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Nanomaterials as Stationary Phases and Supports in Liquid Chromatography: A Review

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Abbreviations

ALD Atomic-layer deposition
BuMA Butyl methacrylate
CNTs Carbon nanotubes
EDMA Ethylene dimethacrylate
GLAD Glancing angle deposition
GMA Glycidyl methacrylate
GMM Glyceryl methacrylate
GNP Gold nanoparticle
HILIC Hydrophilic interaction liquid chromatography

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Abstract

The development of various nanomaterials over the last few decades has led to many applications for these materials in liquid chromatography (LC). This review will look at the types of nanomaterials that have been incorporated into LC systems and the applications that have been explored for such systems. A number of carbon-based nanomaterials and inorganic nanomaterials have been considered for use in LC, ranging from carbon nanotubes, fullerenes and nanodiamonds to metal nanoparticles and nanostructures based on silica, alumina, zirconia and titanium dioxide. Many ways have been described for incorporating these nanomaterials into LC systems. These methods have included covalent immobilization, adsorption, entrapment, and the synthesis or direct development of nanomaterials as part of a
chromatographic support. Nanomaterials have been used in many types of LC. These applications have included the reversed-phase, normal-phase, ion-exchange, and affinity modes of LC, as well as related methods such as chiral separations, ion-pair chromatography and hydrophilic interaction liquid chromatography. Both small and large analytes (e.g., dyes, drugs, amino acids, peptides and proteins) have been used to evaluate possible applications for these nanomaterial-based methods. The use of nanomaterials in columns, capillaries and planar chromatography has been considered as part of these efforts. Potential advantages of nanomaterials in these applications have included their good chemical and physical stabilities, the variety of interactions many nanomaterials can have with analytes, and their unique retention properties in some separation formats.

Key words: Nanomaterials; Nanoparticles; Liquid chromatography; Planar chromatography

1 Introduction

The term “nanoscience” refers to the study and use of structures and materials with sizes in the range of approximately 1-100 nm [1]. These nanomaterials frequently have chemical and physical characteristics that are distinct from those for their macroscopic counterparts, which can be exploited in many fields of science and technology [2,3]. These properties are determined by the size, morphology and phase of the nanomaterials and can often be tailored to adjust the final properties of these materials [4]. For instance, a nanoparticle’s size is determined by such parameters as the temperature, pressure, concentration, or other reaction conditions that lead to particle nucleation and growth [5]. As a nanomaterial particle decreases in size, the ratio of its surface area to volume rapidly increases, which can have a significant influence on its properties as a support for chemical separations [6,7].
The application of nanoparticles and nanomaterials in separation science has been of great interest in recent years due to the unique physical properties of these substances, their large surface area-to-volume ratios, and their ability to be employed with a variety of surface chemistries [7-10]. This review will discuss several types of nanomaterials that have been employed in liquid chromatography (LC) and will explore some analytical applications for these materials. Nanomaterials to be considered will include carbon-based or organic materials (e.g., carbon nanotubes, fullerenes, nanodiamonds, and some types of organic polymers), as well as inorganic materials (e.g., silica nanoparticles or gold nanoparticles). Techniques for placing these nanomaterials on LC supports or in LC systems will then be discussed. The use of these nanomaterials in various types of LC will next be examined, including their applications in reversed-phase LC, normal-phase LC, ion-exchange chromatography, and chiral separations, among others. The potential advantages or disadvantages of the resulting materials and applications will also be considered, as compared to more conventional LC supports and stationary phases. Most of this discussion will focus on column-based applications; however, the use of nanomaterials in packed capillaries or in planar formats for LC will also be examined.

2 Types of Nanomaterials used in LC

A number of nanomaterials have been explored for use as supports and stationary phases in column, capillary or planar LC methods. As is indicated in Table 1, both carbon-based nanomaterials and inorganic nanomaterials have been used in LC applications [3,11-13]. Much of this work was been done through the use of columns, however, nanomaterials have also been employed in packed capillaries and in planar techniques such as thin-layer chromatography (TLC) and ultrathin-layer chromatography (UTLC) (see Sections 4-8).
2.1 Carbon-Based Nanomaterials

Various carbon-based nanomaterials have been used in LC [14-20]. Nanomaterials that fall within this group are carbon nanotubes, fullerenes, and nanodiamonds [7,14,17-20]. Advantages of these materials include their unique adsorption properties and their good chemical or physical stabilities [3,15,16].

Carbon nanotubes, or CNTs, are made of sp² hybridized carbon atoms that are present in cylinders with diameters in the nanometer range and with lengths that can extend up to tens of micrometers [21]. There are two main types of CNTs: single-walled CNTs (SWCNTs) and multi-walled CNTs (MWCNTs) [3,22,23]. SWCNTs are formed when a single layer of graphene is folded onto itself and the edges along its length are joined; this gives a material with lengths varying from hundreds of nanometers to several micrometers and with diameters that range up to 2 nm [24]. MWCNTs are created when several additional graphene tubes are present around the core tube of a SWCNT; this type of material has an interlayer spacing of 3.4 Å and a diameter that is typically around 10-20 nm [3]. The ends for both of these structures are usually capped by fullerene-like structures [25]. CNTs have been of interest as stationary phases because of their good stability and their ability to be used as either individual nanoparticles or as aggregates that can undergo various interactions with solutes, including dipole-dipole interactions, hydrogen bonding, π-π stacking, and non-polar interactions, among others [26,27]. These properties have been found in a number of studies to provide stationary phases based on CNTs with excellent long-term stabilities and the ability to provide improved retention, resolution, and analysis times for a number of compound classes when compared to traditional LC columns [26,28-34], such as those containing C₁₈- or aminopropyl-silica (e.g., see Sections 4-5) [26,28,29,31,32].

Fullerenes are hollow carbon cages that are made of pentagonal and hexagonal rings [3,11,14,35]. The sp² bonds that hold together the structure of a fullerene provide this agent...
with relative strong and selective binding to analytes that contain phenyl groups [14,36,37]. Some advantages of using fullerenes as stationary phases in LC are their low reactivity, their good mechanical stability, and their good hydrolytic stability when compared to common inorganic supports (e.g., silica and alumina) [14]. It is also possible to further modify these agents, such as by adding an amine group, for their attachment to an LC support [11,14]. These properties have been found to provide fullerene-based stationary phases with selectivities and retention properties that are complementary to those of common supports that are used in reversed-phase LC or hydrophilic interaction liquid chromatography (HILIC) [38-40].

Like regular diamonds, nanodiamonds (or NDs) contain a lattice of sp³–hybridized carbon atoms [3,41]. Nanodiamonds have been of interest in LC because of their mechanical and thermal stabilities, their hydrolytic stability over a wide pH range, and the absence of shrinking or swelling by these nanomaterials in many common mobile phases. In addition, nanodiamonds can be arranged in micro-dispersed structures that can be used directly in LC [42]. There are number of ways to synthesize nanoscale diamonds, with the size of a nanodiamond particle being dependent on the method that is utilized [41,43]. For example, nanodiamonds between 4 and 25 nm can be synthesized by means of shockwave methods, while nanoparticles with sizes up to 50 nm can be produced by mechanical milling [43]. Nanodiamonds have been used to make stationary phases for reversed-phase LC [44], normal-phase LC [42], and ion-exchange chromatography [45], with several of these materials showing unique retention behavior when compared to more traditional stationary phases for these methods [42,45].

Organic polymer-based nanomaterials have also been explored for use in LC [3,33,40,46-48]. For instance, nano-latex agglomerated stationary phases have been used as ion-exchange resins [3]. Polymer-based monoliths can be formed inside capillaries or
microfluidic channels, making them of interest for use in miniaturized systems [46] and as
general platforms for the entrapment or immobilization of nanomaterials in LC [33,40,48].
Hybrid materials have also been described that incorporate silica particles coupled with latex
particles (e.g., for use in ion-exchange chromatography) [47], as well as latex-modified silica
monoliths and organic monoliths [46,48].

2.2 Inorganic Nanomaterials

A number of inorganic-based nanomaterials have been used in LC [3,36,49-52]. As is
ture with traditional columns, silica is of interest as a nanomaterial for LC because of its
relatively high surface area, its availability in various particle sizes and pore diameters, and
the ease with which its surface can be modified to contain a wide range of functional groups
[36]. These properties have led to the use of silica nanoparticles and related materials (e.g.,
silica nanofibers) in making stationary phases or supports for such methods as reversed-phase
chromatography, HILIC, and UTLC [52-55]. However, silica also has limitations in terms of
its thermal and chemical stability, such as the relatively narrow pH range over which it can be
utilized [36]. Alternative nanomaterials based on alumina, zirconia, or titanium oxide can be
used to overcome some of these limitations [3,36]. In addition, support materials based on a
composite of silica microspheres and metal oxide nanoparticles have been reported that
combine the chemical stability of these metal oxides with the ease of surface modification
that is provided by silica [36,50-52,56].

Metal nanoparticles that have been explored for use in LC and related separation
methods have included gold nanoparticles, which are also known as GNPs or AuNPs [57-66].
The use of gold nanoparticles is appealing due to their ease of preparation, their controllable
particle size and narrow size distribution, their good solubility, and the ability to conveniently
modify their surfaces [62,64,65]. For instance, chemicals or biomolecules that contain thiol
groups can be adsorbed spontaneously onto gold and used to prepare or generate well-organized, self-assembled monolayers [63]. This feature has made gold nanoparticles useful in work with thiol-containing reagents to create new stationary phases that can be used in reversed-phase or normal-phase LC [61,64,65].

3 Incorporation or Immobilization of Nanomaterials in LC Systems

There are a variety of approaches that have been used to immobilize or place nanomaterials in LC systems. Covalent immobilization has often been utilized in such work because of its ability to prepare stationary phases that are chemically stable and that provide good long-term stabilities. For instance, a number of studies have immobilized CNTs onto silica-based supports that contain groups such as amines [26,67]. An example is the use of glutardialdehyde as a bifunctional linker to couple amine-containing CNTs to aminopropyl silica [26,29]. The covalent immobilization of fullerenes onto various inorganic and organic LC supports, including silica, has also been described through the use of N-hydroxysuccinimide, epoxy groups, and amine groups [38,39,68,69]. For instance, an amine-containing C\textsubscript{60} fullerene was immobilized onto glycidoxypropyl silica [70], and brominated fullerenes have been covalently attached to amine-containing silica [69]. In addition, the coupling of fullerenes to silica has been investigated through use of perfluorophenyl azide, as is illustrated in Figure 1 [71,72]. One study used polybutadiene to modify silica and to immobilize MWCNTs in the presence of γ-radiation [28]. Another report involved the esterification of carboxylated MWCNTs with an allyl alcohol, followed by grafting of the modified nanoparticles onto polystyrene-divinylbenzene beads [73].

Covalent immobilization has been used with inorganic nanomaterials as well. For instance, bifunctional linkers such as alkane thiols, cysteamine and polyethyleneimine have been used to couple metal nanoparticles onto metallic [74] or polymeric supports [75]. Gold
nanoparticles have been coated on aminopropyl silica and then covered with \textit{n}-octadecanethiol for use in reversed-phase LC [64]. A monolith made with a co-polymer of glycidyl methacrylate (GMA) and ethylene dimethacrylate (EDMA) was treated with cystamine and tris(2-carboxylethyl)phosphine to generate free thiol groups on this support, which were then used to bind gold nanoparticles [76]. Gold nanoparticles have also been attached to thiol-activated particles of graphene oxide-modified silica for use in reversed-phase LC [12]. Monoliths based on poly(4-methylstylene-\textit{co}-vinylbenzene chloride-\textit{co}-divinylbenzene) have been coated with gold nanoparticles through thiol groups and used in LC [77].

Another strategy that has been used for charged nanoparticles has been to adsorb them onto a support or surface by means of electrostatic interactions [12,67,76,78-81]. Immobilization through adsorption is often easier to carry out than covalent immobilization, although it can result in lower stability for the stationary phase and some loss in reproducibility when a column containing such a material is used over long periods of time [67]. Another advantage of using adsorption is it can allow multiple layers of nanoparticles to be placed onto a support [67], which can lead to improved retention and resolution [31]. Adsorption through electrostatic interactions has been used for immobilizing materials such as gold nanoparticles [12,76,78], nanomaterials based on zirconia and titanium oxide [52,56,79], and latex nanoparticles [80] onto silica [12,31,32,50,52,56,81] or organic monoliths [46,48,76]. A few specific examples are the adsorption of gold nanoparticles onto silica that has been stabilized with L-cysteine or L-cysteine methyl ester [66] and the adsorption of zirconia nanoparticles directly, or through alternating layers of these nanoparticles and sodium dodecyl sulfate, on the surface of silica particles [56,81].

Other non-covalent interactions (e.g., dipole-dipole interactions and dispersion interactions) have also been utilized to adsorb nanoparticles onto or within supports such as
silica and organic monoliths [67]. Examples for carbon-based nanomaterials include the adsorption of SWCNTs to aminopropyl silica or related materials [27,32,82], the layering of hydroxyl-MWCNTs on silica [31], and the adsorption of oxidized and carboxylated MWCNTs on an amine-activated GMA/EDMA co-polymer [83].

Some nanomaterials have been placed within supports by means of entrapment [33,83,84]. This is a general approach that has been used directly with many types of nanomaterials or modified forms of nanomaterials (e.g., carboxylated MWCNTs) [33,73,83,84]. A possible advantage of this technique is it may lead to an increase in the amount of immobilized nanomaterials (versus covalent immobilization) and in the corresponding surface area for the stationary phase. However, the stability of the resulting stationary phase is often less than what is seen for covalent coupling methods [67]. One report placed carboxylated MWCNTs in polystyrene-divinylbenzene beads by adding these nanomaterials to the polymerization mixture used to make this support [73]. MWCNTs have also been entrapped within monoliths based on GMA and EDMA [83] or glyceryl methacrylate (GMM) and EDMA [33]. Fumed silica nanoparticles have been incorporated within a GMM/EDMA monolith during the polymerization of this support [84].

In some cases the nanomaterial can be made and used directly as an LC support [42,85,86]. Although the situations in which this method can be used are limited, such an approach could potentially result in a large amount of the nanomaterial being placed into a system, leading to high retention [42,86]. This approach was employed when micro-dispersed sintered detonation nanodiamonds were prepared and packed into a column for use in normal-phase LC [42]. In addition, silica nanoparticles with an average diameter of 670 nm and containing C_{18} groups have been prepared and evaluated for use as a support and stationary phase in ultra-high pressure LC (UPLC) [86].
Yet another possible route is to create the nanomaterial directly on or within a chromatographic support [13,61,87,88-90]. For example, micron-sized aggregates of gold nanoparticles treated with \( n \)-octadecanethiol have been used in capillary reversed-phase LC [61]. The direct placement of nanomaterials on supports has been of particular interest for use in planar chromatography and UTLC as a way for quickly making and modifying these materials [13,88-90]. Vapor deposition methods have been used to fabricate or place nanomaterials on supports for UTLC [13,87]. Atomic-layer deposition (ALD) has also been used to deposit nanomaterials based on aluminum oxide or zirconium oxide onto silica for UTLC-based separations [13]. Glancing angle deposition (GLAD) has been recently employed to deposit silver nanoparticles and silica-based nanomaterials onto substrates such as glass for UTLC [88-90]. Silver nanorods have been prepared for use in UTLC by employing oblique angle deposition (OAD) [88]. Other work has used electrospinning to create nanomaterials for UTLC. This last method utilized electrostatic forces to produce nanofibers from a polymeric solution and its contents, and was found to be a simple and cost-effective approach for making these nanomaterials [53].

4 Nanomaterials in Reversed-Phase LC

Given the widespread popularity of reversed-phase LC, it is not surprising that many types of nanomaterials have been considered for use in this separation mode. Reversed-phase LC makes use of the partitioning of compounds into a non-polar stationary phase [44,55]. Advantages of reversed-phase LC include its ability to work directly with aqueous samples and its ability to retain a wide range of compounds based on the general property of their polarity. Both carbon-based nanomaterials and inorganic nanomaterials have been examined as tools to alter the retention or selectivity of reversed-phase separations and the physical or
chemical properties of the supports that can be used in these separations [12,31,32,38,55,56,83].

4.1 Carbon-Based Nanomaterials in Reversed-Phase LC

CNTs have been used in many applications involving reversed-phase LC. Several of these reports have involved SWCNTs or MWCNTs that were attached to silica particles for the separation or analysis of small analytes [28,31]. Jiang and co-workers used layer-by-layer self-assembly to place hydroxyl-MWCNTs on silica for use in reversed-phase LC [31]. This support was packed into a 150 mm × 4.6 mm i.d. column and compared to traditional C_{18}-silica for use in separating organic acids, amines, substituted aromatic compounds, and polyaromatic hydrocarbons in the presence of aqueous mobile phases or aqueous/organic mixtures. A series of supports with different numbers of layers (from 1-5) were prepared as part of this work, with an increase of up to five layers giving an improvement in the separation of polyaromatic hydrocarbons and baseline resolution for these analytes [31]. A report by Speltini et al. described the preparation of MWCNT-modified silica, which was then placed into a 300 mm × 3.9 mm I.D. column and used in the presence of mobile phases containing water and acetonitrile to separate barbiturates and retain simple aromatics, aromatic acids, or aromatic amines. This support was found to work particularly well for benzene-related compounds, which were more easily separated than when using a traditional C_{18} column [28].

Guillaume and co-workers reported the combination of CNTs with silica for separating medium-to-large biomolecules. SWCNT-modified silica was evaluated for use in a 50 mm × 4.6 mm I.D. column and in the separation of twelve dipeptides in the presence of various water/acetonitrile mixtures as the mobile phase; this support gave good stability over an extended period of time and significantly higher efficiency than a traditional C_{18} stationary
phase [29]. The same research group used SWCNTs that had been adsorbed onto amine-modified superficially porous silica for reversed-phase LC [32]; this material was utilized in resolving a series of monoclonal antibodies that are biopharmaceutical agents. These antibodies co-eluted on a superficially porous C18 column but were resolved on the SWCNT column under the same chromatographic conditions [32].

CNTs have also been placed onto non-silica supports for use in LC. Many of these other supports have been based on organic polymers. For example, several approaches have been described and compared by Zhu and co-workers for placing carboxylated MWCNTs into or onto polystyrene-divinylbenzene beads for use in chromatographic separations [73]. These materials were stable over a wide pH range. Some of these supports and stationary phases were used in 50 mm × 4.6 mm I.D. columns for reversed-phase LC with water/methanol mobile phases to retain a variety of neutral, acidic and basic organic compounds. This type of stationary phase resulted in better resolution for these analytes when compared to a polystyrene-divinylbenzene column and was stable over more than 800 injections. The chromatographic performance depended on the amount of MWCNTs that were added to the support, with higher amounts improving mechanical stability but also leading to a possible decrease in column efficiency [73].

A number of studies have examined the use of CNTs with monolithic supports for reversed-phase LC. Frechet and co-workers encapsulated MWCNTs within 170-200 mm × 100 μm I.D. capillaries containing GMA/EDMA monoliths for use in reversed-phase LC and the separation of uracil and alkylbenzenes [83]. The same study examined the use of oxidized and carboxylated MWCNTs that were adsorbed onto amine-activated GMA/EDMA monoliths in 100 mm × 4.6 mm I.D. columns for use in such separations. These columns were stable for more than 6,000 column volumes (and with a relative change in retention of less than 1.6%), confirming that there was no significant amount of leaching for the
MWCNTs from the monolith [83]. Guillaume and co-workers adsorbed SWCNTs within a 100 mm × 4.6 mm I.D. aminopropyl silica monolith, which was used to resolve a series of aromatic solutes that included phenol, aniline, catechol, 3-nitrophenol, and various alkyl phenols, dinitronaphthalenes and dihydroxynaphthalenes [82]. Sharp and symmetric peaks were obtained for all compounds on the SWCNT monolith, while no separation was obtained on a C18 monolith [82]. In work by El Rassi’s group, MWCNTs have been placed within a 250 mm × 4.6 mm I.D. column C18 organic monolith and employed for the separation of alkylbenzenes or proteins (see examples in Figure 2), with an improvement in the protein separation being noted due to additional π-interactions with the MWCNTs. The same study entrapped MWCNTs within a GMM/EDMA monolith, which was examined for use in retaining alkylbenzenes, anilines, phenols, and phenoxyacid herbicides [33].

Both silica and monolithic supports have been used with fullerenes in the reversed-phase mode of LC. For instance, fullerene-modified silica was used along with graphite-like phases in two-dimensional LC separations [38]. This approach allowed for the separation of fourteen furanocoumarin compounds in 10 min, with the fullerene-modified silica giving selectivity that was orthogonal to that obtained with traditional reversed-phase supports and graphite-like phases [38]. Chambers et al. prepared 53-108 mm × 100 μm I.D. capillaries that contained GMA/EDMA or butyl methacrylate (BuMA)/EDMA monoliths and that incorporated a C60-methacrylate monomer as part of the polymeric mixture. These capillary monoliths were then tested for use in the reversed-phase separation of alkylbenzenes [40]. The addition of the C60-methacrylate monomer to the monolith increased the column efficiency by 18-fold for benzene (or 85,000 plates/m) versus the parent monolith [40].

Linford and co-workers reported a layer-by-layer approach for immobilizing nanodiamonds on microdiamond core particles for use in reversed-phase LC [91]. This material was packed into a 300 mm × 4.6 mm I.D. column and gave near baseline resolution.
for a series of alkylbenzenes. This type of column could also separate the pesticides cyanazine and diazinon, although it did not have good stability and degraded after overnight flushing with the mobile phase [91]. Later work by the same group with this layer-by-layer approach for making nanodiamond supports instead began with a carbon core and was followed by treatment with 1,2,7,8-diepoxyoctane, resulting in good efficiency and stability even at pH 2.7 and pH 13.0 [44].

4.2 Inorganic Nanoparticles in Reversed-Phase LC

Metal nanoparticles can be used for reversed-phase separations by modifying them to contain non-polar groups. Yan and co-workers have treated micron-sized aggregates of gold nanoparticles with n-octadecanethiol for use as a reversed-phase packing material in a 250 mm section of a 100 μm I.D. capillary [61]. This material was tested for its ability to retain several small organic compounds (e.g., benzene, naphthalene, 2-methylnaphthalene and acenaphthene), giving good mechanical stability at pressures up to 52 MPa and being stable at pH 12 for up to 140 h. Acidic and neutral compounds both gave good peak shapes in this type of capillary; however, peak tailing was observed for basic compounds [61]. The same group prepared gold nanoparticles that had been coated on non-porous 1.3 μm diameter silica and coated with n-octadecanethiol [64]. It was again found that this material could be used at pressures up to 52 MPa and that this support was stable over a pH range of 1-12 for at least 60 h. A 200 mm section of a 100 μm I.D. capillary that was packed with this material gave efficient separations for a number of neutral, acidic and basic organic compounds. Up to around 66,500 plates/m were seen when this support was used in capillary LC and up to $2.5 \times 10^5$ plates/m were observed when this support was used in pressurized capillary electrochromatography [64].
Svec and co-workers utilized GMA/EDMA monoliths in 89-119 mm × 100 μm I.D. capillaries that were treated with cystamine and tris(2-carboxylethyl)phosphine to generate free thiol groups of this support. These groups were used to bind gold nanoparticles, which were then treated with 1-octanethiol or 1-octadecanethiol to create reversed-phase supports for the separation of proteins. This synthetic approach could be used to increase the pore surface coverage of the gold nanoparticles to over 60% (w/w), with monoliths that contained 15-30 nm gold nanoparticles giving the best pore surface coverage and separations [76]. Gold nanoparticles have also been attached to thiol-activated graphene oxide-silica particles and tested for use in reversed-phase LC and the separation of alkylbenzenes, isomerides, amino acids, nucleosides, and nucleobases [12].

One report described the development of segmented monoliths in 100 μm I.D. capillaries, with an initial region that contained immobilized gold nanoparticles followed by a second region that was used for reversed-phase separations [92]. The first zone contained gold nanoparticles that were placed on monoliths that had been activated with amine groups through photo-grafting or photo-masking, and the second region had monoliths that contained non-polar residues such as lauryl groups for use as a non-polar stationary phase. These segmented supports were examined for use in the on-column entrapment and separation of several model proteins or peptides, which included bovine serum albumin, carbonic anhydrase, insulin, and ribonuclease B [92].

Silica nanoparticles with an average diameter of 670 nm and containing C\textsubscript{18} groups were prepared for reversed-phase UPLC through a one-step sol-gel process. These particles were put into an approximately 90 mm × 50 μm I.D. capillary and evaluated for use with a test mixture of ascorbic acid, hydroquinone, resorcinol, catechol, and 4-methylcatechol, giving separation efficiencies of up to 500,000 plates/m [86]. These columns were tested at column pressures of roughly 50,000 psi and were found to be stable under both acidic and
basic conditions (i.e., pH < 1 or pH > 11, respectively) [86]. In work by Aydogan et al.,
fumed silica nanoparticles were functionalized to contain vinyl groups and added as co-
polymerization agents during the formation of GMM/EDMA monoliths in 100 mm × 4.6 mm
i.d. columns. The surface of this support was modified with C\textsubscript{18} groups and used to separate
alkylbenzenes, phenols and aniline derivatives, as well as a series of model proteins [55].

Chen’s group developed zirconia nanoparticles that were adsorbed onto micron-sized
silica particles and modified with octadecyltrichlorosilane for use in LC [56]. The resulting
hybrid support was packed into a 150 mm × 4.6 mm I.D. column. This column had good
selectivity in the reversed-phase mode for alkylbenzenes and aromatic hydrocarbons. The
hybrid support was stable up to a pH of 11, and it had a higher surface area and pore volume
than other zirconia-based supports [56]. Composite particles that had titanium oxide
nanoparticles coated onto a silica core were prepared and modified with octadecyltrichlorosilane for use in reversed-phase LC and 150 mm × 4.6 mm I.D. columns [51,52]. These stationary phases exhibited several favorable characteristics for use in LC,
including a narrow pore size distribution, a high surface area, and a high pore volume. This
material also had good pH stability and was evaluated for use in separating alkylbenzenes,
simple aromatics, and basic aromatic compounds [51,52].

5 Nanomaterials in Normal-Phase LC and HILIC

Nanomaterials have also been utilized in the modes of normal-phase LC and HILIC
[26,39,50,66,77,84]. These techniques are both based mainly on the partitioning of analytes
into a polar stationary phase, although the exact nature of this phase and of the corresponding
mobile phase does differ in these two methods [77,84]. These separation modes are
particularly useful in resolving polar compounds which do not have strong retention in
reversed-phase LC.
5.1 Carbon-based Nanomaterials in Normal-Phase LC and HILIC

Various carbon-based nanomaterials have been employed in separations for normal-phase LC. As an example, amine-modified SWCNTs have been immobilized by the Guillaume group onto aminopropyl silica by using glutardialdehyde as a cross-linking agent [26]. The resulting support was used in 50 mm × 4.6 mm i.d. columns and compared with traditional aminopropyl silica. These columns were used with mobile phases based on mixtures of toluene and cyclohexane to separate terpenes such as geraniol, linalool, α-terpineol and thymol, as well as various ortho-substituted isomers of polychlorinated biphenyls. The amine-modified SWCNT support gave larger retention factors than aminopropyl silica and was stable for half a year under the given separation conditions [26]. SWCNTs have been adsorbed through non-covalent interactions onto aminopropyl silica; the resulting support was used in a 125 mm × 2.1 mm i.d. column with toluene as the mobile phase and compared with aminopropyl silica, as well as with C18 silica and a graphite column, for retaining benzene and various polyaromatic hydrocarbons [27]. There was only a small difference in retention times for these analytes on the aminopropyl silica or C18-silica columns; however, there was clear difference in retention on the SWCNT-treated column. In addition, the affinity of this material towards linear polyacenes was much higher than it was for non-linear analogs, an effect which was suggested to be the result of stacking by such compounds with the SWCNTs [27].

Nanodiamonds and fullerenes have been used in some work with normal-phase LC. Micro-dispersed sintered detonation nanodiamonds have been examined by Nesterenko et al. for use as the stationary phase and support in a 150 mm × 4.0 mm i.d. column for normal-phase LC [42]. This material gave higher retention and a better efficiency for alkylbenzenes than was seen for more common normal-phase supports. The strong retention seen on the
nanodiamond support was attributed to the formation of hydrogen bonds between polar analytes and the surface hydroxyl and carboxyl groups on the nanodiamonds [42]. An oxidized fullerene was immobilized to aminopropyl silica and used as a stationary phase for HILIC by Liu et al. [39]. This material was used to separate such compounds as nucleosides, nucleobases, water soluble vitamins, amino acids and saccharides. It was demonstrated that the separation of these analytes occurred through an ion-exchange effect. In addition, a 150 mm × 4.6 mm i.d. column that contained this fullerene-based stationary phase gave better efficiency in the separation of amino acids and saccharides than columns that contained aminopropyl silica [39].

5.2 Inorganic Nanomaterials in Normal-Phase LC and HILIC

Several studies have used metal nanoparticles for normal-phase LC or HILIC. A report by Elfimova et al. used gold nanoparticles that were adsorbed onto silica and employed in a 100 mm × 4.6 mm i.d. column for normal-phase LC and the separation of substituted aminopyridines; this stationary phase gave increased retention for these compounds when compared to non-modified silica [66]. Gold nanoparticles were placed by Wang and co-workers onto thiol-activated graphene oxide-modified silica; this material was examined for use in a 150 mm × 4.6 mm i.d. column and HILIC with such analytes as alkylbenzenes, isomerides, amino acids, nucleosides, and nucleobases [12]. In a report by Svec and co-workers, monoliths based on poly(4-methylstyrene-co-vinylbenzyl chloride-co-divinylbenzene) and coated with gold nanoparticles through thiol groups were tested in 159-171 mm × 100 μm i.d. capillaries for use in separating nucleosides or peptides by HILIC [77]. These monoliths were prepared by hyper-crosslinking and had a large surface area (i.e., 430 m²/g), with gold nanoparticles being densely covered on the pore surface. The gold nanoparticle-modified monoliths were treated with polyethyleneimine, which acted as a
spacer to add a second layer of gold nanoparticles that were then functionalized with cysteine. The two layers of gold nanoparticles resulted in a stationary phase that gave better separations for the test analytes than a monolith that contained only a single layer of gold nanoparticles [77].

Gold nanoparticles have been placed by Liang et al. within thiol-activated GMA/EDMA monoliths in 100 mm × 250 μm i.d. capillaries and modified for use in both HILIC and enzymatic treatment (Figure 3) [93]. The gold nanoparticles were modified with cysteine for use in HILIC and glycopeptide enrichment, or were modified with peptide-N-glycosidase F for the on-line deglycosylation of glycopeptides. It was possible with this method to identify 196 N-linked glycopeptides from 122 glycoproteins in human plasma, making this approach of interest for large-scale glycoproteomic research [93].

Fumed silica nanoparticles have been incorporated by Aydogan and El Rassi within a GMM/EDMA monolith during the polymerization of this support [84]. This material was used in a 150 mm × 4.6 mm i.d. column for HILIC and characterized for its retention and separation of polar solutes such as toluene, acrylamide, dimethylformamide, and thiourea; nucleosides; nucleotides; and hydroxybenzoic acids. The resulting column had both good mechanical stability and reproducibility [84].

Li et al. developed a support for normal-phase LC consisting of zirconia nanoparticles that were coated on micron-sized silica particles and then calcinated at a high temperature [50]. A pH of 6.0 was used to make this material, at which the silica had a negative charge and allowed electrostatic attraction of the negatively-charged zirconia nanoparticles. This hybrid support was placed in a 150 mm × 4.6 mm i.d. column and used with organic solvent mixtures as the mobile phase. This column was tested for its ability to separate a series of neutral aromatic compounds (i.e., benzene, toluene, xylene, biphenyl and naphthalene) and nitroaniline isomers, giving good peak shapes and an efficiency of around 5,000 plates/m.
This type of column was also stable over the application of up to 3 L of the mobile phase, with no significant change being noted in analyte retention under these conditions [50].

6 Nanomaterials in Ion-Exchange Chromatography or Related Separation Modes

The separation of charged analytes by techniques such as ion-exchange chromatography or ion-pair chromatography can also be accomplished by using nanomaterials. Carbon-based nanomaterials and inorganic nanomaterials have both been modified and explored for use in such separation modes [30,45,65,73,81,94,95].

6.1 Carbon-Based Nanomaterials in Ion-Exchange Modes

The use of carbon-based nanomaterials for ion-exchange chromatography can be achieved through the modification of these materials to contain charged groups. For instance, Huang et al. derivatized the surface of MWCNTs to contain quaternary ammonium groups, onto which sulfonated polystyrene-divinylbenzene beads (PS-DVB) were adsorbed for the separation of inorganic ions (e.g., bromide, chloride, fluoride, nitrate, and nitrite) [30]. The use of this PS-DVB/MWCNT composite in a 100 mm × 4.0 mm i.d. column gave similar chromatographic behavior and separation efficiencies to those seen with commercial anion-exchange columns. A separation of various inorganic ions with the MWCNT-containing column gave an efficiency of 20,000 plates/m and good permeability, allowing for potential use of this column at high flow rates [30]. In work by the same group, PS-DVB beads containing MWCNTs were placed into a 150 mm × 4.6 mm i.d. column and used with tetrabutylammonium hydroxide as an ion-pairing agent to separate eleven organic acids by ion-pair chromatography (see Figure 4) [73].

Nanodiamonds have also been used for ion-exchange chromatography. For instance, Nesterenko and co-workers made 50-150 mm × 4.0 mm i.d. columns containing micro-dispersed sintered detonation nanodiamonds that were used to resolve alkali, alkaline earth,
transition metal cations, phenols and benzoic acids; these columns gave selectivities similar to those seen for carboxylate-type cation-exchange columns [45,94,95]. The ion-exchange capability of this support was proposed to be due to the carboxyl and hydroxyl groups on the surface of nanodiamonds [45,94], with changes in the amount of hydroxyl groups making it possible for this support to exhibit either anion- or cation-exchange properties [45].

6.2 Inorganic Nanomaterials in Ion-Exchange Modes

Several reports have used inorganic nanomaterials in mixed-mode columns that can be employed for either ion-exchange chromatography or other modes of LC [81,65]. For example, the Svec group incorporated gold nanoparticles onto organic monoliths to prepare mixed-mode stationary phases that could be used for either reversed-phase LC or ion-exchange chromatography [65]. This report used 120 mm × 100 μm i.d. capillaries containing GMA/EDMA monoliths that had been modified with cystamine and tris(2-carboxylethyl)phosphine (TCEP) to create thiol groups for coupling with gold nanoparticles. After gold nanoparticles had been placed within these monoliths, the nanoparticles were further coated with alkanethiols, mercaptoalkanoic acids or various mixtures of these agents. The resulting monoliths were evaluated against commercial C₈ and C₁₈ columns as well as ion-exchange columns, in which it was demonstrated that the gold nanoparticle-monoliths were capable of separating protein mixtures in a more efficient manner than these other columns [65].

A support was made by Liang et al. for use in either ion-exchange or normal-phase LC by coating silica core particles with zirconium nanoparticles and then modifying this material with N-methylimidazolium [81]. The silica had previously been modified with poly-(sodium-p-styrenesulphonate) to allow electrostatic adsorption of zirconia nanoparticles to this core. At an acidic pH (e.g., near or below the pKₐ of 4.0 for the protonated surface
groups on zirconia), the support could be used as an anion-exchanger. Under these conditions, this material could be used in 150 mm × 4.6 mm i.d. columns to separate various inorganic and organic anions. When the same material was used with ethanol/hexane as a mobile phase, normal-phase conditions were created that could be used to retain and separate text mixtures of basic organic compounds or hydroxybenzenes [81].

7 Nanomaterials in Affinity Chromatography and Chiral Separations

A few reports have examined the use of nanomaterials in affinity chromatography and related systems that employ molecular recognition or a biologically-related binding agent as a means for creating a separation [70,51]. This has also included the creation of stationary phases based on nanomaterials for chiral separations [97], which has long been a challenging area in LC [96-98].

7.1 Carbon-Based Nanomaterials in Affinity Chromatography and Chiral Separations

Carbon-based nanomaterials have been used in only a few reports for affinity-based separations. One such report examined the reciprocal molecular recognition between cyclodextrins and fullerenes and used this effect to resolve cyclodextrin rings of various sizes by using a C_{60} fullerene that was immobilized onto silica and placed into a 250 mm × 4.0 mm i.d. column [70]. This approach was found by Bogdanski et al. to be complementary in its behavior to work in which the reverse format was employed with immobilized cyclodextrins to separate C_{60} and C_{70} fullerenes [70].

It has been noted that CNTs can have the ability for chiral recognition [99], a feature that is thought to be due to the helicity or spiral alignment (i.e., either left- or right handed) of the rings along the axis of the CNT [100]. Mayadunne and El Rassi used MWCNTs that were entrapped in a 100 mm × 4.6 mm i.d. GMM/EDMA monolith to separate the enantiomers of bupivacaine, 2,4-dichlorophenoxypropionic acid, dansyl-phenylalanine, and
dansyl-methionine, resulting in peak resolutions in the range of 0.9-1.3 and selectivity factors of 1.4-3.9 [33]. SWCNTs entrapped in various organic monoliths in 200 mm × 150 μm i.d. capillaries have been used in a similar manner by Ahmed et al. to separate and give baseline resolution (i.e., a peak resolution of 1.5 or greater) for the chiral forms of drugs such as sulconazole, nomifensine, etozoline, chlorpheniramine, and celiprolol (see Figure 5) [101]. A partial separation (i.e., a peak resolution between 1.0 and 1.5) was also noted for the enantiomers of acebutolol, aminoglutethimide, atenolol, carprofen, metoprolol, pindolol, teboconazole, and tocaindide [101].

7.2 Inorganic Nanomaterials in Affinity Chromatography and Chiral Separations

Titanium oxide nanoparticles have been coated by Wu et al. onto silica spheres by means of liquid phase deposition and employed in metal oxide affinity chromatography to retain phosphopeptides or adenosine phosphate compounds [51]. This material was used in a 100 μm i.d. capillary to separate adenosine monophosphate, adenosine diphosphate and adenosine triphosphate. This separation made use of Lewis acid-base interactions between the titanium oxide and phosphate groups of these compounds. The same material was also utilized to enrich phosphorylated peptides that were obtained from digests of α-casein and β-casein (see Figure 6) [51].

Metal nanoparticles have been used to form chiral stationary phases by combining them with various binding agents. This was accomplished by Liu et al. [102] through the adsorption of gold nanoparticles to thiol-activated silica, followed by modification of remaining sites on the gold nanoparticles to form a self-assembled monolayer that could be used to immobilize bovine serum albumin (BSA) by means of a carbodiimide coupling reaction. The resulting BSA support was packed into a 50 mm × 4.6 mm i.d. column and used to separate the enantiomers of dansyl-norvaline. A maximum peak resolution of 1.3 was
observed, and the enantioselectivity of BSA was retained even after it had been immobilized to the gold nanoparticles [102].

8 Nanomaterials in Planar Liquid Chromatography

A number of reports have explored the use of nanomaterials as supports or stationary phases in LC modes based on planar chromatography. This work has included not only traditional TLC but also the newer technique of UTLC [13,89,103,104]. UTLC is a type of planar chromatography which uses thinner layers with finer pores than TLC, thus enabling faster separations over shorter distances and with better detection sensitivity [13,89]. Both carbon-based nanomaterials and inorganic nanomaterials have been used in this research.

8.1 Carbon-Based Nanomaterials in Planar Chromatography

Several reports have considered the use of carbon-based nanomaterials in UTLC. One study by Clark and Olesik used electrospinning and pyrolysis to make glassy carbon nanofibers with diameters of around 200-350 nm and to give a stationary phase mat with a thickness of approximately 15 µm [104]. The resulting UTLC plates had tunable retention, gave an efficiency of over 10,000 plates, and could be used over at least 35 runs. In addition, the selectivity of this material could be adjusted by pyrolyzing the electrospun nanofibers at various temperatures, and these supports were tested for use in the separation of some dyes and amino acids, resulting in good resolution for these analytes (see Figure 7) [104]. Another report by the Olesik group used electrospinning to prepare nanofibrous materials for UTLC that combined MWCNTs or edge-plane carbon nanorods with polyacrylonitrile. This type of material was tested by using it to separate several dyes and a series of five polyaromatic hydrocarbons [34]. When compared to plates containing only polyacrylonitrile, both the
plates containing MWCNTs and edge-plane carbon nanorods gave enhanced resolution during the separation of polyaromatic hydrocarbons [34].

Other nanomaterials for UTLC have also been prepared by electrospinning. This approach has been used by Lu and Olesik to make nanofibers based on polyvinyl alcohol (PVA), which have been examined for use in separating fluorescein-labeled amino acids [105] and hydrolysis products of aspartame [105]. ULTC plates containing the PVA nanofibers gave much smaller plate heights and higher efficiencies than commercial plates containing silica for use in high-performance TLC, with this efficiency being dependent on such factors as the mat thickness and sample concentration or application conditions [105]. A variation on electrospinning that can produce aligned nanofibers has been used in work by Beilke et al. with polyacrylonitrile, with the resulting UTLC supports being used for the separation of β-blockers and steroids [106]. These UTLC plates provided 2- to 2.5-times faster analysis times, better reproducibility, and up to 100-times greater efficiency when compared to non-aligned UTLC plates made by electrospinning [106]. Electrospun nanofibers based on polyacrylonitrile have also been used in planar electrochromatography by Newsome and Olesik [107]. Plate heights down to 11 μm were obtained with this material. The thickness of the nanofiber layer was easily tunable in this work, with thicker layers being more suitable for the separation of complex mixtures and thinner layers producing faster separations. It was further demonstrated how this type of plate could be used in a two-dimensional separation for a dye mixture, as based on UTLC followed by planar electrochromatography [107].

Other carbon- or organic-based nanomaterials have also been considered for use in planar chromatography. For instance, a brush-gel polymer based on GMA and diethylene glycol dimethacrylate was prepared by Costantini et al. and modified to contain primary amine groups for use in TLC. This support was utilized to separate a mixture of fluorescent
dyes and ochratoxin, where the separation efficiency could be adjusted by varying the polymerization conditions [108]. Carboxylated MWCNTs have been spread onto TLC plates containing carboxymethylcellulose and used by Huang et al. for the chiral separation of ofloxacin enantiomers [109]. Silica gels that were impregnated with oxidized MWCNTs and β-cyclodextrin by Chen et al. have been used in TLC for separating the enantiomers of propranolol [110].

8.2 Inorganic Nanomaterials in Planar Chromatography

Several inorganic nanomaterials have been considered for use in planar chromatography. Shapalova et al. used gold nanoparticles that had been placed on commercial silica or alumina supports for TLC and modified with L-cysteine as a chiral binding agent; these materials were used to separate chiral analytes such as tetrahydrozoline, hydroxyzine, ketoprofen, fenoprofen, pindolol, and fluoxetine [103]. Arrays of silver nanorods have been employed by Chen and co-workers as supports/stationary phases for UTLC and to aid in analyte detection by surface enhanced Raman spectroscopy (SERS) [88,111]. One study used this combination to carry out detection during the separation of fluorescent dyes such as melamine and Rhodamine 6G, with spatially-resolved spectra being obtained by SERS along the direction of the separation that occurred on the UTLC plate [88]. The same general approach was used to analyze polyaromatic hydrocarbons in cooking oils, giving a chip-based technique that could be used for both sample cleanup and analysis [111]. In addition, gold nanoparticles have been placed onto aminopropyl silica by Amoli-Diva and Pourghazi for use in planar chromatography and the separation of steroids in human urine [112].

Brett and co-workers developed nanostructured thin films made by GLAD and based on silica for use in UTLC; these thin films have been tested for use in the separation of
lipophilic dyes by the normal-phase mode of LC [89]. An unusual feature of these materials was the effect of anisotropy in the nanostructures on movement by the analytes and on the system’s efficiency [89]. This effect was examined in more detail by the same group with the aid of modeling studies [113]. Another report by Brett and co-workers used nanostructured UTLC plates based on silica and made by GLAD, which were then modified by reactive ion etching and annealing to vary the migration velocity, retention, and separation performance that were obtained for lipophilic dyes [54]. In a study by Kirchert et al., UTLC plates made with GLAD and containing silicon dioxide nanostructured thin films were coupled with electrospray ionization mass spectrometry to separate and characterize sugars [114]. UTLC plates have been made by Hall et al. that contained silica nanopillars made by GLAD; these plates were reacted with octadecyltrichlorosilane and treated with oxygen plasma to tune the polarity of the plates for use in reversed-phase separations, as evaluated by using dye mixtures [90].

Some nanomaterials for UTLC have been made by combining the methods of GLAD and ALD [13,115]. It was found in one report by Brett and co-workers that the surface area of the resulting media depended on the ALD coating thickness of aluminum oxide, as examined through its effect on the separation of lipophilic dyes [13]. UTLC plates have also been made by Peev et al. using GLAD to deposit microstructures of silica as slanted columnar thin films on glass, followed by the use of ALD to coat these structures with alumina. These materials have been used in combination with anisotropic contrast optical microscopy to simultaneously separate and detect lipophilic dyes on small UTLC plates [115].

A number of alternative techniques have been explored for making inorganic nanomaterials for planar chromatography. Silica nanopillar arrays have been made for this purpose by Kirchner et al. through the use of electron beam lithography and the thermal
dewetting of platinum \[116\]. These nanopillars were modified with octadecyltrichlorosilane for use in the reversed-phase separation of fluorescent dyes, some derivatized amines, and the drugs daunorubicin and adriamycin \[116\]. This type of stationary phase was found to exhibit unique behavior in terms of transport of the solvent and analytes, which resulted in low volume separations to be achieved that also had high efficiencies \[116\]. Silica nanofibers have been placed in polyvinylpyrrolidone by Newsome and Olesik and utilized to make silica/polymer nanofibers by electrospinning. These nanomaterials were then used directly in UTLC or treated further with heat to provide cross-linking or calcination prior to their use in the separation of dye mixtures or amino acids, with an efficient separation for the amino acids being obtained in a travel distance of only 15 mm on the UTLC plates containing calcined nanofibers \[53\].

9 Conclusion

This review examined the types and applications of nanomaterials that have been explored for use as stationary phases or supports in LC. As shown in Section 2, much of this work has used carbon-based nanomaterials such as CNTs, fullerenes, or nanodiamonds, along with some organic polymer-based nanomaterials. However, a number of inorganic nanomaterials have also been employed, including those based on silica, alumina, zirconia or titanium oxide, as well as gold or silver nanoparticles. Both small and large mass analytes have been used to evaluate the potential applications of nanomaterials in LC. These analytes have ranged from dyes, drugs and various classes of organic compounds to amino acids, peptides and proteins. Some advantages noted in these applications have been the variety of interactions certain nanomaterials can have with analytes (e.g., as occurs with CNTs, fullerenes and nanodiamonds) \[27,33,34,38-40,42,45\] and the unique retention or elution properties of nanomaterials in some separation formats (e.g., in mixed-mode or multi-
dimensional separations and UTLC) [30,45,89,107]. The ability to control the surface area or amount of such a material in an LC system can also be useful in adjusting retention or efficiency [31,73,76,77,104,105,107,108]. Many nanomaterials have been demonstrated to have good chemical and physical stabilities when used in LC [29,61,64,73,83,86], although this has not been true in all situations [91].

Several means for incorporating nanomaterials in LC systems were described in Section 3. These methods included covalent immobilization, adsorption, entrapment, and the synthesis or direct development of nanomaterials as part of a chromatographic support [3,31,36,67-69,73-84,87-90]. The direct use of some nanomaterials as LC supports has also been possible in a few situations [42,86]. It was noted that each of these approaches has its advantages and disadvantages. For instance, covalent immobilization tends to produce more stable supports than when using adsorption or entrapment; however, adsorption and entrapment tend to be easier to use [33,67,83,84] and entrapment can be used directly with many nanomaterials, or after only minor modifications of these materials [33,73,83,84].

Several techniques for creating nanomaterials directly on or within a support have also been considered, and particularly in work with planar methods such as UTLC [31,87-90]. Many of these latter methods require specialized equipment [13,53,88-90] and only some (e.g., electrospinning) have been evaluated in terms of their cost and general ease-of-use [53]. Thus, it is expected that future research will not only continue to explore new approaches for placing nanomaterials in LC systems but will also require a closer evaluation of how such methods can be used to routinely produce stable supports in a convenient and cost-effective manner.

Much of the work with carbon-based nanomaterials in LC has involved their use in reversed-phase LC and as alternatives to traditional supports containing C_8 or C_{18} groups as the stationary phase (see Section 4). Several of these reports have made use of the additional
interactions a number of these nanomaterials can have with analytes and the ability to make some of these nanomaterials with high surfaces or large amounts of material in the column. This, in turn, has led to a possible increase in retention, efficiency and/or selectivity for these media when compared to standard reversed-phase supports [28,29,31-33,38,73,82]. Many inorganic nanomaterials have also been considered for use in reverse-phase LC. Supports containing modified gold nanoparticles have received the most attention [61,64,76,12,92]; however, some reports have examined the use of modified silica nanoparticles [55,86], zirconia nanoparticles [56] or titanium oxide nanoparticles [51,52] in reversed-phase separations.

A number of nanomaterials have also been explored for applications based on normal-phase LC, ion-exchange chromatography, and affinity chromatography (see Sections 5-7). This has included work with related separation modes such as HILIC, ion-pair chromatography and chiral separations. Most of the research in all LC modes has involved the use of nanomaterials in a traditional column format, as described throughout Sections 4-7, followed by reports that have used nanomaterials in planar techniques such as TLC and UTLC (see Section 8) [13,89,103,104]. Several reports have used nanomaterials in capillaries or monoliths [40,61,76,83,92,93], and one study has employed silica nanoparticles for UPLC [86]. Other studies have examined the use of nanomaterials in capillary electrochromatography [64], mixed-mode techniques [65,81] and multi-dimensional separations [107]. Because of the advantages of these nanomaterials and the variety of separation modes or formats in which they can be used, it is expected that even more applications and formats will appear in the future as work continues in the creation of new forms for nanomaterials and in improved ways for using them within LC systems.

10 Acknowledgments
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11 Conflict of interest statement

The authors have no financial or commercial conflicts of interest to declare regarding the material presented in this paper.

References


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Figure Legends

Figure 1. Immobilization of a fullerene onto a silica support that was activated with a silane containing perfluorophenyl azide (PFPA). Reproduced with permission from Ref. [71].

**Figure 2.** Reversed-phase separations obtained at 1 mL/min for mixtures of (a) alkylbenzenes or (b) proteins on 100 mm × 4.6 mm I.D. C$_{18}$ organic monoliths that contained entrapped MWCNTs. The solutes in (a) were separated under isocratic conditions and were as follows (1) toluene, (2) ethylbenzene, (3) propylbenzene, (4) butylbenzene, (5) amylbenzene, (6) hexylbenzene, and (7) heptylbenzene. The proteins in (b) were separated using a linear acetonitrile gradient and had the following identities: (1) ribonuclease A, (2) cytochrome C, (3) lysozyme, (4) transferrin, (5) bovine serum albumin, (6) β-lactoglobulin A, and (6) ovalbumin. Adapted with permission from Ref. [33].
**Figure 3.** Scheme for the use of gold nanoparticles immobilized within thiol-activated GMA/EDMA monoliths for (left) the HILIC-based enrichment of glycopeptides from protein digests and (right) the on-line deglycosylation of these glycopeptides by using immobilized peptide-N-glycosidase F (PNGase F). Reproduced with permission from Ref. [93].
**Figure 4.** Separation of organic acids by ion-pair chromatography on a 100 mm × 4.6 mm I.D. column containing polystyrene-divinylbenzene beads that contained entrapped MWCNTs. These results were obtained at 1.0 mL/min and in the presence of a 80:20 water:acetonitrile mobile phase mixture that contained 5.0 mM tetrabutylammonium hydroxide as an ion-pairing agent. The peaks shown in this chromatogram were due to the following analytes: (1) ascorbic acid, (2) p-hydroxybenzoic acid, (3) D-mandelic acid, (4) 3,4-dimethoxyhydrocinnamic acid, (5) 3,4-dimethoxycinnamic acid, (6) 4-toluene sulfonyle chloride, (7) 2-methyl-3-nitrophenyl acetic acid, (8) salicylic acid, (9) m-nitrocinnamic acid, (10) p-iodobenzoic acid, and (11) p-chlorocinnamic acid. Adapted with permission from Ref. [73].
Figure 5. (a) Chiral separation for the enantiomers of chlorpheniramine on a monolith column containing SWCNTs and (b) proposed mechanism for this separation based on the interactions of chlorpheniramine with a SWCNT. The results in (a) were obtained in a 45:55 methanol/water mobile phase mixture containing 0.1% trifluoroacetic acid and on a 200 mm × 150 µm I.D. column at a flow rate of 0.3 µL/min. Adapted with permission from Ref. [101].
Figure 6. Mass spectra acquired for phosphorylated peptides that were isolated from a tryptic digest of α-casein as obtained by using (a) a column containing titanium dioxide (TiO₂) nanoparticles coated onto a core of silica spheres or (b) commercial titanium dioxide spheres. The mass peaks due to phosphopeptides are indicated by asterisks (*), and the number of the asterisks by each peak indicate the number of sites that were phosphorylated on the given peptide. Oxidized phosphopeptides are denoted by quotation marks (“”), and the internal standard is indicated by “I.S.” Adapted with permission from Ref. [51].
Figure 7. Use of ULTC plates containing electrospun glassy carbon nanofibers for the separation of (a) a mixture of the dyes sulforhodamine 640 (S640), rhodamine 610 perchlorate (Rh610P), and pyromethene 597 (P597); or (b) separation of the amino acids lysine (Lys), phenylalanine (Phe), and threonine (Thr). The total development distance was (a) 15 mm or (b) 20 mm and the development time was approximately 5 min. Adapted with permission from Ref. [104].
Table 1. Nanomaterials used as stationary phases or supports in liquid chromatography

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<th>General type of nanomaterial(^a)</th>
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\(^a\)The figures shown for the carbon-based nanomaterials are (top) C\(_{60}\) fullerene and (bottom) single-walled or multi-walled carbon nanotubes. The figures shown for the inorganic nanomaterials are (top) nanostructured columns coated with a material such as aluminum.
oxide and (bottom) gold nanoparticles coated on graphene-modified silica. These figures were adapted with permission from Refs. [3,11-13].