polyethylene oxide (PEO) acting as a porogen to form the macropores and micropores in the silica gel, and a catalyst, which can be an acid catalyst (such as acetic acid or nitric acid) [13] or a base catalyst (such as N-methylimidazole or dimethylaminopyridine) [14] or a binary catalyst, acid and base in sequence[15]. The hydrolysis of the alkoxysilane precursor (or its alkyl/aryl derivative) produces the silanol groups (Si-OH) [16]. This is followed by water or alcohol condensation to produce polycondensed species containing siloxane linkages (-Si-O-Si-) between two silane molecules, forming a three-dimensional network of sol-gel polymer [17]. The mixture is commonly stirred at 0 °C for 30 min and then the homogeneous solution is poured into a mould and allowed to react at an elevated temperature [18]. A three-dimensional network will be created when the hydrolysis and polycondensation reactions are completed [8]. The resulting monolithic silica is treated with a basic environment, produced by thermal decomposition of urea or ammonium hydroxide solution at elevated temperature to tailor the mesopore structure. Figure 2 shows the SEM micrograph of the silica-based monolith showing the high porosity of the monolith. Silica-based monoliths consist of relatively large through-pores, which are important for fast flow and can provide high column efficiency, and small-sized skeletons, containing the mesopores which give high surface area [19].
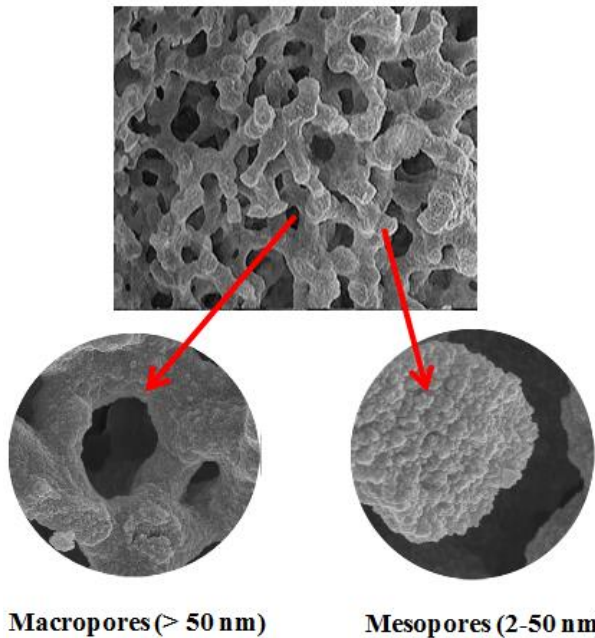

Figure 2: SEM micrograph of the porous structure of a silica-based monolith [20]

B. Modification the surface of the monoliths

The surface of the silica monolith can be easily derivatised with many functional moieties leading to additional efficiency and selectivity [21]. Modification of the surface of the silica-based monolith has been carried out by bonding the silica surface with another chemical species in order to get the desired selectivity for HPLC column or SPE sorbent. The most common reaction for derivatisation of the silica surface with chemical species is organosilanisation, which is based on using a derivatisation reagent [22]. The modification of the silica surface commonly occurs in the mesopores (2-50 nm) since the mesopores are more accessible for the derivatisation reagent as well as for the analytes. In contrast, micropores (>2 nm) are inaccessible and the silanol groups in these pores cannot be modified because they are blocked by the bonded moieties in the mesopores, [21] as can be seen in Figure 3.
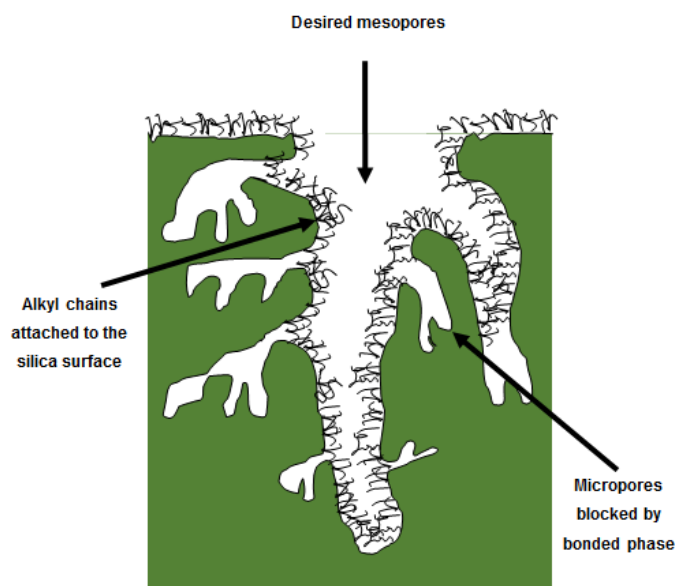

Figure 3 : Schematic diagram shows the porous silica-based surface, showing the inaccessibility of some micropores [21]

Some of the silanol groups in the mesopores stay without modification. If the attached organic moieties are small groups, their movement is restricted and the neighbouring silanol groups are not blocked, while if the attached organic moieties are large, such as octadecyl groups, then their movement blocks the neighbouring silanol groups and prevents other organic moieies from reaching these silanol groups thus having a number of free silanol groups [21]. These free silanol groups have a negative effect on separation and extraction because they can cause a second ion-exchange interaction between the free silanol groups and the analytes. In chromatography, this interaction can cause tailing peaks and decrease the resolution of separation, while the effect of the free silanol groups in SPE is that they can retain the polar functional groups and make elution more difficult [23].

In order to decrease the effect of the secondary interaction, the number of free silanol groups needs to be

decreased. This can be performed by using a second "end capping" reaction, which involves bonding smaller silane-type molecules such as trimethylchlorosilane (TMCS), or hexamethyldisilazane (HMDS) between the larger bonded moieties [24].

Conditioning of the modified silica surface before loading the sample solution is important for many reasons. The most common bonded material is octadecylated silica sorbent, and before conditioning, the sorbent (in its dry form), the bonded moieties in the octadecyl bonded phase material are completely randomly oriented on the surface of the sorbent. If the sample solution is applied, the environment surrounding the bonded moieties would be highly polar and this environment is not compatible with octadecyl groups. As can be seen in Figure 4 (A), if the octadecyl bonded phase sorbent is not conditioned, the alkyl groups will be aggregated between bonded groups in order to minimise exposure to the polar medium by forming clusters between them, and this can cause a decrease in the efficiency of the sorbent and decrease the extraction recovery of the target analytes. To solve this problem, the organic groups need to be conditioned with an organic solvent such as methanol. In this situation, the octadecyl bonded phase will be less aggregated, and ready for the interaction with the sample, Figure 4 (B). The ideal situation happens when octadecyl bonded silica sorbent is treated with a solvent less polar than methanol such as acetonitrile or tetrahydrofuran, Figure 4 (C). In this case, the octadecyl bonded phase will be more opened and fully condititioned. Therefore, the selection of the conditioning solvent is important, which depends on the type of the bonded organic moiety [21, 25, 26].
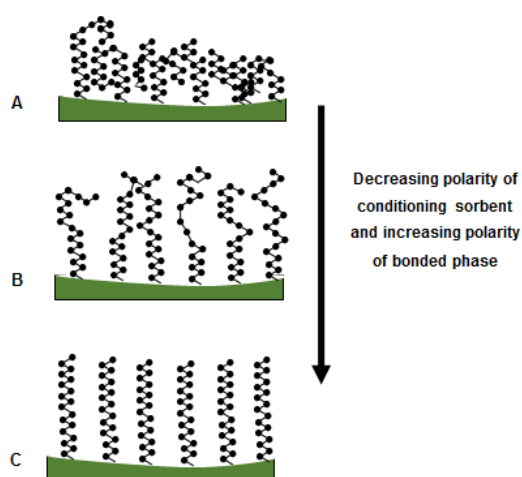
Figure 4 : Schematic diagram showing the effect of conditioning step on octadecyl bonded silica: (A) without conditioning, (B) partially conditioned, and (C) fully conditioned [21].

C. Advantages and disadvantages of the silica-based monoliths

Porous inorganic silica-based monolithic materials can overcome the drawbacks of polymer-based monoliths since they have high mechanical strength, relatively high thermal stability, and high porosity [4]. Moreover, they are not affected by organic solvents in the same way as polymer-based monoliths, which can shrink or swell in the presence of organic solvents [3, 27]. Silica-based monoliths contain a distribution of both macropores that can increase the liquid flow through the monolith without increasing the backpressure, and mesopores that can increase the surface area giving a good interaction with analytes and maximising the loadability of the sorbent [28, 29]. In addition, the surface of a silica-based monolith contains silanol groups (Si-OH) that can be used for immobilisation of different functional groups [5], and the attachment of groups on the silica surface is easier than on an organic polymer support since it has a high number of crosslinking bonds which require hours to reach equilibrium for surface activation [30].

However, there are some disadvantages of silica-based monoliths; for example, the monolithic silica columns should be prepared and chemically modified independently, which means preparation of silica-based monoliths is time consuming and difficult to control, leading to poor reproducibility. Moreover, the monolithic silica rods need to be wrapped in column materials, such as PEEK, when they are prepared in a mould [31]. Additionally, fabrication of a sol-gel monolith directly inside a microchip is difficult because the location of the monolith inside the extraction chamber cannot be defined since the preparation of the silica-based monolith depends on using thermal initiation [32]. Another negative aspect of the use of a sol-gel monolith in microchips is the shrinkage that happens as the monolith is formed during the condensation reaction, which can cause voids between the silica network and the microchip wall, resulting in a reduced surface area for sample adsorption and can cause the silica monolith to crack [13]. The main problem with using silica-based monoliths is that silica is not stable at high pH values [33].

D. Applications

Synthetic silica-based monolithic materials have been introduced as porous monolithic separation media in HPLC, GC, and CEC [15, 34]. In addition, they have been used as immobilised enzymatic reactors [35] and as sorbents in solid phase extraction [36]. Although silica-based monoliths are becoming increasingly popular as sorbents, there are few papers describing their use as

materials for protein extraction; therefore, the use of the silica-based monolith as a sorbent in different applications will be reviewed.

In 2002, Landers et al. [37] fabricated different silica matrices onto a microchip for use as a sorbent for DNA. The silica matrix was fabricated using silica beads (15 µm), silica-based monolith using a sol-gel process, or a combination of silica beads and monolithic silica material, which was fabricated by adding the silica beads to the sol-gel precursor mixture before the condensation reaction. Figure 5 (A) and (B) shows images of the microchamber packed with silica beads. The extraction recovery of DNA using the silica beads was as high as 57.1 %; however, the DNA extraction was highly irreproducible (standard deviation 43.1 %). Figure 5 (C) shows the silica sol-gel monolith that was fabricated using TEOS, PEO, and an aqueous solution of nitric acid. The monolithic silica material was cracked and the extraction recovery of DNA was very low (33.2 %). The best extraction recovery of DNA was achieved using the silica bead/sol-gel hybrid matrix (70.6 %), Figure 5 (D) and (E), and with acceptable reproducibility (standard deviation < 3%).
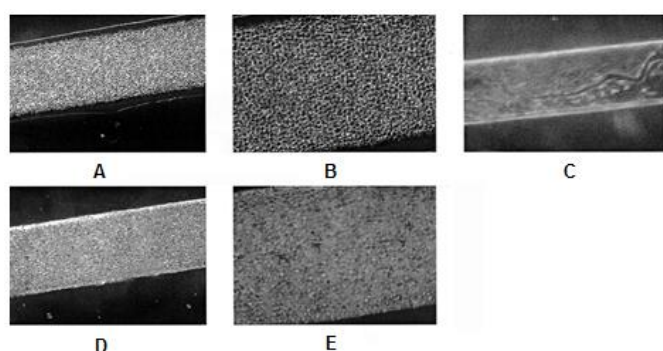
Figure 5 : Silica materials in microfabricated chambers: (A) silica beads, (B) silica beads at 10× magnification, (C) silica-based monolith using sol-gel, (D) two-step silica beads and sol-gel, and (E) two-step silica beads and sol-gel at 10× magnification [37].

Miyazaki et al. [38] in 2004 developed a monolithic silica extraction tip for the analysis of peptides and proteins. The silica-based monolith was fabricated using TEOS, PEO with average molecular mass 100,000 g mol-1 as a porogen, and an aqueous solution of nitric acid. After fabrication, the monolithic silica rod was cut and fixed inside a 200 µL pipette tip using supersonic adhesion. Following this, the silica surface was chemically modified with either an octadecyl group in order to make it hydrophobic and enrich proteins, or the silica surface was coated with a titania phase and used

for the concentration of phosphorylated peptides. The results show that the C18-bonded monolithic silica extraction tip has the ability to purify different standard proteins varying in molecular weight up to 40000 Da; however, the extraction of proteins decreased with increasing molecular weight of the proteins. In addition, the fabricated monolith was not optimised nor its porous structure characterised.

In 2006, Wu et al. [39] fabricated a glass microchip containing a TMOS-based silica-gel monolith for DNA extraction from clinical samples and from bacterial colonies. The problem of shrinkage of the inorganic monolithic material was minimised by treating the inner walls of the glass microchip with a 1 M sodium hydroxide solution (NaOH) for 12 hours to hydrolyse the walls of the glass microchip before fabrication of the monolith. This allowed the surface to participate in the condensation reaction resulting in the attachment of the silica skeleton to the glass walls. The average extraction recovery of DNA using the underivatised silica monolith was 85%. However, shrinkage of the silica monolith was not completely avoided and the monolith contained large interstitial voids. In addition, pore blockage of the monolithic material was observed during repeat extraction on a single device, as confirmed by a decrease in the extraction recovery.

In 2008, Xu and Lee [40] fabricated a hybrid organic-inorganic silica-based monolith using two monomers that were TMOS and 3-mercaptopropyltrimethoxysilane (MPTS) to yield thiol groups. This was followed by oxidation of the hybrid silica monolith using hydrogen peroxide (H2O2) for 24 hours at room temperature to derivatise mercapto groups into sulfonic acid groups for use in cation-exchange purification. The effect of the amount of PEO in the polymerisation mixture was investigated. Increasing the amount of PEO resulted in changing the final monolith from opaque to transparent, and the macropores of the monolith, which were studied by SEM observations, were decreased (Figure 6). The performance of the mercapto-modified monoliths was tested for in-tube microextraction of four anaesthetics (procaine, lidocaine, tetracaine, and bupicaine) in spiked human urine, and the extraction recovery was greater than 92%.

In 2010, Nema et al.[36] fabricated a silica-based monolith using TMOS and PEO (MW 10,000 g mol-1)

in the presence of 0.01M acetic acid as a catalyst. A monolithic silica rod was fixed in a 2 mL syringe installed in SPE vacuum manifold (Figure 7). The unmodified silica-based monolith with ionisable silanol groups was used in off-line cation exchange for preconcentration of polar analytes (epinephrine, normetanephrine, and metanephrine) from a urine sample. The technique was simple and robust. Although the extraction recovery of metanephrine was approximately 105 %, the extraction recoveries of epinephrine and normetanephrine were approximately 60 % and further optimisation is required.
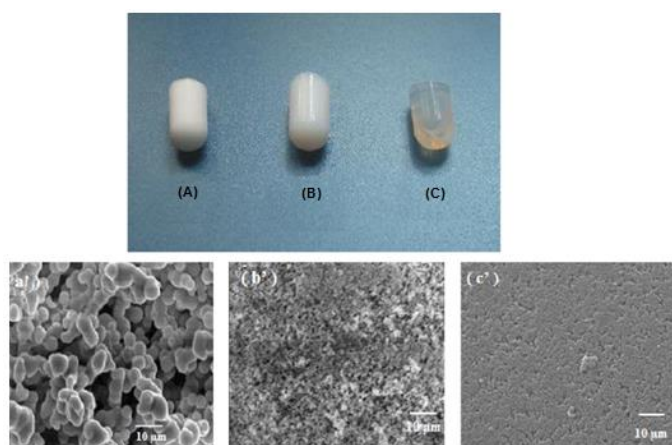
Figure 6: Photograph and SEM micrographs of silica-based monoliths prepared using different PEO content: (A) 0.1 g, (B) 0.2 g, and (C) 0.4 g [40]

E. Comparison with the organic-based monoliths

Based on the literature, each monolith has its own individual advantages and disadvantages, as indicated in the summary of previous monolith research in Table 1, which shows comparison data for organic and inorganic monoliths.
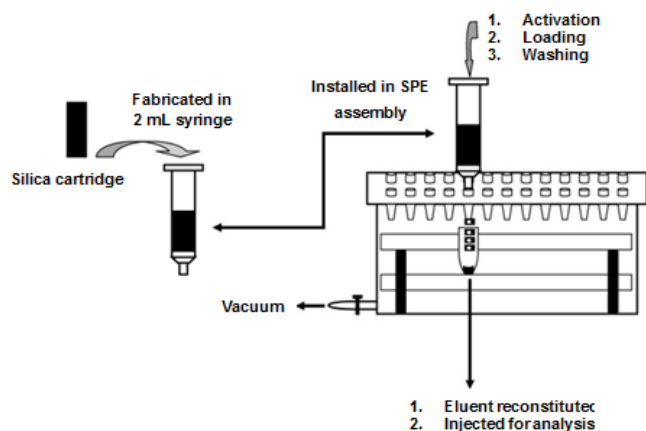
Figure 7: Extraction steps using non-modified silica monolith on SPE manifold [36].

Table 1.1 : Summary of the advantages and disadvantages of organic and inorganic monoliths based on literature review.

Comparison	Organic monoliths	Inorganic monoliths
Simple preparation method	Yes.	Yes.
Preparation time	Short, since the fabrication of the organic monolith is a single-step process.[66, 67]	Long, since they should be prepared and chemically modified independently.[55]
Option for stationary phase	They can be fabricated by using a wide range of monomers and crosslinking agents enabling the porous properties of the monolith to be controlled.[75]	No.
Surface modification of the monolith	They have a high number of crosslinking bonds, which require hours to reach equilibrium for surface activation.[120]	They can be easily derivative with many functional moieties leading to additional efficiency and selectivity.[31, 120]
Fabrication inside microchip	Easy, since the initiation of a polymerisation reaction can be performed by photo initiation (light).[69, 70]	Difficult, since the location of the monolith inside the microchip cannot be defined because their fabrication depends on using thermal initiation.[103, 121]
Surface area	Although some attempts have been made to increase the surface area of organic monoliths, the fabricated monoliths in previous reports showed relatively low surface areas.[89, 90]	High.[35, 118, 119]
Permeability	Moderate.[86, 87]	High.[35, 118, 119]
Affected by temperature and/or solvents	They are affected by temperature and/or organic solvents causing shrinking or swelling.[86, 87]	They have high mechanical strength, and relatively high thermal stability.[115]
Stable over the whole pH range	They are stable over a wide range of pH values.[282]	They are not stable at high pH values.[25]
Use for protein extraction	They have found widespread use in protein extraction in capillary and microfluidic chip formats.[65]	Few papers describing their use as materials for extraction.[49]
Protein extraction efficiency	High.[93, 94]	The ability to purify different standard proteins varying in molecular weight up to 40 kDa.[125]

III. CONCLUSION

Porous inorganic silica-based monolithic materials have many advantages such as they have high mechanical strength, relatively high thermal stability, high porosity, and they are not affected by organic solvents. In addition, the surface of a silica-based monolith contains silanol groups that can be used for immobilisation of different functional groups.

IV. REFERENCES

[1] Frantisek, S., Less common applications of monoliths: Preconcentration and solid-phase extraction. Journal of Chromatography B, 2006. 841(1–2): p. 52-64.

[2] Lion, N., et al., Why the move to microfluidics for protein analysis? Current Opinion in Biotechnology, 2004. 15(1): p. 31-37.

[3] Lin, Z., et al., Preparation and evaluation of a macroporous molecularly imprinted hybrid silica monolithic column for recognition of proteins by high performance liquid chromatography. Journal of Chromatography A, 2009. 1216(49): p. 8612-8622.

[4] Gambero, A., et al., Use of Chemically Modified Silica with β-Diketoamine Groups for Separation of α-Lactoalbumin from Bovine Milk Whey by Affinity Chromatography. Journal of Colloid and Interface Science, 1997. 185(2): p. 313-316.

[5] Cohen, S.L. and B.T. Chait, Influence of Matrix Solution Conditions on the MALDI-MS Analysis of Peptides and Proteins. Analytical Chemistry, 1996. 68(1): p. 31-37.

[6] Siouffi, A.M., Silica gel-based monoliths prepared by the sol–gel method: facts and figures. Journal of Chromatography A, 2003. 1000(1–2): p. 801-818.

[7] Fields, S.M., Silica Xerogel as a Continuous Column Support for High-Performance Liquid Chromatography. Analytical Chemistry, 1996. 68(15): p. 2709-2712.

[8] Zou, H., et al., Monolithic stationary phases for liquid chromatography and capillary electrochromatography. Journal of Chromatography A, 2002. 954(1–2): p. 5-32.

[9] Alzahrani, E., Fabrication of Monolithic Silica Microchip for Efficient Monolithic DNA Purification. International Journal of Advanced Engineering and Nano Technology, 2014. 2(13-18).

[10] 10. Minakuchi, H., et al., Octadecylsilylated Porous Silica Rods as Separation Media for Reversed-Phase Liquid Chromatography. Analytical Chemistry, 1996. 68(19): p. 3498-3501.

[11] Lee, S.H., et al., Influence of ionic liquids as additives on sol–gel immobilized lipase. Journal of Molecular Catalysis B: Enzymatic, 2007. 47(3–4): p. 129-134.

[12] Kato, M., K. Sakai-Kato, and T. Toyo'oka, Silica sol-gel monolithic materials and their use in a variety of applications. Journal of Separation Science, 2005. 28(15): p. 1893-1908.

[13] Ishizuka, N., et al., Monolithic silica columns for high-efficiency separations by high-performance liquid chromatography. Journal of Chromatography A, 2002. 960(1-2): p. 85-96.

[14] Frolova, A.M., et al., Producing of Monolithic Layers of Silica for Thin-Layer Chromatography by Sol-Gel Synthesis. The Open Surface Science Journal 2009. 1: p. 40-45.

[15] Adam, T., et al., Towards the column bed stabilization of columns in capillary electroendosmotic chromatography: Immobilization of microparticulate silica columns to a continuous bed. Journal of Chromatography A, 2000. 887(1-2): p. 327-337.

[16] Xu, L., Z. Shi, and Y. Feng, Porous monoliths: sorbents for miniaturized extraction in biological analysis. Analytical and Bioanalytical Chemistry, 2010. 399(10): p. 3345-3357.

[17] Allen, D. and Z. El Rassi, Capillary electrochromatography with monolithic silica column: I. Preparation of silica monoliths having surface-bound octadecyl moieties and their chromatographic characterization and applications to the separation of neutral and charged species. Electrophoresis 2003. 24: p. 408–420.

[18] Keeling-Tucker, T., et al., Controlling the Material Properties and Biological Activity of Lipase within Sol−Gel Derived Bioglasses via Organosilane and Polymer Doping. Chemistry of Materials, 2000. 12(12): p. 3695-3704.

[19] Siouffi, A., Silica gel-based monoliths prepared by the sol-gel method: facts and figures. Journal of Chromatography A, 2003. 1000(1-2): p. 801-818.

[20] Rieux, L., et al., Silica monolithic columns: Synthesis, characterisation and applications to the analysis of biological molecules. Journal of Separation Science, 2005. 28(14): p. 1628-1641.

[21] Simpson, N., Solid-Phase Extraction: Principles, Techniques, and Applications 2000, New York: Marcel Dekker.

[22] Sandoval, J.E. and J.J. Pesek, Hydrolytically stable bonded chromatographic phases prepared through hydrosilylation of olefins on a hydride-modified silica intermediate. Analytical Chemistry, 1991. 63(22): p. 2634-2641.

[23] Herrero-Martínez, J.M., et al., Characterization of the acidity of residual silanol groups in microparticulate and monolithic reversed-phase columns. Journal of Chromatography A, 2004. 1060(1–2): p. 135-145.

[24] Sanagi, M., et al., Preparation and Application of Octadecylsilyl-Silica Adsorbents for Chemical Analysis. Malaysian Journal of Analytical Sciences, 2001. 7: p. 337-343.

[25] Thurman, E.M. and M.S. Mills, Solid-Phase Extraction: Principles and Practice. 1998, New York: Wiley.

[26] Larrivee, M.L. and C.F. Poole, Solvation parameter model for the prediction of breakthrough volumes in solid-phase extraction with particle-loaded membranes. Analytical Chemistry, 1994. 66(1): p. 139-146.

[27] Girault, H., et al., Why the move to microfluidics for protein analysis. Current Opinion in Chemical Biology, 2004. 15(1): p. 31-37.

[28] Kelea, M. and G. Guiochon, Repeatability and reproducibility of retention data and band profiles on six batches of monolithic columns. J. Chromatogr., A, 2002. 960: p. 19–49.

[29] Hennion, M.C., Solid-phase extraction: method development, sorbents, and coupling with liquid chromatography. J. Chromatogr., A, 1999. 856: p. 3–54.

[30] Jal, P.K., S. Patel, and B.K. Mishra, Chemical modification of silica surface by immobilization of functional groups for extractive concentration of metal ions. Talanta, 2004. 62(5): p. 1005-1028.

[31] Guiochon, G., Monolithic columns in high-performance liquid chromatography. Journal of Chromatog. A, 2007. 1168: p. 101-168.

[32] Alzahrani, E. and K. Welham, Fabrication of an octadecylated silica monolith inside a glass microchip for protein enrichment. Analyst, 2012. 137(20): p. 4751-4759.

[33] Poole, C.F., New trends in solid-phase extraction. TrAC Trends in Analytical Chemistry, 2003. 22(6): p. 362-373.

[34] Luo, Q., et al., Preparation of 20-μm-i.d. Silica-Based Monolithic Columns and Their Performance for Proteomics Analyses. Analytical Chemistry, 2005. 77(15): p. 5028-5035.

[35] Ota, S., et al., High-throughput protein digestion by trypsin-immobilized monolithic silica with pipette-tip formula. Journal of Biochemical and Biophysical Methods, 2007. 70(1): p. 57-62.

[36] Nema, T., E. Chan, and P. Ho, Application of silica-based monolith as solid phase extraction cartridge for extracting polar compounds from urine. Talanta, 2010. 82(2): p. 488-494.

[37] Wolfe, K.A., et al., Toward a microchip-based solid-phase extraction method for isolation of nucleic acids. Electrophoresis 2002. 23(5): p. 727-733.

[38] Miyazaki, S., et al., Development of a monolithic silica extraction tip for the analysis of proteins. Journal of Chromatography A, 2004. 1043(1): p. 19-25.

[39] Wu, Q., et al., Microchip-Based Macroporous Silica Sol-Gel Monolith for Efficient Isolation of DNA from Clinical Samples. Anal. Chem. , 2006. 78: p. 5704-5710.

[40] Xu, L. and H.K. Lee, Preparation, characterization and analytical application of a hybrid organic–inorganic silica-based monolith. Journal of Chromatography A, 2008. 1195(1–2): p. 78-84.