CHAPTER

10 USING OF NANOMATERIALS TO ENHANCE THE SEPARATION EFFICIENCY OF MONOLITHIC COLUMNS

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1 MOTIVATION BEHIND HIGH-EFFICIENCY COLUMNS

Chromatographic methods have been the most effective techniques to separate, isolate, and purify chemicals. From crushed bricks introduced by Tswett to microchips [1], separation science has developed in varied branches, each of which has its advantages, limitations, and application fields. Many of these developments reached maturity and became standard routine techniques for the analyst. Today, chromatography is a family of dominant and widely used techniques in the analytical laboratory. The literature on chromatographic methods has exploded and almost all laboratories that deal with analytical problems have at least one or more chromatographic instruments. Chromatographic techniques especially liquid chromatography (LC) and gas chromatography (GC) systems are now being used routinely for every type of molecules from the smallest ions to the largest molecules. In chemistry and many other sciences, chromatographic techniques have been found to be powerful analytical tools in various areas such as environmental, biological, pharmaceutical, industrial, food, clinical, forensic, agricultural, organic synthesis, toxicological, and petrochemical researches [2–6].

Many science and technology fields are becoming more sophisticated and facing new and continuous challenges; hence, the necessity to analyze more complex substances is an urgent and critical issue. Furthermore, the need to reduce the analysis time and cost, to develop green analytical methods, and the necessity to enhance the sensitivity and resolution of the analysis have increased accordingly. On the other hand, such needs may increase as more and more materials and products are discovered and developed. However, the development of powerful separation techniques is mainly attached to the chemical, physical, and technical challenges of columns fabrication. Modern chromatographic developments are concerned with creating new types of columns and improving the existing columns' efficiency, reliability, and reproducibility. Great efforts have been made and a lot of useful columns and stationary phases have been reported and have become commercially available. These columns seem promising for the separation of enormous chemicals range, including biological, environmental, pharmaceutical, and other fields.

Although it is the smallest component, the analytical column is considered the heart and the most important part in any chromatographic system; it is the only device where the actual separation of the analyte mixture takes place. Stationary phase materials are the media essentially producing the

Nanomaterials in Chromatography. http://dx.doi.org/10.1016/B978-0-12-812792-6.00010-8

separation; therefore, the properties of the column packing materials are of primary importance for successful separations. The selectivity, capacity, and efficiency of the column are all affected by the nature of the packing material or the materials of construction [7]. Great varieties of different columns are provided by dozens of companies nowadays and therefore, for selecting a suitable column for a certain separation, the chromatographer should be able to decide what column type is needed for certain applications, and what are the desired characteristics of the selected column.

Generally, four distinct characteristics could be used for packing materials classification: type (nonporous, porous, monolithic, and core–shell), geometry (surface area; particle size, shape and distribution; pore size, volume and diameter; etc.), surface chemistry (type of bonded ligands, bonding density, structural stability, and surface reactivity, etc.), and type of base material (silica, polymeric, alumina, zirconia, etc.). All these parameters are interrelated in their effect on the chromatographic quality of the column: the type of base material can significantly affect the adsorbent surface structure, the porosity of the packing material affects both the bonding density and the adsorbent surface area, which are the properties of significant importance, the geometrical properties are strongly related to the column efficiency and flow resistance, while the surface chemistry is mainly responsible for the analyte retention and separation selectivity. However, the performance of the separation column is a subjective factor, which is dependent on many other variables such as the types of analytes, column dimensions (length and internal diameter), and even on the chromatographic conditions and detections used for the evaluation of the overall quality.

2 MONOLITHIC COLUMNS: PAST AND PRESENT

Chromatographic techniques have grown to be the most popular and versatile of all analytical techniques in laboratories today. One of the key and powerful advantages is that these techniques are extremely flexible and can be adapted to accomplish the analytical needs in the development processes stages [8]. Many chromatographic problems have been resolved or minimized through developments in the last few decades. Understanding modern column trends is important since column technologies continue to evolve rapidly, resulting in new products with higher performance and more consistency [9,10]. Chromatographic systems and especially columns, have seen many innovations and developments in various directions. The rapid development of high-performance columns led to the success of separation processes. However, the development of successful separation methods is closely linked to the chemical and technical challenges related with designing and preparation high-resolution columns and packing materials. Stationary phases are now available in many varieties of packings as well as chemical structures and can be functionalized for added specificity.

In comparison with the particulate stationary phases, monoliths represent a relatively new and innovative type of packing materials for chromatographic analysis. The concept of monolithic columns has been identified as an alternative to particle packed columns [11,12]. In contrast to conventional and traditional columns and stationary phases, monoliths are formed from a block of continuous single piece materials made of highly porous rods with two types of pore structures (macropores and mesopores of different sizes), giving them the most important characteristics—greater porosity and permeability—that enable them to work well at fast mobile phase flow rates without causing high backpressure and reducing mass transfer resistance [13]. In 1967, Kubin used the first monolithic material for separation [14]; several attempts were then reported in the literature since the early 1990s.

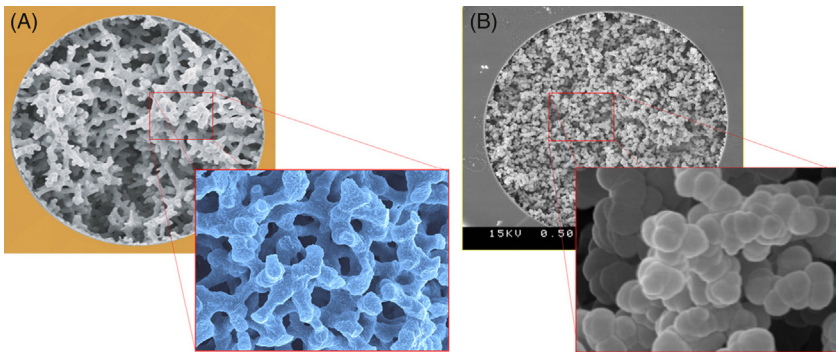

FIGURE 10.1

SEM micrographs showing difference in morphology of monolithic capillary columns prepared from silica (A) (left) and poly(butyl methacrylate-ethylene dimethacrylate) (B) (right) [27].

In summary, two basic classes of monolith supports have been developed for chromatographic applications based on the nature of the basic chemistry of the monolith structure [15]: (1) organic-based polymer monolithic columns produced by a simple molding process [11,12,16], and (2) inorganic-based silica monolithic columns made by the sol–gel approach [17]. These monolithic phases are essentially synthesized from silica or organic monomers such as styrene, acrylamide, acrylate, and methacrylate derivatives [11,18–22]. However, hybrid organic–inorganic monoliths having intermediary properties of both basic types have also been prepared using various approaches and have become increasingly popular [23–25]. Although organic and inorganic monoliths have some similar properties such as the presence of large through pores that enable high permeability to flow with low column backpressure, the structural morphologies and pore size distributions of both types are completely different as shown in Fig. 10.1 [26,27]. While the organic polymer-based monolith structure consists of interconnected nonporous microglobules with a surface area that does not exceed tens of $m^2\ g^{-1}$ inorganic silica-based monoliths are formed from mesoporous bicontinuous structure and their surface area lies in the range of hundreds of $m^2\ g^{-1}$ [27]. For this reason, organic-based monoliths showed to be outstanding for the rapid separation of large molecules such as proteins, peptides, synthetic polymers, and nucleic acids, while inorganic-based monoliths exhibit fast separations of small molecules. In addition to the variation in application and chemistry, organic and inorganic monoliths also differ in the fabrication techniques and conditions.

In general, monolithic columns have been prepared by polymerization mixtures consisting of monomers, porogenic solvent and initiator, this reaction typically leads to macroporous media with large through pores. The polymerization conditions such as time and temperature also affect the resulting structure. Several attempts have been made to overcome the inherent drawbacks of the polymer-based monoliths for the separation of small analytes. Various groups tried to change the type and even the content of the monomeric mixture including monomers, porogenic solvents, and initiators, while other groups studied the effect of the polymerization temperature and time [19,28–30], introduced a new hyper-crosslinking reaction [31], terminated the polymerization reaction at an early stage [32], used a single crosslinker [33], or incorporated nano- or microparticles into the porous monolith structure; these will all be discussed in the next sections. Most reports focused on increasing the surface area and improving the homogeneity of the monolith' structure.

Since polymer chemistry and materials science are highly rich in options, this trend will continue, and explorations will certainly lead to continues and unlimited innovations of novel materials with unexpected properties and a wide variety of potential applications, which make them attractive and carry excessive promise for the future of separation science. The manufacture of monoliths is currently still under progress, and thus will require more and more theoretical and experimental studies to improve their preparation and performance.

3 MONOLITHS CONTAINING NANOMATERIALS

Nanomaterials or nanoparticles are a class of substances of particles with 1–100 nm size or feature in at least one of their dimensions. Nanomaterials have attracted great attention in various fields, such as materials, chemistry, physics, environment, electronics, medicine, and optics, due to their exceptional chemical and physical properties [34–40]. The intrinsic and unique properties of nanomaterials are mostly because of their high surface atom fraction, which means more active atoms are available to interact with other materials in comparison to microscale or bulk materials [41].

The implementation of nanomaterials in separation science started with the frenetic evolution of nanotechnology although they were suggested to have been used in analytical chemistry since 1982 [42]. The structural and surface characteristics of nanomaterials combined with their large surface-to-volume ratios, high adsorption capacity, easy functionalization, and ability to undergo various noncovalent forces, such as hydrogen bonding, π–π interaction, dispersion forces, and hydrophobic interactions, have provided many interests in analytical chemistry applications. Nowadays, using nanomaterials in chromatography and separation science is growing rapidly, and as expected, they also found their way into the monolithic arena. If monolithic column properties such as their high porosity, permeability, and diverse functionality are combined with the amazing properties of nanomaterials such as their high specific surface area, it is possible to obtain hybrid columns possessing very powerful and selective characteristics, which will provide good opportunities to improve and develop not only separation science but also most other analytical chemistry branches. Therefore, porous polymer monoliths containing nanomaterials have recently gained much research importance.

According to the literature, there are two basic approaches that have been developed to functionalize the monolithic structure with the nanomaterials. In general, nanoparticles can be introduced into monoliths by mixing with the monomeric mixture before polymerization, or by linking onto the pore surface of the monolithic structure. In the first approach, nanomaterials such as carbon nanotubes, gold nanoparticles, and silica nanoparticles were incorporated into the typical monomeric mixture consisting of a monomer, crosslinker, porogenic solvents, and initiator. The monomeric mixture was then mixed into a homogenous solution, the uniform mixture was then filled into an empty and previously activated column in the case of fused silica tubings, and the polymerization took place by heating or some other polymerization process. In this relatively simple protocol, selection of the porogenic solvents is very important to disperse the nanoparticles and maintain a stable and uniform stationary phase matrix inside the column.

The nanoparticles in the second protocol were chemically attached to the monolith structure using specific interaction processes. In this approach, the nanomaterials may be linked to the monomeric mixture either before or after the polymerization reaction; the nanoparticles could be attached to the monomer before polymerization through covalent bonds, electrostatic interactions, or hydrogen bonds. The mixture was then filled into the empty conditioned column for polymerization. By another method,

the monolithic column could be regularly prepared with an unmodified monomer and crosslinker. The nanomaterials are then dispersed in a suitable solvent and pumped through the column to attach with the monolith structure.

Various types of nanoparticles were used to enhance the selectivity and separation efficiency of the monolithic columns such as polymeric latex nanoparticles, carbonaceous nanomaterials, metallic nanoparticles, and silica nanoparticles. This chapter covers the latest advances and applications of nanoparticle-based monoliths and focuses on the carbonaceous nanomaterials as efficient and promising stationary phases for various chromatographic separation modes. A variety of scenarios for introducing nanoparticles into different monolithic structures are described in the next two sections.

4 CARBONACEOUS NANOMATERIAL-BASED MONOLITHS

Since the first introduction of the zero-dimensional (0D) fullerene C60 in 1985 by Smalley' group [43], and later the one-dimensional (1D) carbon nanotube (CNT) [44] and the two-dimensional (2D) graphene [45], the analytical applications of carbon-based nanomaterials began and gained significant impacts in various applications. The power of carbon nanomaterials is mostly used to enhance the performance of the polymer monolithic columns for the separation of small molecules. Monoliths containing carbonaceous nanomaterials are mainly focused on CNTs, graphene, graphene oxides, and fullerenes.

4.1 CARBON NANOTUBES

The first carbon-based nanomaterial incorporated into the polymer monolith was the single-walled carbon nanotubes (SWNTs) in 2005 [46]. In this initial effort, SWNTs were incorporated into an organic polymer monolith containing vinylbenzyl chloride and ethylene dimethacrylate for use in micro-high-performance liquid chromatography and capillary electrochromatography. To prepare a homogenous separation column, SWNTs should be well dispersed in the monomeric mixture before polymerization. For this reason, an acid oxidative method was developed; SWNTs were treated with a mixture of two aqueous acids solution consisted of 98% H_2SO_4 and 30% H_2O_2 (9:1) to increase their solubility through the introduction of hydroxyl moieties on their surface. The treated SWNT were then dispersed in 2-propanol and used as a porogen. This treatment exhibited a stable suspension matrix with no precipitation observed after 2 weeks as shown in Fig. 10.2A. Because of the hydrophobic characteristics of SWNT, a monolithic column incorporated with SWNT enhanced the chromatographic retention of small neutral molecules in reversed-phase LC. Fig. 10.2B shows a comparison of four neutral compound (uracil, phenol, *N*,*N*-diethyl-*m*-toluamide, and toluene) separations using a poly(vinylbenzyl chloride-ethylene dimethacrylate) control monolithic column at 0.50 μL min^{-1} flow rate and a treated poly(vinylbenzyl chloride-ethylene dimethacrylate-SWNT) monolithic column at a flow rate of 0.40 μL min^{-1} under isocratic elution conditions with 50% aqueous acetonitrile + 0.1% (v/v) TFA as the mobile phase. The results revealed that the incorporation of SWNTs into the monolithic stationary phase improved peak efficiency and influenced chromatographic retention.

In one of the most important work in this field, multiwalled carbon nanotubes (MWNTs) were entrapped into the monolithic polymer prepared from glycidyl methacrylate as monomer and ethylene dimethacrylate as crosslinker to afford stationary phases with enhanced chromatographic performance of small molecules [47]. MWNTs were incorporated in the monolith using two approaches. The first

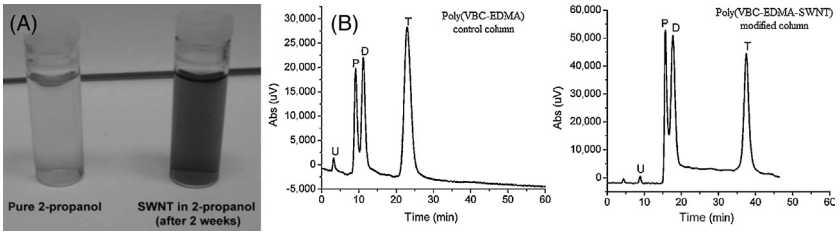

FIGURE 10.2

(A) SWNT suspension in 2-propanol after H_2SO_4 and H_2O_2 pretreatment. (B) Chromatograms of a reversed-phase test mixture separated on poly(vinylbenzyl chloride-ethylene dimethacrylate) control monolith and poly(vinylbenzyl chloride-ethylene dimethacrylate-SWNT) monolith under isocratic elution (50% aqueous/acetonitrile + 0.1% (v/v) TFA) at flow rates of 0.50 and 0.40 μL min^{-1}, respectively. Conditions: column dimensions, 0.075 mm i.d. × 400 mm; room temperature; 214 nm detection wavelength. Peaks: (*U*) uracil, (*P*) phenol, (*D*) *N*,*N*-diethyl-*m*-toluamide, (*T*) toluene.

Reprinted with permission from [46] Y. Li, Y. Chen, R. Xiang, D. Ciuparu, L.D. Pfefferle, C. Horwath, J.A. Wilkins, Incorporation of single-wall carbon nanotubes into an organic polymer monolithic stationary phase for μ-HPLC and capillary electrochromatography, Anal. Chem. 77 (2005) 1398–1406.

approach involved direct addition of MWNTs in the polymerization mixture, followed by polymerization. 1-Dodecanol and cyclohexanol were selected as porogenic solvents, which led to maintaining a homogeneous mixture as the MWNT remain dispersed for several days. While the control column (with no MWNTs) exhibited an efficiency of only 1800 plates/m for benzene, the addition of only 0.25 wt% MWNTs (with respect to the monomers) to the polymerization mixture increased the efficiency to over 15,400 and 35,000 plates m^{-1} at 1.00 and 0.15 μL min^{-1} mobile phase flow rates, respectively, as demonstrated by the separation of alkylbenzenes shown in Fig. 10.3.

In the alternative approach, poly(glycidyl methacrylate-ethylene dimethacrylate) monolith surface was functionalized with ammonia, and then shortened CNTs, bearing carboxyl functionalities, were attached to the pore surface through the aid of electrostatic interactions with the amine functionalities. Reducing the pore size of the monolith enhanced the column efficiency for the retained benzene to 30,000 plates m^{-1} at a flow rate of 0.25 μL min^{-1}. The two fabrication approaches demonstrated that the addition of MWNTs significantly increased both retention time of the analytes and column efficiency of the prepared columns.

A similar trend was also observed by Aqel et al. for monolithic columns prepared in capillaries from benzyl methacrylate and ethylene dimethacrylate after the addition of very small amounts of MWNTs [48]. In this work, the porogen was optimized to obtain a stable and homogeneous suspension inside the capillary columns, which was a mixture of three solvents composed of cyclohexanol, 1,4-butandiol, and butanol. By using this ternary mixture, the MWNTs were well dispersed and the suspension was homogenous and stable, and no precipitation was observed for about 2 h after mixing, as can be seen in Fig. 10.4A. Since the precipitation and also segregation of the MWNTs from the polymerizing mixture is very slow in comparison with the polymerization process, the MWNTs do not have enough time to precipitate and to separate from the monolith phase. In contrast to other related works, the prepared capillary columns were tested for the separation of relatively polar mixtures consisting of ketones and phenols as demonstrated in Fig. 10.4B and C. The strongly hydrophobic character of MWNTs again

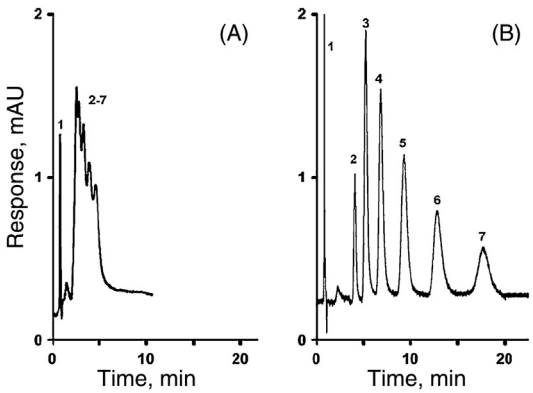

FIGURE 10.3

Separation of uracil and alkylbenzenes using a monolithic poly(glycidyl methacrylate-ethylene dimethacrylate) capillary column (A) and its counterpart containing 0.25 wt% entrapped MWNT (B), both columns were prepared at 55 °C. Conditions: column dimensions, 180 mm × 100 μm i.d.; mobile phase, 45% acetonitrile, 5% THF, 50% water; flow rate, 1.0 μL min^{-1}; back pressure, 16 MPa; 254 nm detection wavelength. Peaks: (1) uracil, (2) benzene, (3) toluene, (4) ethylbenzene, (5) propylbenzene, (6) butylbenzene, and (7) amylbenzene [47].

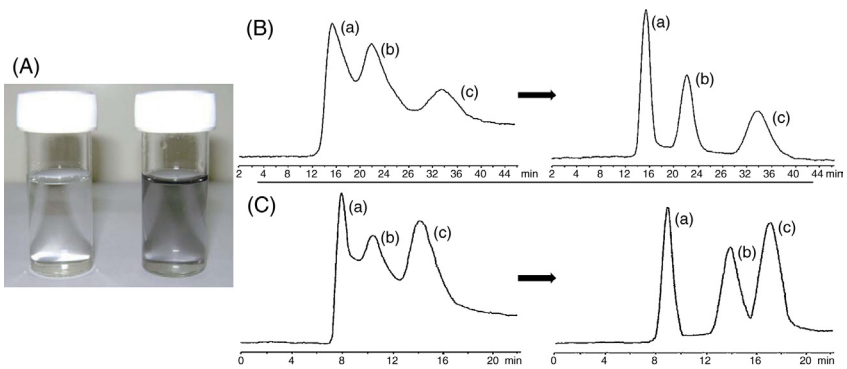

FIGURE 10.4

(A) Porogenic mixture (cyclohexanol, 1,4-butandiol, and butanol, 20/40/40% v/v/v), (left) without MWNT, (right) with incorporated 1.0 mg mL^{-1} MWNT. (B) Chromatograms on control column (left) and incorporated MWNT column (right) of ketones with a binary acetonitrile/water (50:50, v/v) with 1.0% FA mobile phase at 1.0 μL min^{-1}, where (a) acetone, (b) acetophenone, and (c) butyrophenone. (C) Chromatograms on control column (left) and incorporated MWNTs column (right) of phenols using acetonitrile/water (50:50, v/v) with 1.0% FA mobile phase at 1.5 μL min^{-1}, where (a) aminophenol, (b) nitrophenol, and (c) chlorophenol.

Reprinted with permission from A. Aqel, K. Yusuf, Z.A. Al-Othman, A.Y. Badjah-Hadj-Ahmed, A.A. Alwarthan, Effect of multi-walled carbon nanotubes incorporation into benzyl methacrylate monolithic columns in capillary liquid chromatography, Analyst 137 (2012) 4309–4317.

played an important role in the retention behavior of the modified column. The results showed that the incorporation of MWNTs slightly affected the retention while it enhanced the column efficiency by increasing the column efficiency by a factor of up to 9. This effect corresponded to an improved resolution and full separation of the solutes.

In an advanced study, Mayadunne and El-Rassi prepared hybrid CNT-monolith columns in two approaches [49]. First, an optimized amount of CNTs were incorporated into octadecyl monolithic columns and prepared at a larger scale in a stainless-steel tubing of 4.6 mm internal diameter and 10 cm length. As a result of this incorporation, the column performance improved in terms of pressure and also showed improved separation of seven proteins: ribonuclease A, cytochrome *c*, bovine serum albumin, ovalbumin, β-lactoglobulin A, lysozyme, and transferrin. This work also revealed that the optimum incorporated amount of MWNTs depends on the physical characteristics of the CNTs such as nanotube outer diameter. The larger outer diameter nanotubes provided sharper peaks for proteins. In the second approach, a neutral monolith was coated with CNTs for the separation of a wide range of small and large solutes including some chiral compounds. An inert and relatively hydrophilic monolith (glyceryl monomethacrylate and ethylene glycoldimethacrylate) was selected for this task, which proved to be the most suitable support enhancing the homogeneity of the hydroxyl functionalized nanotubes. In this case, the monolith serves as an ideal support to prepare a real CNT stationary phase.

Poly(glycidyl methacrylate-ethylene glycol dimethacrylate) was incorporated with carboxylated SWNTs and successfully applied for the separation of ten chiral drugs in capillary electrochromatography [50]. To acquire a homogeneous solution, SWNTs was satisfactorily dispersed in 1-propanol after it was acidified by a H_2SO_4:HNO_3 (3:1) mixture to prepare the carboxylated SWNTs. As a chiral selector, pepsin was bonded to the carboxylated SWNT-incorporated monoliths via epoxide groups as reactive sites and glutaraldehyde as the spacer. The results suggested that the carboxylated SWNTs played a significant role in improving the separation efficiency, although pepsin was the dominant element in determining the chiral recognition ability of the monolith. Fig. 10.5 proves the influence of carboxylated SWNTs and pepsin on the enantioseparation of the (±)-nefopam drug on the polymer monoliths.

The main goal of adding CNTs into the monoliths in all mentioned works was to enhance the retentive property of the monolith by providing more interaction sites since the graphite sheets rolled up tubes, CNTs are carbon allotropes with cylindrical nanostructures, are hydrophobic in nature [51,52], and also would establish π–π interactions with aromatic and π-bond rich solutes. Fig. 10.6A shows the effect of the incorporated CNT amounts on the retention factors of alkylbenzenes [49]. As can be seen in this figure, by increasing the amount of nanotubes from 1.0 to 12.5 mg, and using isocratic elution at 1.0 mL min^{-1} with a mobile phase at 35% v/v acetonitrile in water, the retention factor values increased by about 20% for all alkylbenzenes. Furthermore, most studies suggested that the incorporated nanotubes cross the through pores of the monolith structure [47,48], as can be clearly seen by scanning ion conductance micrograph of the internal monolith structure shown in Fig. 10.6B. On the other hand, the specific surface area of the incorporated monoliths determined by the Brunauer–Emmett–Teller method was increased when compared with that of the unmodified monoliths [48,50].

4.2 GRAPHENE AND GRAPHENE OXIDE

Graphene possesses a single-layer or a few layers thickness of two-dimensional structure consisting of sp^2-hybridized carbons. Graphene sheet provides the possibility of functionalizing its chemical structure; oxidation to graphene oxide is considered the most important method to enhance the functionality

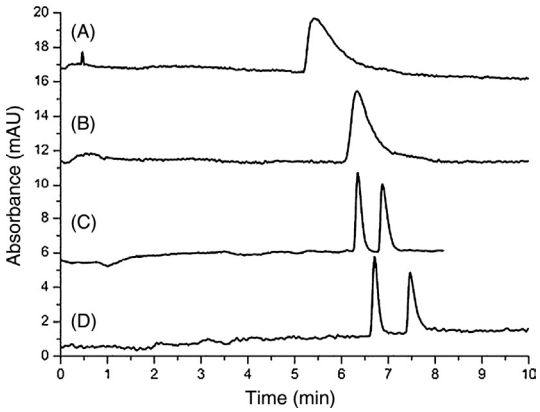

FIGURE 10.5 Influence of carboxylated-SWNTs and pepsin on the enantioseparation of (±)-nefopam on the polymer monoliths.

(A) poly(glycidyl methacrylate-ethylene dimethacrylate) monolith, (B) poly(glycidyl methacrylate-carboxylated-SWNTs-ethylene dimethacrylate) monolith, (C) pepsin-immobilized poly(glycidyl methacrylate-ethylene dimethacrylate) monolith, (D) pepsin-immobilized poly(glycidyl methacrylate-carboxylated-SWNTs-ethylene dimethacrylate) monolith. Conditions: running buffer, 15 mmol L^{-1} acetic acid-ammonium acetate (pH 6.0); injection, 10 kV, 1 s; applied voltage, 10 kV; temperature, 15 °C; polymer monolith length, 22 cm; total length, 33 cm [50].

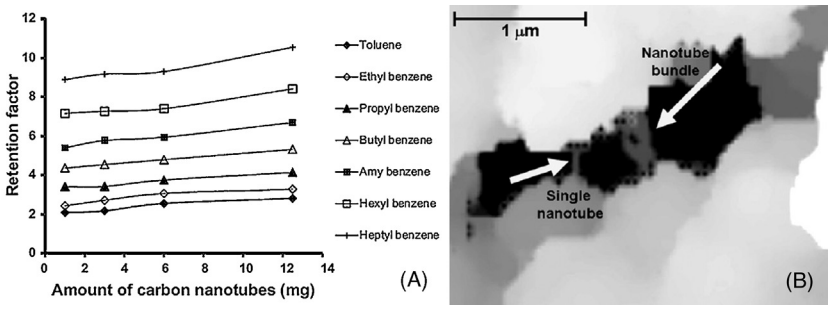

FIGURE 10.6

(A) Retention factors of alkylbenzenes obtained on the poly(glyceryl monomethacrylate-ethylene glycoldimethacrylate) monolithic columns incorporating various amounts of OH-MWNTs [49]. (B) Scanning ion conductance microscopy image of MWNT bridging the through pore of a poly(glycidyl methacrylate-ethylene dimethacrylate) monolith containing entrapped MWNTs [47].

of graphene [53]. Having most of the CNTs' advantages, graphene and graphene oxide have gained the attention of many analytical chemistry researchers in preconcentration and separation methods.

Several groups have tried to introduce graphene and graphene oxide to solve the deficiency of the bare polymer-based monoliths. Wang and Yan fabricated a monolithic stationary phase consisting of poly(methacrylic acid-ethylene dimethacrylate) and graphene oxide via one-step room temperature polymerization for capillary electrochromatography [54]. Graphene oxide sheets were dissolved in

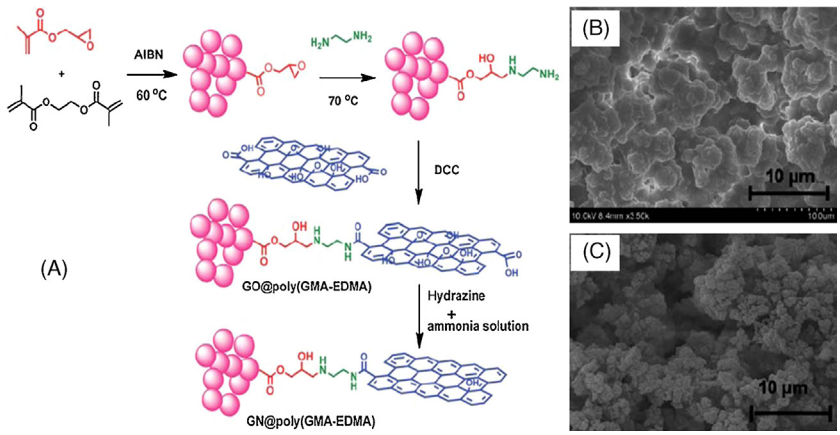

FIGURE 10.7

(A) Illustration of the procedure for preparing graphene oxide@poly(glycidyl methacrylate-ethylene dimethacrylate) and graphene@poly(glycidyl methacrylate-ethylene dimethacrylate) monoliths. (B) SEM micrograph of the internal structure of graphene oxide@ poly(glycidyl methacrylate-ethylene dimethacrylate) monolithic column. (C) SEM micrograph of poly(glycidyl methacrylate-ethylene dimethacrylate) monolithic column.

Reprinted with permission from S. Tong, X. Zhou, C. Zhou, Y. Li, W. Li, W. Zhou, Q. Jia, A strategy to decorate porous polymer monoliths with graphene oxide and graphene nanosheets, Analyst 138 (2013) 1549–1557.

cyclohexanol depending on the presence of functional groups in graphene oxide sheets, which improves their solubility [53]. Cyclohexanol was also used as a porogen to create a homogeneous dispersion before introducing the monomeric mixture into the preconditioned fused silica capillary. The reaction was carried out based on nitric acid–initiated polymerization at room temperature for 24 h. The prepared column gives excellent performance for capillary electrochromatography separation of both neutral and polar compounds (alkyl benzenes and polycyclic aromatic hydrocarbons). Graphene oxide is an electron-rich material; the intrinsic characteristic of graphene oxide such as the hydrophobicity and $\pi-\pi$ electrostatic stacking property, in addition to the ultrahigh specific surface area, makes the graphene oxide attractive as the stationary phase for capillary electrochromatography separation [55,56].

In contrast to previous incorporation methodology, Tong et al. succeeded to attach graphene and graphene oxide to the pore surface of the monolithic structure via a chemical bonding method [57]. Monolith stationary phases synthesized from glycidyl methacrylate and ethylene dimethacrylate were allowed to react through the epoxide groups with ethylenediamine. The amine-modified monolithic columns were linked with graphene oxide by coupling the amine groups of the polymer with the carboxyl groups of graphene oxide. Finally, the graphene-monolith column was obtained via chemical reduction of graphene oxide-monolith column by hydrazine. The scheme for the synthesis of the columns is illustrated in Fig. 10.7A, and the SEM image of the graphene oxide-monolith column (Fig. 10.7B) clearly demonstrates the change in the untreated monolithic column morphology (Fig. 10.7C) caused by modification with multilayer graphene oxide nanosheets. The images shown in Fig. 10.7B and C confirm that microglobules are encapsulated in graphene oxide and their size increases from 0.5–1.0 μm to 2–5 μm with little collapse of the polymer bed after loading graphene oxide. The synthesized

columns were employed for the extraction of sarcosine. This phase exhibited excellent stability and enrichment performance as a solid phase extraction material.

Depending on the graphene nanosheets' excellent properties, such as high loading capacity, rich π-electron system, and easy modification, the same group developed a poly(butyl methacrylate-ethylene dimethacrylate) monolithic column functionalized with graphene nanosheets by a one-step in situ polymerization procedure [58]. The monolith was successfully used for microextraction and high enrichment capacity of glucocorticoids (including dexamethasone, betamethasone, prednisolone, triamcinolone, triamcinolone acetonide, cortisone, hydrocortisone, fluocinonide, and beclomethasone dipropionate) from cosmetic samples using liquid chromatography-mass spectrometry for analysis.

Compared with other carbonaceous nanomaterials such as CNTs and C60 fullerene, graphene oxide exhibits properties of more facile modification, hydrophilicity, and good dispersibility in a variety of solvents due to the presence of various oxygen functional groups including hydroxyl, epoxy, and carboxylic acid groups located on their basal planes and the sheet edges [59,60]. In a unique scenario, graphene oxide nanosheets have been functionalized with 3-(trimethoxysilyl)propylmethacrylate and copolymerized with glycidyl methacrylate and ethylene dimethacrylate as a functional crosslinker [61]. The chromatographic performance of the monolithic column was evaluated by separation of small molecules of hydrophobic steroids (including hydrocortisone, prednisone acetate, and medroxyprogesterone acetate) and polar aromatic amines (including *o*-phenylenediamine, aniline, *p*-nitroaniline, and β-naphthylamine) in the isocratic reversed-phase mode. The results demonstrate that copolymerization of functionalized graphene oxide into porous polymer monolith enhanced the separation performance over those on the parent monolith.

4.3 FULLERENES AND NANODIAMONDS

Although fullerenes and nanodiamonds have been used for the functionalization of various silica particles as particulate stationary phases and in many other analytical fields [62–66], only one single paper has combined monoliths and fullerenes, and to date no work has been devoted to the use of nanodiamond material to enhance the separation efficiency of monoliths.

Porous monoliths of glycidyl methacrylate and butyl methacrylate with ethylene dimethacrylate standard monomers copolymerized with C60 fullerene moiety-bearing monomer, as an example, [6,6]-phenyl-C_{61}-butyric acid 2-hydroxyethyl methacrylate ester was used for the fast and high efficient separation of small molecules in the reversed-phase mode [67]. The structure of the C60 fullerene-based monomer [6,6]-phenyl-C_{61}-butyric acid 2-hydroxyethyl methacrylate ester is provided in Fig. 10.8A. While no separation of alkylbenzenes has been obtained using the parent poly(glycidyl methacrylate-ethylene dimethacrylate) monolithic column with only 4400 plates m^{-1} for benzene (Fig. 10.8B, left), a good separation with a rather high efficiency of 72,000 plates m^{-1} for the retained compound benzene at a flow rate of 0.15 μL min^{-1} is achieved using poly(glycidyl methacrylate-ethylene dimethacrylate) containing 1.0 wt% C60 fullerene-based monomer (Fig. 10.8B, middle). Further improvement of the column efficiency for benzene to 85,000 plates/m and reduction in the tailing from peaks asymmetry were observed by the addition of 2.5% tetrahydrofuran (THF) to the mobile phase (Fig. 10.8B, right). The same improvement was observed by the addition of the C60 fullerene-based monomer into the poly(butyl methacrylate-ethylene dimethacrylate) monolithic column. The resulting butyl methacrylate-incorporated monolithic column provided 120,000 plates m^{-1} for benzene at a flow rate of 0.10 μL min^{-1} and a retention factor of 4.2.

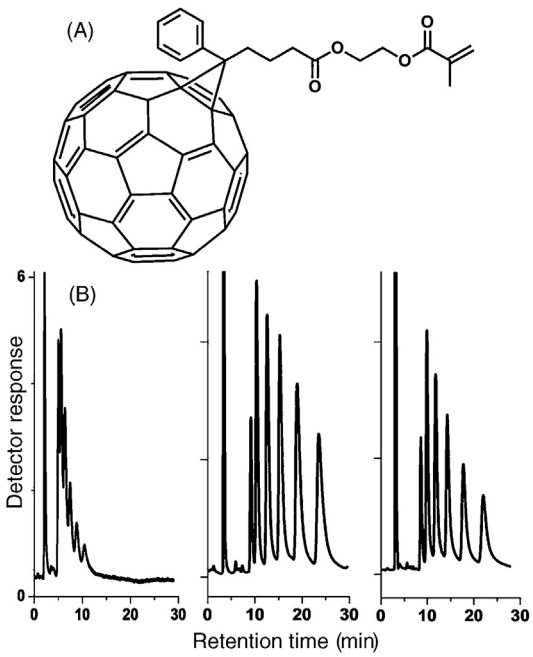

FIGURE 10.8

(A) Structure of [6,6]-phenyl-C_{61}-butyric acid 2-hydroxyethyl methacrylate ester. (B) Separation of uracil and alkylbenzenes using a parent monolithic poly(glycidyl methacrylate-ethylene dimethacrylate) column (left) and using a column containing 1.0 wt% C60 fullerene-based monomer (middle, right), both prepared at a temperature of 70 °C. Conditions: column dimensions (53 mm × 100 μm i.d.), 0.15 μL min^{-1} flow rate, UV detection at 254 nm; (left) mobile phase 50:50 vol% acetonitrile-water, backpressure 15 MPa (middle) mobile phase 50:50 vol% acetonitrile-water, backpressure 25 MPa; (right) mobile phase 47.5:2.5:50 vol% acetonitrile–THF–water, backpressure 27 MPa. Peaks in order of elution: uracil, benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, and amylbenzene.

Reprinted with permission from S.D. Chambers, T.W. Holcombe, F. Svec, J.M.J. Frechet, Porous polymer monoliths functionalized through copolymerization of C60 fullerene-containing methacrylate monomer for highly efficient separations of small molecules, Anal. Chem. 83 (2011) 9478–9484.

5 OTHER NANOPARTICLES-BASED MONOLITHS

Using methods similar to those mentioned in the previous sections, many groups introduced several nanoparticles into the polymer and silica-based monoliths. The first work using nanoparticles immobilized in the pore surface of the monolithic columns concerned the attachment of quaternary amine functionalized polymer latex nanoparticles in 2004 [68]. Nowadays, numerous types of nanomaterials have emerged to improve the separation characteristics of monolithic columns and gained considerable and wide attention. In addition to the carbon-based nanomaterials mentioned in the previous section, these nanomaterials can be divided into polymeric (latex) nanoparticles; metal nanoparticles such as gold, silver, and iron oxide nanoparticles; inorganic nanoparticles such as silica and hydroxyapatite nanoparticles; and others. Table 10.1 summarizes the most relevant examples of nanoparticle-based monoliths

Table 10.1 Examples of Nanoparticles Incorporated Into the Monoliths Stationary Phases and Their Applications

Nanoparticles	Monoliths System	Applications and Achievements	References
Polymeric Latex Nanoparticles			
Quaternary amine functionalized (60 nm) latex particles	Poly(butyl methacrylate-ethylene dimethacrylate-2-acrylamido-2-methyl-1-propanesulfonic acid)	Separation of seven saccharides: D(+)galactose, D(+)glucose, D(+)xylose, D(+)mannose, maltose, D(−)fructose, and sucrose in less than 10 min Separation of saccharides obtained by the enzymatic hydrolysis of corn starch as a complex sample	[68]
Quaternary ammonium functionalized (65 nm) latex particles	Poly(butyl methacrylate-ethylene dimethacrylate-2-acrylamido-2-methyl-1-propanesulfonic acid)	Separation of seven inorganic anions: bromide, nitrate, iodide, iodate, bromate, thiocyanate, and chromate over a period of 90 s in capillary electrochromatography	[69]
		Highly reproducible and rapid separation of seven analyte anions: iodate, bromate, nitrite, benzoate, nitrate, benzene sulfonate, and toluene sulfonate in less than 2 min using micro-ion chromatography	[70]
Quaternary ammonium functionalized (65 nm) latex particles	Poly(glycidyl methacrylate-ethylene glycol dimethacrylate)	High capacity monolithic support for agglomerated ion-exchange materials Separation of seven inorganic anions: $IO_3^-, BrO_3^-, NO_2^-, Br^-, NO_3^-, I^-$, and benzene sulfonate	[71]
Quaternary ammonium functionalized (70 nm) latex particles	Silica monolith	Separation of inorganic anions: $Br^-, I^-, Cl^-, BrO_3^-, IO_3^-, SCN^-, CrO_4^{2-}, NO_3^-$ *and* $S_2O_3^{2-}$ using ion-exchange capillary electrochromatography	[72]
Three different alkyl quaternary ammonium functionalized latex nanoparticles; AS9-SC (105 nm), AS12A (140 nm) and DNApac (151 nm)	Silica monolith	Fast separation of anions: acetate, formate, nitrate, bromate, thiocyanate, and iodide in about 2.5 min	[73]
Metallic Nanoparticles			
Gold nanoparticles (20 nm)	Poly(butyl methacrylate-ethylene dimethacrylate)	High capacity with very dense and homogeneous coverage was obtained at the monolith surface	[74]
Gold nanoparticles (15 nm)	Poly(glycidyl methacrylate-ethylene glycol dimethacrylate)	Selective capture of cysteine containing peptides to reduce the complexity of peptide mixtures prior to their chromatographic separation	[75]

(*Continued*)

Table 10.1 Examples of Nanoparticles Incorporated Into the Monoliths Stationary Phases and Their Applications (*cont.*)

Nanoparticles	Monoliths System	Applications and Achievements	References
Gold nanoparticles (15 and 10 nm)	Poly(glycidyl methacrylate-ethylene dimethacrylate)	Separation of three peptides; Tyr–Gly, Tyr–Gly–Gly, and Tyr–Gly–Gly–Phe–Leu in capillary electrochromatography mode Separation of proteins; ribonuclease A, cytochrome *c*, and myoglobin in nano-high pressure liquid chromatography and ion exchange modes	[76]
Gold nanoparticles (different sizes varying from 5 to 40 nm)	Poly(glycidyl methacrylate-ethylene dimethacrylate)	Reversed phase separation of simple protein mixtures (ribonuclease A, cytochrome *c*, and myoglobin) The best separations were obtained using monoliths modified with 15, 20, and 30 nm nanoparticles since these sizes produced the most dense coverage of pore surface with nanoparticles	[77]
Gold nanoparticles (15 nm)	Poly(glycidyl methacrylate-ethylene dimethacrylate)	Separations of proteins: ribonuclease A, cytochrome *c*, myoglobin, and lysozyme using mixed chromatographic modes: reverse phase and ion exchange	[78]
Gold nanoparticles (average diameter of about 15 nm)	Silica monolith	Separation of thiourea; alkylbenzenes; toluene, ethylbenzene, propylbenzene, butylbenzene in 13 min and phenols; resorcinol, phenol,4-methoxyphenol, *p*-cresol in 10 min using capillary electrochromatography	[79]
Gold nanoparticles (about 15 nm)	Poly(glycidyl methacrylate-ethylene dimethacrylate)	Screening of α-glucosidase inhibitor from natural products extracts by capillary electrophoresis	[80]
Gold nanoparticles (about 16 nm)	Silica monolith	Enantioseparation of 10 pairs phenylthiocarbamyl amino acids in capillary electrochromatography within 18 min	[81]
Gold nanoparticles (5, 10, and 15 nm)	Poly(4-methylstyrene-vinylbenzyl chloride-divinylbenzene)	Separation of mixtures of nucleosides: thymine, adenosine, cytidine, cytosine, and guanosine, and peptides: Phe–Gly–Phe–Gly, Val–Try–Val, Gly–Phe, Gly–Leu, Gly–Try, Lys–Val, and Gly–Gly–Gly in hydrophilic interaction chromatography mode	[82]
Ionic liquid-gold nanoparticles	Silica monolith	Separation of five hydrophobic *n*-alkylbenzenes: benzene, toluene, ethylbenzene, *n*-propylbenzene, and *n*-butylbenzene in 12 min Separation of four PAHs: naphthalene, acenaphthene, fluorine, and pyrene in about 8 min Separation of five phenols: hydroquinone, 2-aminophenol, phenol, 4-methoxyphenol, and *p*-cresol in about 8 min Separation of five aromatic amines: *o*-phenylenediamine, 2-nitroaniline, aniline, *p*-toluidine, and 1-naphthylamine in less than 10 min Separation of three basic compounds: caffeine, atenolol, and metoprolol in about 7 min	[83]

Gold nanoparticles (average diameter about 9.5 ± 2.5 nm) modified with β-cyclodextrin	Poly(glycidyl methacrylate-ethylene dimethacrylate)	Separation of three pairs of enantiomer drugs: chlorpheniramine, zopiclone, and tropicamide using capillary electrochromatography	[84]
Gold nanoparticles (about 10 nm) functionalized with boron nitride nanotubes	Poly(glycidyl methacrylate-ethylene dimethacrylate)	Isocratic mode separation of a series of benzene and naphthalene derivatives: benzene, parabens (methyl paraben, ethyl paraben, propyl paraben, and butyl paraben) and naphthalene derivatives: naphthalene, naphthalene-1-thiol, naphthalene-2-thiol, dithionaphthalene, and dimethyl(amino)naphthalene-1,8Bis	[85]
Gold nanoparticles (20 nm)	Poly(butyl methacrylate-ethylene dimethacrylate) and poly(lauryl methacrylate-ethylene glycol dimethacrylate)	Reversed-phase separation of a mixture of four standard proteins: ribonuclease B, insulin, carbonic anhydrase, and bovine serum albumin	[86]
Silver nanoparticles (50 nm)	Poly(glycidyl methacrylate-trimethylolpropane triacrylate)	Used as high sensitivity surface enhanced Raman scattering detection of Rhodamine 6G with excellent signal stability and detection of label-free biomolecules: bradykinin and cytochrome *c* May be used for biomolecule adsorption	[87]
Silver nanoparticles (<100 nm)	Poly(lauryl methacrylate-ethylene dimethacrylate-[2-(methacryloyloxy)ethyl] trimethyl ammonium chloride)	Separation of neutral compounds: sterols, fatty acid methyl esters, tocopherols, and polyaromatic hydrocarbons in capillary electrochromatography with lower *C*-term	[88]
Silver nanoparticles (4 nm)	Poly(glycidyl methacrylate-ethylene dimethacrylate)	Reversed phase separation medium for proteins Used as precolumns to remove the excess radioiodine (radioactive waste) from a radiolabeled pharmaceutical May be used for high capacity fishing-out iodide and iodine	[89]
Silver nanoparticles (ranging from 5 to 10 nm)	Silica monolith	Powerful separation tool for hydrocarbons bearing different number, position, and configuration of unsaturated bonds Separation of benzene, nitrobenzene, and *o*-nitroanisole in about 3 min Separation of aromatic hydrocarbons: benzene, naphthalene, anthracene, and phenanthrene in less than 3 min	[90]
Iron oxide nanoparticles (20 nm)	Poly(glycidyl methacrylate-ethylene dimethacrylate)	Efficient and selective enrichment of single or multiphosphorylated peptides from peptide mixtures of α- and β-casein digests	[91]
Iron oxide nanoparticles (10 nm)	Poly(acrylamide,*N*,*N*'-methylene-bis-acrylamide-allyl glycidyl ether) cryogel monolith	Efficient and selective adsorption of proteins: bovine serum albumin Separation of biomolecules from stock feeds in downstream processes	[92]
Iron oxide nanoparticles (20 nm)	Poly(2-hydroxyethyl methacrylate-ethylene dimethacrylate)	Efficient and selective enrichment of phosphopeptides from peptide mixtures of α- and β-casein digests	[93]

(*Continued*)

Table 10.1 Examples of Nanoparticles Incorporated Into the Monoliths Stationary Phases and Their Applications (*cont.*)

Nanoparticles	Monoliths System	Applications and Achievements	References
Three different nanoparticles; core–shell silica Fe_3O_4@SiO_2/NH_2, wormlike and hexagonal SBA-15 silica	Poly(butyl methacrylate-ethylene dimethacrylate)	Separation of organic acids: isophthalic acid, phthalic acid, sulfanilic acid, salicylic acid, and *o*-iodobenzoic acid in capillary electrochromatography mode Separation of aqueous extract of rhizoma gastrodiae	[94]
Titanium dioxide nanoparticle (10–20 nm)	Poly(ethylene glycol dimethacrylate)	Selective enrichment of specific proteins or peptides	[95]
Titanium dioxide/zirconium dioxide mixed nanoparticles (<100 nm)	Poly(divinylbenzene)	Selective retention of approximately 20 phosphopeptides from an α-casein tryptic digest	[96]
Alumina nanoparticles (10–20 nm)	Poly(*N*-isopropylacrylamide-*N*,*N*'-methylene bisacrylamide)	Prevent the swelling of the organic polymer and enhanced the loading capacity Effective determination of synthetic food dyes; tartrazine, sunset yellow, Allura Red, and azorubine in soft drink samples	[97]
Alumina nanoparticles (10–20 nm)	Poly(*N*-isopropylacrylamide-glycidyl methacrylate-ethylene dimethacrylate)	Preconcentration and determination of Sudan I–IV dyes in red wine samples	[98]
Platinum/palladium nanoflowers (20 nm)	Poly(glycidyl methacrylate-ethylene dimethacrylate) and poly(butyl methacrylate-ethylene dimethacrylate)	Used as microreactor to process some traditional chemical reactions (reduction of iron(III) to iron(II) and oxidation of NADH to NAD^+) Eliminated the need for removal of the nanoparticles from the product (centrifugation step after the reaction)	[99]
Inorganic Nanoparticles			
Fumed silica nanoparticles (about 12 nm)	Poly(glyceryl monomethacrylate-ethylene dimethacrylate)	Separations of small polar molecules such as nucleosides, nucleotides, and hydroxybenzoic acids using hydrophilic interaction mode Separation of toluene, acrylamide, DMF, and thiourea in 4 min Separation of five nucleosides in about 10 min Separation of six hydroxybenzoic acids in about 8 min Separation of four nucleotides in about 19 min	[100]
Fumed silica nanoparticles (about 12 nm)	Poly(glyceryl monomethacrylate-ethylene dimethacrylate)	Separation of three types of small solutes including the alkylbenzenes homologous series, weak acidic compounds (phenols), and basic compounds (anilines) in reversed phase chromatography mode Separation of six standard proteins; ribonuclease A, cytochrome *c*, carbonic anhydrase, lysozyme, myoglobin, and α-chymotrypsinogen A in about 10 min	[101]

Silica nanoparticles (7–40 nm)	Poly(acrylamide-*N*,*N*-methylene-bis-acrylamide) cryogel monolith	Protein chromatography; adsorption of lysozyme May be applied to the separation of biomolecules in downstream processes	[102]
Silica nanoparticles	Organic–silica hybrid	Separation of alkylbenzenes, anilines, and phenols in capillary electrochromatography based on reversed-phase and cation exchange interactions mechanisms Separation of bovine serum albumin tryptic digests	[103]
Hydroxyapatite nanoparticles (<200 nm)	Poly(2-hydroxyethyl methacrylate-ethylene dimethacrylate)	Efficient and selective enrichment of phosphopeptides from peptide mixtures of α- and β-casein digests	[93]
Hydroxyapatite nano-rods (50 nm × 150 nm)	Poly(2-hydroxyethyl methacrylate-ethylene dimethacrylate)	Separation of a model mixture of proteins: ovalbumin, myoglobin, lysozyme, and cytochrome *c* in 6 min Separation of a mixture of protein A/IgG2 antibody complex Selective enrichment of phosphopeptides from tryptic digests of ovalbumin, β- and α-casein	[104]

for chromatographic applications. It is worth noting that various nanoparticle-based monoliths have been prepared for sample preparation, pretreatment, preconcentration, detection, and other purposes [41,105–110]. In simple words, according to all these publications, the application of nanoparticles for chromatography and analytical chemistry has proven to be very useful and promising.

6 CONCLUSIONS

As with the "heart" for humans, the column "where separation takes place" is considered the most important part in any column chromatography system. During tens of years and until now, most of the chromatography development has focused on the design of many different ways to enhance the columns' stationary phase properties and efficiencies. A huge amount of stationary phase materials has been prepared, developed, and reported to deal with the complexity and variability of a tremendous number of different applications. Monoliths, in chromatographic terms, are continuous porous rods of macroporous structures usually created by the in situ polymerization of different monomers. Monolithic stationary phases are relatively new materials and attract increasing interest due to the easy preparation and modification processes, fast mass transfer kinetics between the mobile and stationary phases, high column permeability that reduces the backpressure, rapid chromatographic separation speed, and the variation in chemistry. Monolithic supports are now widely used and have rapidly become highly popular as separation media in all chromatographic methods including capillary electrochromatography, high-performance liquid chromatography, capillary liquid chromatography, and even GC for various applications such as environmental, food, pollutants, ions, and even chiral analysis.

Based on the chemistry of the monolith backbone, all monolithic structures fit in three main categories; (1) organic, (2) inorganic, and (3) hybrid organic–inorganic monoliths. In addition to the variation in the chemical structures and preparation methodologies, each type of monolith has its advantages, limitations, and application. The hybrid organic–inorganic monoliths combine the advantages of both basic organic and inorganic types. The coupling of nanoparticles and especially carbon-based nanomaterials such as CNT, graphene oxide, and fullerene with the porous polymer monoliths hold the potential to overcome the weaknesses of monoliths and to design highly robust and efficient columns for the separations of small molecules. The amazing chemistry of monolithic materials together with the unique physical and chemical properties of nanostructure materials, besides the wide variability and ease of preparation, will undoubtedly evolve and widen to contribute and provide more solutions to the continuous problems not only for separation science and chromatography but also for various application areas such as sample preparation and preconcentration. In this chapter, we reviewed the latest developments and applications of nanoparticle-based monoliths and specifically carbon nanostructures as promising stationary phases for chromatographic separations.

ACKNOWLEDGMENT

The author would like to extend his sincere appreciation to the Deanship of Scientific Research at King Saud University for funding this research group No. RGP-1437-011.

REFERENCES

[1] Roeraade J. From crushed bricks to microchips. In: Issaq HJ, editor. A Century of Separation Science. New York, USA: Marcel Dekker Inc; 2002. p. 27–48.

[2] Heftmann E, editor. Fundamentals and Applications of Chromatography and Related Differential Migration Methods, Part B: Applications. Elsevier B.V.; 2004. Amsterdam, 69B.

[3] Naushad M, Khan MR, editors. Ultra Performance Liquid Chromatography Mass Spectrometry: Evaluation Applications in Food Analysis. New York, USA: CRC Press, Taylor, Francis Group; 2014.

[4] Shibamoto T, editor. Chromatographic Analysis of Environmental and Food Toxicants. New York, USA: Marcel Dekker Inc.; 1998.

[5] Nollet LM, editor. Food Analysis by HPLC. New York, USA: Marcel Dekker Inc.,; 2000.

[6] Kazakevich Y, Lobrutto R, editors. HPLC for Pharmaceutical Scientists. NJ, USA: John Wiley & Sons Inc.; 2007.

[7] Weston A, Brown PR, editors. HPLC and CE principles and practice. San Diego, California, USA: Academic Press; 1997.

[8] Bynum KC. Pharmaceutical development: from pre-clinical to post approval. In: Ahuja S, Rasmussen H, editors. HPLC Method Development for Pharmaceuticals, Vol 8 of Separation Science and Technology. Amsterdam: Elsevier Inc; 2007. p. 298.

[9] Majors RE. HPLC column packing design. LC-GC Eur. 2003;16:8–13.

[10] Majors RE. Highlights of HPLC 2004. LC-GC North Am. 2004;22:870–82.

[11] Hjerten S, Liao J, Zhang R. High-performance liquid chromatography on continuous polymer beds. J Chromatogr. A 1989;473:273–5.

[12] Svec F, Frechet J. Continuous rods of macroporous polymer as high-performance liquid chromatography separation media. Anal. Chem. 1992;64:820–2.

[13] Lubda D, Cabrera K, Kraas W, Schaefer C, Cunningham D. New developments in the application of monolithic HPLC columns. LC-GC Europe 2001;14:730–2.

[14] Kubin M. A generalized exponential function as a model of polymer distribution curves. Collec. Czech. Chem. Commun. 1967;32:1505–17.

[15] Unger K, Skudas R, Schulte M. Particle packed columns and monolithic columns in high-performance liquid chromatography-comparison and critical appraisal. J. Chromatogr. A 2008;1184:393–415.

[16] Tennikova TB, Svec F, Belenkii BG. High-performance membrane chromatography: a novel method of protein separation. J. Liquid Chromatogr. 1990;13:63–70.

[17] Minakuchi H, Nakanishi K, Soga N, Ishizuka N, Tanaka N. Octadecylsilylated porous silica rods as separation media for reversed-phase liquid chromatography. Anal. Chem. 1996;68:3498–501.

[18] Al-Othman ZA, Aqel A, Al Abdelmoneim HA, Badjah-Hadj-Ahmed AY, Alwarthan AA. Preparation and evaluation of long chain alkyl methacrylate monoliths for capillary chromatography. Chromatographia 2011;74:1–8.

[19] Aqel A, ALOthman ZA, Badjah-Hadj-Ahmed AY, Yusuf K, Alwarthan AA. Preparation and evaluation of benzyl methacrylate monoliths for capillary chromatography. J. Chromatogr. Sci. 2014;52:201–10.

[20] Wang Q, Svec F, Frechet J. Macroporous polymeric stationary-phase rod as continuous separation medium for reversed-phase chromatography. Anal. Chem. 1993;65:2243–8.

[21] Minakuchi H, Nakanishi K, Soga N, Ishizuka N, Tanaka N. Effect of domain size on the performance of octadecylsilylated continuous porous silica columns in reversed-phase liquid chromatography. J. Chromatogr. A 1998;797:121–31.

[22] Alshemari H, Aqel A, ALOthman ZA. Preparation of high porous poly(2-ethylhexyl methacrylate-*co*-ethylene glycol dimethacrylate) monolithic columns for fast separation of small molecules. Asian J. Chem. 2014;26:8223–8.

[23] Chen ML, Zheng MM, Feng YQ. Preparation of organic–inorganic hybrid silica monolith with octyl and sulfonic acid groups for capillary electrochromatograhpy and application in determination of theophylline and caffeine in beverage. J. Chromatogr. A 2010;1217:3547–56.
[24] Jandera P, Urban J, Skerikova V, Langmaier P, Kubickova R, Planeta J. Polymethacrylate monolithic and hybrid particle-monolithic columns for reversed-phase and hydrophilic interaction capillary liquid chromatography. J. Chromatogr. A 2010;1217:22–33.
[25] Lin H, Ou J, Liu Z, Wang H, Dong J, Zou H. Facile construction of macroporous hybrid monoliths via thiol-methacrylate Michael addition click reaction for capillary liquid chromatography. J. Chromatogr. A 2015;1379:34–42.
[26] Svec F, Frechet JMJ. Kinetic control of pore formation in macroporous polymers. Formation of "molded" porous materials with high flow characteristics for separations or catalysis. Chem. Mater. 1995;7:707–15.
[27] Svec F. Quest for organic polymer-based monolithic columns affording enhanced efficiency in high performance liquid chromatography separations of small molecules in isocratic mode. J. Chromatogr. A 2012;1228:250–62.
[28] Moravcova D, Jandera P, Urban J, Planeta J. Comparison of monolithic silica and polymethacrylate capillary columns for LC. J. Sep. Sci. 2004;27:789–800.
[29] Aoki H, Kubo T, Ikegami T, Tanaka N, Hosoya K, Tokuda D, Ishizuka N. Preparation of glycerol dimethacrylate-based polymer monolith with unusual porous properties achieved via viscoelastic phase separation induced by monodisperse ultra high molecular weight poly(styrene) as a porogen. J. Chromatogr. A 2006;1119:66–9.
[30] Meyer U, Svec F, Frechet JMJ, Hawker CJ, Irgum K. The use of stable free radicals for the sequential preparation and surface grafting of functionalized macroporous monoliths. Macromolecules 2000;33:7769–75.
[31] Urban J, Svec F, Frechet JMJ. Efficient separation of small molecules using a large surface area hypercrosslinked monolithic polymer capillary column. Anal. Chem. 2010;82:1621–3.
[32] Nischang I, Teasdale I, Bruggemann O. Towards porous polymer monoliths for the efficient, retention-independent performance in the isocratic separation of small molecules by means of nano-liquid chromatography. J. Chromatogr. A 2010;1217:7514–22.
[33] Lubbad SH, Buchmeiser MR. Fast separation of low molecular weight analytes on structurally optimized polymeric capillary monoliths. J. Chromatogr. A 2010;1217:3223–30.
[34] Henglein A. Physicochemical properties of small metal particles in solution: "microelectrode" reactions, chemisorption, composite metal particles, and the atom-to-metal transition. J. Phys. Chem. 1993;97:5457–71.
[35] Martin CR, Mitchell DT. Nanomaterials in analytical chemistry. Anal. Chem. 1998;70:322A–7A.
[36] Hubler A, Osuagwu O. Digital quantum batteries: energy and information storage in nanovacuum tube arrays. Complexity 2010;15:48–55.
[37] Banica FG. Chemical Sensors and Biosensors. Chichester, UK: John Wiley and Sons Ltd; 2012. 135-156.
[38] Luz RS, Iost R, Crespilho F, Crespilho FN, editors. Nanobioelectrochemistry. Berlin, Heidelberg: Springer; 2013. p. 27–48.
[39] Scarselli M, Castrucci P, Crescenzi MD. Electronic and optoelectronic nano-devices based on carbon nanotubes. J. Phys. Condens. Matter. 2012;24:313202–38.
[40] Murray RW. Nanoelectrochemistry: metal nanoparticles, nanoelectrodes, and nanopores. Chem. Rev. 2008;108:2688–720.
[41] Ahmadi M, Elmongy H, Madrakian T, Abdel-Rehim M. Nanomaterials as sorbents for sample preparation in bioanalysis: a review. Anal. Chim. Acta 2017;958:1–21.
[42] Stevens TS, Langhorst MA. Agglomerated pellicular anion-exchange columns for ion chromatography. Anal. Chem. 1982;54:950–3.
[43] Kroto HW, Heath JR, O'Brien SC, Curl RF, Smalley RE. C60: buckminsterfullerene. Nature 1985;318:162–3.
[44] Iijima S. Helical microtubules of graphitic carbon. Nature 1991;354:56–8.

[45] Novoselov KS, Geim AK, Morozov SV, Jiang D, Zhang Y, Dubonos SV, Grigorieva IV, Firsov AA. Electric field effect in atomically thin carbon films. Science 2004;306:666–9.
[46] Li Y, Chen Y, Xiang R, Ciuparu D, Pfefferle LD, Horwath C, Wilkins JA. Incorporation of single-wall carbon nanotubes into an organic polymer monolithic stationary phase for μ-HPLC and capillary electrochromatography. Anal. Chem. 2005;77:1398–406.
[47] Chambers SD, Svec F, Frechet JMJ. Incorporation of carbon nanotubes in porous polymer monolithic capillary columns to enhance the chromatographic separation of small molecules. J. Chromatogr. A 2011;1218:2546–52.
[48] Aqel A, Yusuf K, Al-Othman ZA, Badjah-Hadj-Ahmed AY, Alwarthan AA. Effect of multi-walled carbon nanotubes incorporation into benzyl methacrylate monolithic columns in capillary liquid chromatography. Analyst 2012;137:4309–17.
[49] Mayadunne E, El Rassi Z. Facile preparation of octadecyl monoliths with incorporated carbon nanotubes and neutral monoliths with coated carbon nanotubes stationary phases for HPLC of small and large molecules by hydrophobic and π–π interactions. Talanta 2014;129:565–74.
[50] Miao C, Bai R, Xu S, Hong T, Ji Y. Carboxylated single-walled carbon nanotube-functionalized chiral polymer monoliths for affinity capillary electrochromatography. J. Chromatogr. A 2017;1487:227–34. 025.
[51] Li S, Li H, Wang X, Song Y, Liu Y, Jiang L, Zhu D. Super-hydrophobicity of large-area honeycomb-like aligned carbon nanotubes. J. Phys. Chem. B 2002;106:9274–6.
[52] Aqel A, Abou El-Nour KMM, Ammar RAA, Alwarthan A. Carbon nanotubes, science and technology part (I): structure, synthesis and characterization. Arab. J. Chem. 2012;5:1–23.
[53] Dreyer DR, Park S, Bielawski CW, Ruoff RS. The chemistry of graphene oxide. Chem. Soc. Rev. 2010;39:228–40.
[54] Wang MM, Yan XP. Fabrication of graphene oxide nanosheets incorporated monolithic column via one-step room temperature polymerization for capillary electrochromatography. Anal. Chem. 2012;84:39–44.
[55] Gulbakan B, Yasun E, Shukoor MI, Zhu Z, You MX, Tan XH, Sanchez HN, Powell DH, Dai HJ, Tan WHJ. A Dual platform for selective analyte enrichment and ionization in mass spectrometry using aptamer-conjugated graphene oxide. Am. Chem. Soc. 2010;132:17408–10.
[56] Liu Q, Shi JB, Sun JT, Wang T, Zeng LX, Jiang GB. Graphene and graphene oxide sheets supported on silica as versatile and high-performance adsorbents for solid-phase extraction. Angew. Chem. Int. Ed. 2011;50:5913–7.
[57] Tong S, Zhou X, Zhou C, Li Y, Li W, Zhou W, Jia Q. A strategy to decorate porous polymer monoliths with graphene oxide and graphene nanosheets. Analyst 2013;138:1549–57.
[58] Tong S, Liu Q, Li Y, Zhou W, Jia Q, Duan T. Preparation of porous polymer monolithic column incorporated with graphene nanosheets for solid phase microextraction and enrichment of glucocorticoids. J. Chromatogr. A 2012;1253:22–31.
[59] Morales-Narvaez E, Merkoci A. Graphene oxide as an optical biosensing platform. Adv. Mater. 2012;24:3298–308.
[60] Badri A, Whittaker MR, Zetterlund PB. Modification of graphene/graphene oxide with polymer brushes using controlled/living radical polymerization. J. Polym. Sci. A 2012;50:2981–92.
[61] Li Y, Qi L, Ma H. Preparation of porous polymer monolithic column using functionalized graphene oxide as a functional crosslinker for high performance liquid chromatography separation of small molecules. Analyst 2013;138:5470–8.
[62] Speltini A, Merli D, Profumo A. Analytical application of carbon nanotubes, fullerenes and nanodiamonds in nanomaterials-based chromatographic stationary phases: a review. Anal. Chim. Acta 2013;783:1–16.
[63] Zhang M, Qiu H. Progress in stationary phases modified with carbonaceous nanomaterials for high-performance liquid chromatography. TrAC Trends Anal. Chem. 2015;65:107–21.
[64] Peristyy AA, Fedyanina ON, Paull B, Nesterenko PN. Diamond based adsorbents and their application in chromatography. J. Chromatogr. A 2014;1357:68–86.

[65] Zhang BT, Zheng X, Li HF, Lin JM. Application of carbon-based nanomaterials in sample preparation: a review. Anal. Chim. Acta 2013;784:1–17.
[66] Scida K, Stege PW, Haby G, Messina GA, Garcia CD. Recent applications of carbon-based nanomaterials in analytical chemistry: critical review. Anal. Chim. Acta 2011;691:6–17.
[67] Chambers SD, Holcombe TW, Svec F, Frechet JMJ. Porous polymer monoliths functionalized through co-polymerization of C60 fullerene-containing methacrylate monomer for highly efficient separations of small molecules. Anal. Chem. 2011;83:9478–84.
[68] Hilder EF, Svec F, Frechet JMJ. Latex-functionalized monolithic columns for the separation of carbohydrates by micro anion-exchange chromatography. J. Chromatogr. A 2004;1053:101–6.
[69] Hutchinson JP, Zakaria P, Bowie AR, Macka M, Avdalovic N, Haddad PR. Latex-coated polymeric monolithic ion-exchange stationary phases. 1. Anion-exchange capillary electrochromatography and in-line sample preconcentration in capillary electrophoresis. Anal. Chem. 2005;77:407–16.
[70] Zakaria P, Hutchinson JP, Avdalovic N, Liu Y, Haddad PR. Latex-coated polymeric monolithic ion-exchange stationary phases. 2. Micro-ion chromatography. Anal. Chem. 2005;77:417–23.
[71] Hutchinson JP, Hilder EF, Shellie RA, Smith JA, Haddad PR. Towards high capacity latex-coated porous polymer monoliths as ion-exchange stationary phases. Analyst 2006;131:215–21.
[72] Hutchinson JP, Hilder EF, Macka M, Avdalovic N, Haddad PR. Preparation and characterisation of anion-exchange latex-coated silica monoliths for capillary electrochromatography. J. Chromatogr. A 2006;1109:10–8.
[73] Ibrahim ME, Lucy CA. Mixed mode HILIC/anion exchange separations on latex coated silica monoliths. Talanta 2012;100:313–9.
[74] Connolly D, Twamley B, Paull B. High-capacity gold nanoparticle functionalised polymer monoliths. Chem. Commun. 2010;46:2109–11.
[75] Xu Y, Cao Q, Svec F, Frechet JMJ. Porous polymer monolithic column with surface-bound gold nanoparticles for the capture and separation of cysteine-containing peptides. Anal. Chem. 2010;82:3352–8.
[76] Cao Q, Xu Y, Liu F, Svec F, Frechet JMJ. Polymer monoliths with exchangeable chemistries: use of gold nanoparticles as intermediate ligands for capillary columns with carrying surface functionalities. Anal. Chem. 2010;82:7416–21.
[77] Lv Y, Alejandro FM, Frechet JMJ, Svec F. Preparation of porous polymer monoliths featuring enhanced surface coverage with gold nanoparticles. J. Chromatogr. A 2012;1261:121–8.
[78] Terborg L, Masini JC, Lin M, Lipponen K, Riekolla ML, Svec F. Porous polymer monolithic columns with gold nanoparticles as an intermediate ligand for the separation of proteins in reverse phase-ion exchange mixed mode. J. Adv. Res. 2015;6:441–8.
[79] Ye FG, Lu JY, Wang Y, Zhang AZ, Tian JN, Zhao SL. Preparation and characterization of gold nanoparticle-modified silica monolith for capillary electrochromatography. Chin. J. Anal. Chem. 2011;39:341–5.
[80] Zhang A, Ye F, Lu J, Zhao S. Screening α-glucosidase inhibitor from natural products by capillary electrophoresis with immobilised enzyme onto polymer monolith modified by gold nanoparticles. Food Chem. 2013;141:1854–9.
[81] Lu J, Ye F, Zhang A, Wei Z, Peng Y, Zhao S. Preparation and characterization of silica monolith modified with bovine serum albumin-gold nanoparticles conjugates and its use as chiral stationary phases for capillary electrochromatography. J. Sep. Sci. 2011;34:2329–36.
[82] Lv Y, Lin Z, Svec F. Hypercrosslinked large surface area porous polymer monoliths for hydrophilic interaction liquid chromatography of small molecules featuring zwitterionic functionalities attached to gold nanoparticles held in layered structure. Anal. Chem. 2012;84:8457–60.
[83] Lu J, Ye F, Zhang A, Chen X, Wei Y, Zhao S. Preparation and evaluation of ionic liquid-gold nanoparticles functionalized silica monolithic column for capillary electrochromatography. Analyst 2012;137:5860–5.
[84] Li M, Tarawally M, Liu X, Liu X, Guo L, Yang L, Wang G. Application of cyclodextrin-modified gold nanoparticles in enantioselective monolith capillary electrochromatography. Talanta 2013;109:1–6.

[85] Andre C, Guillaume YC. Boron nitride nanotubes and their functionalization via quinuclidine-3-thiol with gold nanoparticles for the development and enhancement of the HPLC performance of HPLC monolithic columns. Talanta 2012;93:274–8.
[86] Currivan S, Connolly D, Paull B. Production of polymer monolithic capillary columns with integrated gold nano-particle modified segments for on-capillary extraction. Microchem. J. 2013;111:32–9.
[87] Liu J, White I, DeVoe DL. Nanoparticle-functionalized porous polymer monolith detection elements for surface-enhanced Raman scattering. Anal. Chem. 2011;83:2119–24.
[88] Navarro-Pascual-Ahuir M, Lerma-Garcia MJ, Ramis-Ramos G, Simo-Alfonso EF, Herrero-Martinez JM. Preparation and evaluation of lauryl methacrylate monoliths with embedded silver nanoparticles for capillary electrochromatography. Electrophoresis 2013;34:925–34.
[89] Sedlacek O, Kucka J, Svec F, Hurby M. Silver-coated monolithic columns for separation in radiopharmaceutical applications. J. Sep. Sci. 2014;37:798–802.
[90] Zhu Y, Morisato K, Li W, Kanamori K, Nakanishi K. Synthesis of silver nanoparticles confined in hierarchically porous monolithic silica: a new function in aromatic hydrocarbon separations. ACS Appl. Mater. Interfaces 2013;5:2118–25.
[91] Krenkova J, Foret F. Iron oxide nanoparticle coating of organic polymer-based monolithic columns for phosphopeptide enrichment. J. Sep. Sci. 2011;34:2106–12.
[92] Yao K, Yun J, Shen S, Wang L, He X, Yu X. Characterization of a novel continuous supermacroporous monolithic cryogel embedded with nanoparticles for protein chromatography. J. Chromatogr. A 2006;1109:103–10.
[93] Krenkova J, Foret F. Nanoparticle-modified monolithic pipette tips for phosphopeptide enrichment. Anal. Bioanal. Chem. 2013;405:2175–83.
[94] Lei W, Zhang LY, Wan L, Shi BF, Wang YQ, Zhang WB. Hybrid monolithic columns with nanoparticles incorporated for capillary electrochromatography. J. Chromatogr. A 2012;1239:64–71.
[95] Hsieh HC, Sheu C, Shi FK, Li DT. Development of a titanium dioxide nanoparticle pipette-tip for the selective enrichment of phosphorylated peptides. J. Chromatogr. A 2007;1165:128–35.
[96] Rainer M, Sonderegger H, Bakry R, Huck CW, Morandell S, Huber LA, Gjerde DT, Bonn GK. Analysis of protein phosphorylation by monolithic extraction columns based on poly(divinylbenzene) containing embedded titanium dioxide and zirconium dioxide nanopowders. Proteomics 2008;8:4593–602.
[97] Li WJ, Zhou X, Tong SS, Jia Q. Poly(*N*-isopropylacrylamide-*co*-*N*,*N*'-methylene bisacrylamide) monolithic column embedded with α-alumina nanoparticles microextraction coupled with high-performance liquid chromatography for the determination of synthetic food dyes in soft drink samples. Talanta 2013;105:386–92.
[98] Li W, Zhou X, Ye J, Jia Q. Development of a γ-alumina nanoparticle-functionalized porous polymer monolith for the enrichment of Sudan dyes in red wine samples. J. Sep. Sci. 2013;36:3330–7.
[99] Floris PG, Twamley B, Nesterenko PN, Paull B, Connolly D. Agglomerated polymer monoliths with bimetallic nano-particles as flow-through micro-reactors. Microchim. Acta 2012;179:149–56.
[100] Aydogan C, El Rassi Z. Monolithic stationary phases with incorporated fumed silica nanoparticles. Part I. Polymethacrylate-based monolithic column with incorporated bare fumed silica nanoparticles for hydrophilic interaction liquid chromatography. J. Chromatogr. A 2016;1445:55–61.
[101] Aydogan C, El Rassi Z. Monolithic stationary phases with incorporated fumed silica nanoparticles. Part II. Polymethacrylate-based monolithic column with "covalently" incorporated modified octadecyl fumed silica nanoparticles for reversed-phase chromatography. J. Chromatogr. A 2016;1445:62–7.
[102] Xu P, Yao Y, Shen S, Yun J, Yao K. Preparation of supermacroporous composite cryogel embedded with SiO_2 nanoparticles. Chin. J. Chem. Eng. 2010;18:667–71.
[103] Liu Y, Chen Y, Yang H, Nie L, Yao S. Cage-like silica nanoparticles-functionalized silica hybrid monolith for high performance capillary electrochromatography via "one-pot" process. J. Chromatogr. A 2013;1283:132–9.

[104] Krenkova J, Lacher N, Svec F. Control of selectivity via nanochemistry: monolithic capillary column containing hydroxyapatite nanoparticles for separation of proteins and enrichment of phosphopeptides. Anal. Chem. 2010;82:8335–41.
[105] Hong T, Yang X, Xu Y, Ji Y. Recent advances in the preparation and application of monolithic capillary columns in separation science. Anal. Chim. Acta 2016;931:1–24.
[106] Svec F, Lv Y. Advances and recent trends in the field of monolithic columns for chromatography. Anal. Chem. 2015;87:250–73.
[107] Tong S, Liu S, Wang H, Jia Q. Recent advances of polymer monolithic columns functionalized with micro/nanomaterials: synthesis and application. Chromatographia 2014;77:5–14.
[108] Tang S, Guo Y, Xiong C, Liu S, Liu X, Jiang S. Nanoparticle-based monoliths for chromatographic separations. Analyst 2014;139:4103–17.
[109] Connolly D, Currivan S, Paull B. Polymeric monolithic materials modified with nanoparticles for separation and detection of biomolecules: a review. Proteomics 2012;12:2904–17.
[110] Acquah C, Moy CKS, Danquah MK, Ongkudon CM. Development and characteristics of polymer monoliths for advanced LC bioscreening applications: a review. J. Chromatogr. B 2016;1015-1016:121–34.