients in the desired composition was filled in a mould to 2 cm length and incubated at 40 ^{0}C. Monoliths were taken out for compression after 12 h of aging and compressed to 50% and 75% of the original length. The percentages were calculated based on the original length of the monolith. For example, 50% compressed means that monolith was compressed to 1 cm as compared to 2 cm whereas for 75% compression the monolith was compressed to 1.5 cm. The silica monolith was casted in a syringe and this syringe was used for compressing monolith. The compression pressure was applied using hydrothermal press available for FTIR disk preparation, at room temperature. After compression the monolith were again incubated at 40 ^{0}C for 6 h, thus completing 18 h cycle. This was followed by the steps mentioned in section 2.4.1. The resultant monolith was characterized for permeability, surface area, surface morphology using electron microscopy and finally for their performance as solid phase extraction tool. The adsorption capacity of the prepared monolith was taken under consideration and all the levels of characterization were compared to non-compressed monolith.

7.3.2 Adsorption study

The adsorption capacity of the prepared cartridge was demonstrated using morphine, codeine and cocaine as model analytes. The monoliths in the length of 0.5

cm was cut and fixed over 2 mL syringes. The analytes in the concentration range of 1-10 μg/mL was prepared in water and 1 ml of it was allowed to pass through the cartridge. The measurement of three analytes, before and after cartridge passed, was carried out using LC-MS/MS as described in Chapter 5.

7.4 RESULTS AND DISCUSSION

7.4.1 Characteristics of the compressed silica monolith

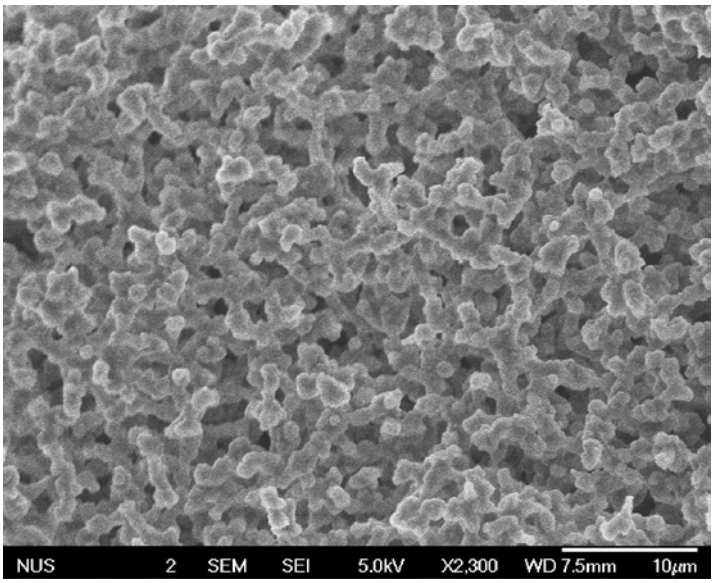
The silica monoliths were obtained through combining the sol gel method and compression technique. The discs have a diameter of about 5 mm and thickness of 5 mm. The prepared monoliths are remarkably stable retaining its mechanical and chemical properties for long time. On visual inspection, the monoliths appeared crack free, opaque (white), harder, denser and in bigger diameter compared to non compressed monolith The advantage of using compression phenomena is that the monolith regain its structure as it aged after structural distortion and compression squeezes out solvent that remain entrapped in the pores of the monolith. This leaves behind more uniform and smaller pores when compared to non-compressed monolith, contributing to its high surface area. To achieve this, syringe was used a mould for casting monolith during compression. The syringe has a narrow opening on the other side to allow the liquid to pass through. Due to its narrow opening, the gelled material was prevented from being squeezed out of the syringe during compression, thus allowing only the solvent to flow through. With the use of compression model, the equilibrium established between the sol-gel reaction was shifted to a forward direction. This enabled a more complete reaction and thus influencing the final structure.

Figure 7-1 showed the SEM images of the prepared silica monolith at various percentages of compression of their original lengths. The figure shows the obvious

increase in the density of the monolithic structure when compressed to 50-75%. However, it was fascinating and interesting that even after compression the structure remained the same. This indicated the regeneration of the structure after compression. In addition the macropores which were more prominent in the non compressed monolith was found to be reduced when compressed from 50-75%.

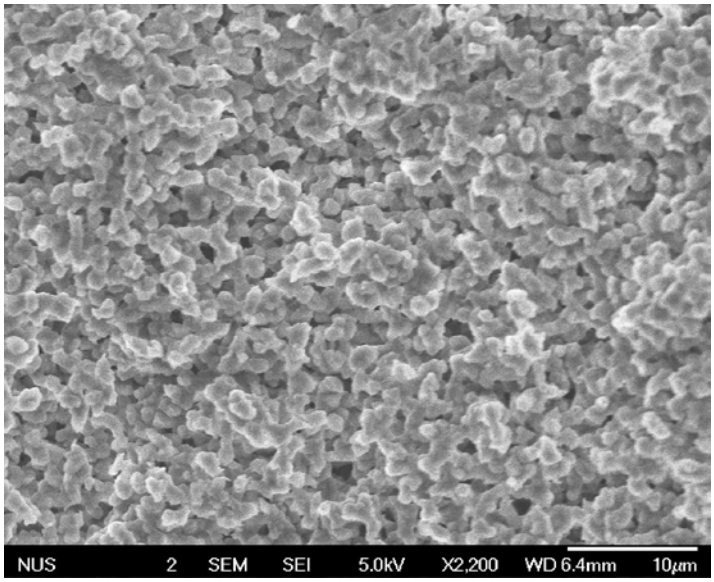
Figure 7-2 demonstrated the TEM images of the prepared monolith and the existence of its ordered structure in mesopores. When the TEM images of the 50% compressed silica monolith was compared to the non compressed monolith, it was found that a more ordered mesopore structure existed on the compression one. This could be attributed to the directed arrangement of pores due to the solvent being squeezed out of the pores leaving a more ordered structure of mesopores as compared to the scattered arranged mesopores in of the non compressed one.

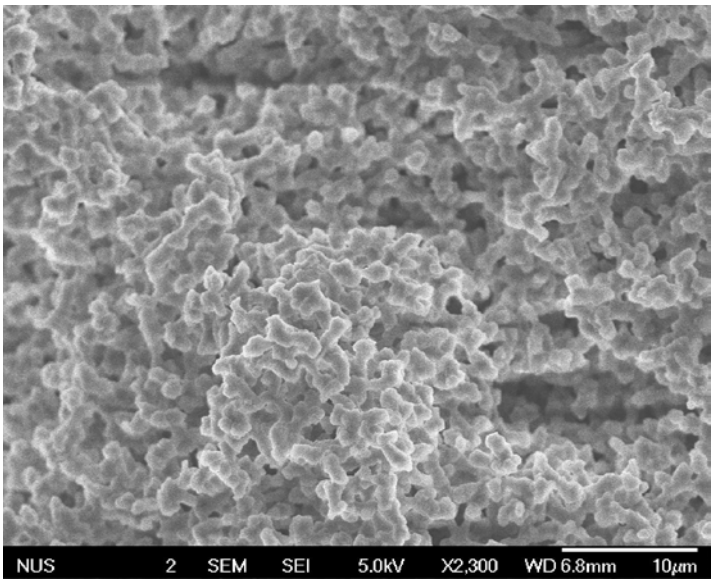
Table 7-1 demonstrated the effect of variable extent of compression on the surface area and pore diameter of the prepared monoliths. The nitrogen adsorption-desorption isotherm of the prepared silica monoliths synthesized with variable compression showed the type IV isotherms with H2 type hysteresis loops, as mentioned in Chapter 2. The obtained isotherm was characteristic for mesoporous material which suggested that the mesoporosity of the silica monolith remained intact after compression. From the nitrogen adsorption study, it was found that after compression to 25% and 75%, silica monolith showed higher surface area but smaller pore diameter as compared to the non compressed silica monolith. This finding was further confirmed by the SEM images.



0% Compressed

50% Compressed





75% Compressed

Fig. 7-1. SEM images of the prepared silica monoliths at various compression length.

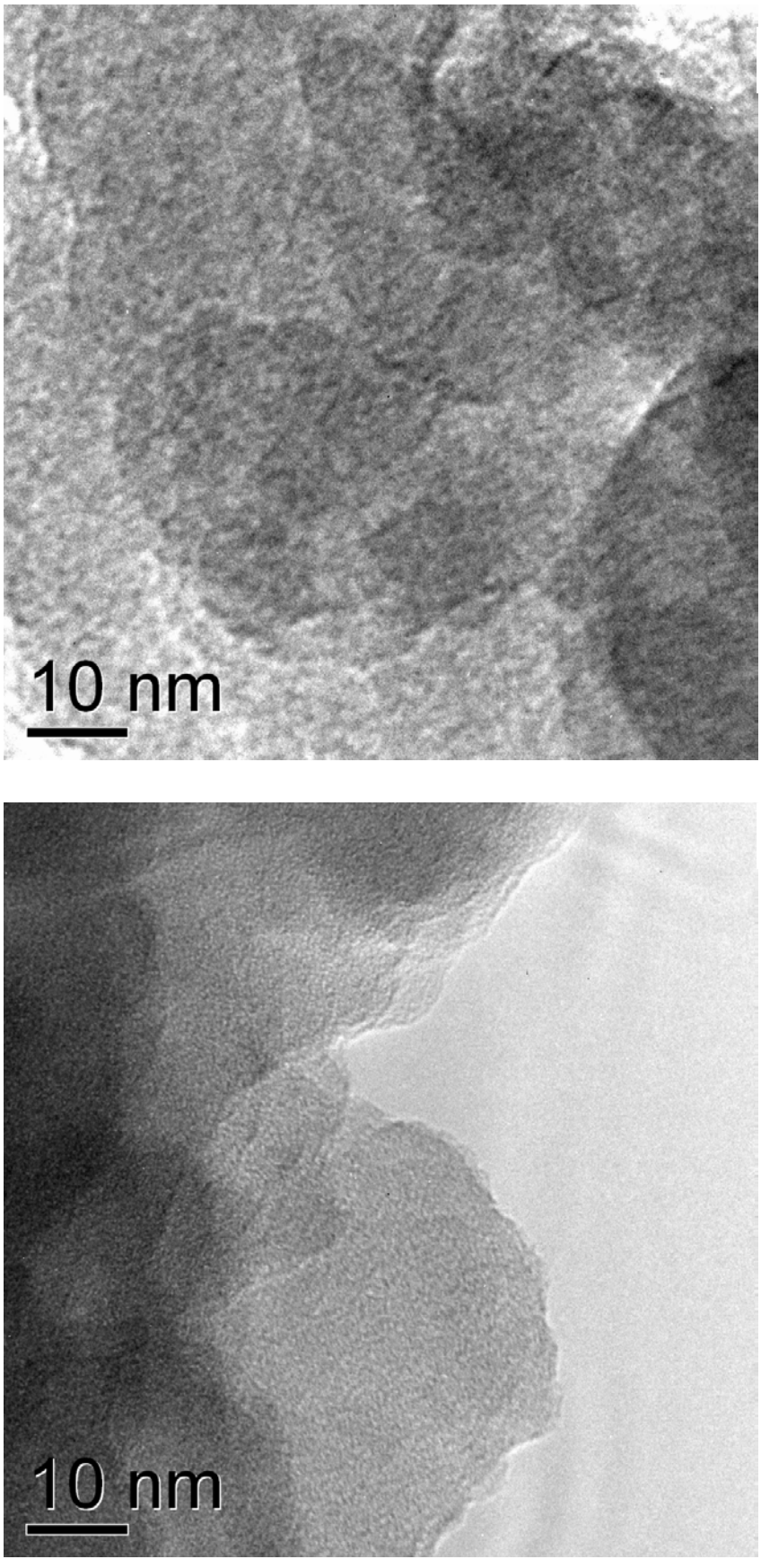


Fig. 7-2. TEM images of silica monolith. (A) 0% compressed; (B) 50% compressed

Table 7-1. Surface characteristics of the prepared silica monoliths.

% compressed	Surface area (m^2/g)	Pore diameter (nm)
0	473.6	9.2
25	579.2	6.3
75	741.2	3.7

Figure 7-3 demonstrates the effect of compression on the permeability of the silica monolith. The back pressure measurement was taken as the benchmark to determine the permeability. The figure shows the increase in the back pressure when compressed from 0% to 75%. With increasing compression strength, the monolithic structure became denser and thus the overall permeability decreased.

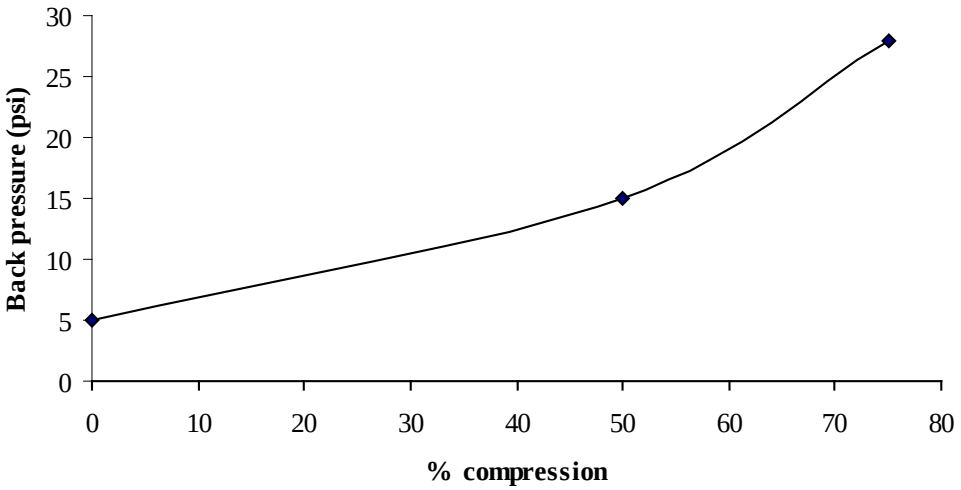


Fig. 7-3. Effect of compression on the permeability of the silica monolith.

7.4.2 Adsorption capacity

The prepared silica monoliths were tested for their adsorption capacity using morphine, codeine and cocaine as the model analytes. The adsorption capacity of the compressed monolith was compared with that of the non compressed silica monolith. It was established (based on experimental results) that compressed monolith possessed high surface, thus it was believed that the capacity of the monolith will be higher as compared to non compressed monolith. In order to verify the proof of

concept, the cartridge was tested at three different concentrations. Table 7-2 showed the adsorption capacity of the cartridge at three concentrations. The concentrations of the analytes in spiked samples were determined using the LC-MS/MS analysis based on the method described in chapter 5. With setting the initial spiked sample to be 100%, as a representative of the concentration, the cartridge passed concentration was determined. It was found that none of the analytes was detected at all three concentrations indicating the high adsorption capacities of the cartridge. However, no difference was detected between the compressed and non compressed monolith. This could be due to the higher capacity of the monoliths and the concentration chosen were not taken to threshold where an actual difference could be visible.

Table 7-2. Adsorption capacity of the prepared silica monoliths.

Concentration (μg/mL)	**Before CP**	**After CP (0% compressed)**	**After CP (50% compressed)**
1	100%	n.d	n.d
5	100%	n.d	n.d
10	100%	n.d	n.d

n.d.- not detected; CP-cartridge passed

7.5 CONCLUSION

Approach incorporating compression in the sol-gel process produced monolith with high surface area and ordered mesopores. The technique could be effectively useful in generating monoliths without destroying the mesoporous nature of the material. Although the permeability of the monolith was compromised, the developed material is expected to be more effective than the non compressed monolith and particle packed cartridges owing to its high surface area. At the same time the structure was preserved after compressing to various lengths. The monolithic cracks generated during the conventional process could be overcome with the more compact structure of the compressed monolith. The material with high surface area could have a wide range of potential applications such as catalysis and separation. In addition to

these, the presence of smaller pores in the compressed monolith as compared to the non compressed monolith could make it useful in entrapping various other molecules like proteins, etc in the monolithic template.

CHAPTER 8

Conclusions and Future Directions

Silica monolithic cartridges have been developed and used for solid phase extraction. In 2004, a solid-phase extraction (SPE) tool with pipette-tip shape was fabricated for purification of bio-samples of various characteristics, utilizing monolithic silica gel as medium [Miyazaki et al., 2004]. The monolithic silica surface was modified with a C-18 phase or coated with titania phase. A C-18-bonded tip and a non-modified tip were used for sample concentration, desaltination and removal of detergents from sample. A titania-coated tip was also applied for purification and concentration of phosphorylated peptides. This novel pre-treatment method using monolithic silica extraction tip is much effective and suitable for protein analysis. Thereafter, various types of pipette tips packed with functionalized monolithic silica become commercially available. They exhibit reversed-phase, normal-phase, or ion-exchange adsorption capacity. Examples of these pipette tips include MonoTip, MonoTip mini, MonoSpin from GL Sciences (Torrance, CA, USA), and OMIX® products from Varian (Palo Alto, CA, USA). The packing materials of OMIX® products are manufactured by a different synthetic procedure, and they have a three-dimensional network skeleton structure made of glass fibre, to which silica gel is bound at high temperature. The above mentioned solid phase extraction cartridges are made of surface modified silica monoliths. Surprisingly, there is little information on the use unmodified silica monolith for solid phase extraction purposes. This thesis is the first report on the use of unmodified silica monolith as a cartridge for solid phase extraction of hydrophilic compounds from biological matrices particularly from urine samples. It has always been a challenge to recover hydrophilic compounds from urine samples.

In this thesis, silica monolith was explored as a solid phase extraction tool, and an attempt was made to further enhance the surface properties of the prepared monolith. Basically the studies described in the thesis were divided into three main areas:

1. **Application of the prepared silica monolith was realized as a sample preparation tool for extracting analytes from urine.**

The prepared and characterized silica monolith was tested for extracting catecholamines (epinephrine, norepinephrine), metanephrine, ketamine and opiates. The classes of the test analytes represented compounds of different physicochemical properties. The success in extracting these analytes with the silica monolith indicated the versatility in application of the prepared monolith. In our cases, catecholamines and metanephrine represented compounds of highly polar group where as ketamine and opiates represented compounds in the mid polar range. The testing the silica monolith using different model analytes were described in the respective chapters. Each chapter presented a progression in approach from the previous one and a constant effort to further refine the process. The preliminary testing (Chapter 3) started with the extraction of catecholamines and metanephrine having high pKa (>11) which are known for their hydrophilicity. 2 cm cartridge was used for extraction taking urine as a biomatrix. The recoveries ranged from 59-105% for the three analytes. The monolith was proved to be effective and the findings were interesting. This led us to explore the potential of silica monolith to prove its diversity in application and the result was presented in Chapter 4. In that chapter, the batch to batch variation in properties of the prepared silica monolith was also investigated. Moreover, the effectiveness of miniaturization was realized and the cartridge length was reduced from 2 cm to 0.5 cm. The analyte was extracted from urine and showed

recovery around 100%. Thus, a more extensive study was required to further demonstrate their effectiveness as solid phase extraction (Chapter 5). This led us to compare their performance with the commercial Oasis HLB in generating clean extracts. Opiates were used as model analytes. The findings showed that the recoveries of the opiates after extraction were around 100%. A full scan LC-MS and GC X QTOF analysis was carried out to demonstrate the matrix effect and the results were compared with the extracts from Oasis HLB. The results demonstrated the efficiency of the cartridge in effectively eliminating the matrix ions. These studies demonstrated the successful application of the unmodified silica monolith for solid phase extraction.

2. Application of silica monolith in desalination.

The mechanism behind the success of silica monolith as SPE was ionic interaction with high surface area. This motivated us to realize the potential of silica monolith for desalination. This study can provide an alternative in advance techniques available for desalination in an economic and environment friendly way.

3. Attempt to improve the surface characteristic of the silica monolith, especially surface area and pore structures.

Compression was selected as a mode to achieve a high surface area and ordered pore structure. The characterization parameters demonstrated the increase in surface characteristics when compared to non compressed monolith. The material structure was preserved after compression and a more compact structure was generated unlike the non compressed monolith. The monolith with high surface area could have a wide range of potential applications such as catalysis and separation.

FUTURE DIRECTIONS

As this was the first time to investigate the application of unmodified silica monolith for solid phase extraction, the content covered in this thesis may not be comprehensive to demonstrate the full potential of silica monolith. The study can be progressed further taking into the following aspects.

1. No attempt was made to modify the surface of silica monolith. With the establishment of the unmodified monolith success, the performance comparison of the modified monolith could help to understand the characteristic of the prepared material. The surface modification utilizing silanol chemistry could be useful in linking various active moieties over its surface which could further diversify their potential. The modified silica monolith could be useful for extracting different classes of compounds with diverse physico-chemical properties.
2. In this study, the silica monolithic disc was inserted into the syringe barrel. Certainly, the fabrication process could be further refined, and the size of the silica monolithic disc could be further reduced. We have succeeded in reducing the size of the silica monolithic disc from 2 cm to 0.5 cm with no compromise in the performance of the cartridge. We have not tested the unmodified silica monolithic cartridges in even smaller sizes than these. We could fabricate the cartridges in the pipette-tip shape or 96-well plate for throughput sample processing [Xu et al., 2007].
3. Our developed unmodified silica monolithic cartridge is very effective in reducing the matrix ions in samples. In this regard, the unmodified silica monolith could be fabricated as cartridge for online cleaning of samples before they enter the mass spectrometer for analysis.

4. Silica monolith for desalination could be extensively explored to know whether the life expectancy of RO membrane can be increased using them as pre-treatment tool. Cartridge regeneration is another issue as currently we have used diluted formic acid to regenerate. The use of temperature can eliminate the acid usage and make it more environmental friendly. A more extensive study to demonstrate its full potential need to be investigated.
5. Surface area enhancement via compression technique can be explored extensively to have a better understanding of its potential and applications. Higher concentrations of the analyte could be tested with the compressed silica monolith to demonstrate to the limit of its extraction capacity.
6. Other techniques of silica monolith preparation can be taken into consideration in order to have a correlation of the current method with other methods.

In summary, the solid phase extraction method is widely used for extraction of target compounds in biological specimens before instrumental analysis. Currently, numerous new matrices for solid phase extraction are being developed. However, in this thesis, the unmodified silica monolith is found to be effective in extracting the hydrophilic analytes from biological samples. We do not eliminate the possibility that some other modified silica monolith could work as effective as or even better than the unmodified ones. It is certain that the silica monolithic materials will emerge as an important medium for sample processing and separation in the field of analysis.

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